Impact of copper sulphate on the general ethology and respiratory surveillance in freshwater exotic carp, *Cyprinus carpio var. communis* of Mansbal lake of Kashmir valley

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Abstract: *The uncontrolled use of pesticides, herbicides and weedicides is the major cause of heavy metal pollution in the Mansbal Lake. Heavy metal pollution in the lake has been found to causes serious ill effects in aquatic organisms and has disturbed the lake ecology to a great extent. The toxicity of copper sulphate to fresh water exotic carp Cyprinus carpio var. communis was studied using static bioassay method. The LC_{50} in 96h was found to be 33µ/L.Ethological changes reported in the exposed fish are, increased opercular movements, excessive mucus secretion ,increased surface behavior, loss of equilibrium, change in body coloration ,irregular swimming activity, rapid jerky movements and aggressiveness. The swimming behavior was in cork-screw palter, rotating along horizontal axis. In sub lethal treatment, the schooling behavior of the fish was also found to disrupt. Significant increase in the ventilation rate was also reported. The fish at 21^{st} day of exposure showed balanced swimming and active feeding and behaved normally. Oxygen consumption by the fish was found to be decreased in lethal concentration(- 22.64 to- 70.13%) ,but in sub lethal concentration decreased trend was found to improve and approached normal level at 21^{st} day(-25.10 to -2.19).The alteration in oxygen consumption is a direct consequence of respiratory distress leading to impairment in oxidative metabolism. When fish were exposed to sub lethal concentrations of copper sulphate , they were found to be under tremendous stress, but that was not fatal.*

Key Words: *Copper sulphate, Toxicity, Ethology, L 50, Cyprinus carpio var. communis , Mansbal Lake.*

1. INTRODUCTION:

The problem of heavy metal pollution in the Mansbal Lake has been a main concern of researchers who investigate the impact of heavy metal pollution on the icthyofauna. This lake has witnessed tremendous change in its ecology from the past one and a half decade due to the heavy usage of pesticides, herbicides and weedicides based on heavy metals particularly on copper. The neighboring villages of the Mansbal Lake spray heavy quantities of pesticides, weedicides and herbicides in their paddy and orchid fields .Most of these are substandard and are known to contain copper sulphate. A good amount of copper sulphate reaches the Mansbal Lake due to run off. This has resulted in the heavy metal pollution of the lake particularly by copper.Copper sulphate is also used as an algaecide for controlling phytoplankton and other weeds(Carbonell & Tarazona, 1882) in the lakes and ponds. Heavy copper intoxication causes mortality of fish fingerlings and weakens their condition, resistance & viability (Jezierska and Witesca). Intensive improved agricultural practices based upon heavy usage of copper sulphate based pesticides, herbicides & weedicides has increased the concentration of copper in Mansbal aquatic ecosystem & effected the fish particularly the *Cyprinus carpio* var. communis.The present study deals with the impact of copper sulphate on the general ethology of the *Cyprinus carpio var. communis* .Respiratory distress as a result of copper sulphate pollution was also a subject of study in the present investigation.

2. MATERIAL AND METHODS:

Adult live fish,*Cyprinus carpio var. communis* were collected from the local fishermen of Hanji Mohalla residing near the Mansbal lake.These fish were brought to the laboratory and were cleaned by using 0.1% $KMno_4$ to avoid any dermal infection. Only healthy fishes (11-14cm in length & 45-50 g in weight) were selected for the experiment. Fishes were acclimatized in the glass aquaria for 15 days and were fed with fish food (earthworms). The water in the aquaria was replaced after every 24^{th} hour.

2.1. Physiochemical parameters of water

The physiochemical parameters of water used for fish bioassay were carried according to the methods described in Standard Methods(APHAAWWA-WEF,1998) .The water quality parameters were as follows : temperature 27±1 $C^{0,}$ salinity 193mg/L ,pH 7.5 , dissolved oxygen 6.8 t0 7.4 mg/L , chloride 45.3mg/L , sodium 1.19mg/L , potassium

30.2mg/L ,calcium 16.04mg/L, magnesium 1mg/L , carbon dioxide 8.0 mg/L, Hardness 113 mg/L, oxygen percentange <R> saturation 57 mg/L and specific gravity 1.00374.

2.2. Toxicant selected and preparation of stock solution

In the present study, copper sulphate($CuSo_4$) was used as a toxicant . The stock solution of copper (100mg/L) was prepared according to the method prescribed in the Standard Methods (APHA-AWWAWEF, 1980).For experimental purpose the required copper concentration was drawn from the prepared stock solution.

2.3. Toxicity Evaluation

The percent mortality of fish in different concentrations of copper sulphate was determined at 96h exposure. For this purpose the experimental fish were divided into batches of ten each, and were exposed to different concentrations of copper sulphate ranging from 10µg/L to 55µg/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 concentrations after 96 hours. A batch of fish was also maintained simultaneously in fresh water medium without copper, 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 LC_{50} copper sulphate was taken in the present study.

Table 1:Toxicity of copper sulphate on *Cyprinus carpio var.communis* of Mansbal lake

S.No	Conc.of toxicant	Log conc. Of toxicant	Number of fish exposed	Alive	Dead	% Mortality	Probit Mortality
1	10	1.0000	10	10	0	0	0.00
2	15	1.1751	10	9	1	10	3.27
3	20	1.3010	10	8	2	20	4.16
4	25	1.3978	10	7	3	30	4.48
5	30	1.4771	10	6	4	40	4.74
6	35	1.5439	10	4	6	60	5.25
7	40	1.6021	10	3	7	70	5.52
8	45	1.6530	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

2.4. Fixation of exposure periods

In order to understand the influence of time over toxicity effect of lethal concentration of copper sulphate on *Cyprinus carpio var. communis* 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 (11-14cm in length & 45-50 g in weight) .They were fed with commercial fish food pellets during acclimatization. After 15 days, if fishes were in normal behavior activity and good health conditions, those species were selected for experimental purpose. The fish were divided into two groups. One group without copper sulphate served as control and the other group was exposed to lethal concentration of copper sulphate for 1,7,14 and 21 days were chosen to observe the long term effects .During this experiment the behavioral changes were critically observed.

3. RESULTS:

***3.1. Toxicity Studies*:**

The percentage mortality of *Cyprinus carpio var.communis* was observed to be 0% and 100% at copper sulphate concentration of 10µg/L and 55µg/L (Table 1). The LC $_{50}$ value obtained was verified using Dragstedt and Behren's equation and was found to be 34 µg/L. Thus the average LC_{50} for 96h was found to be 33µg/L.

3.2. Behavioral observation – Control fish

Normal fish maintained a fairly compact school, covering about one third of the bottom during the first seven days of 21 days experiment. By 7th day , the school became less compact covering up to two third of the tank area. Fishes were found to scrap the bottom surface. When startled, they instantly formed a tight school that was maintained briefly. They were found sensitive to light and moved to the bottom once light was passed into the tank. Except a less *response to form a dense school towards the end of the study, no other extraordinary behavior was observed.*

3.3. Behavioral observations – Exposed fish

The behavioral changes were observed in the fish when exposed to copper sulphate.The fish showed erratic swimming, hyper and hypoactive, imbalance in posture, increase in surface activity, opercular movement, gradual loss in equilibrium and spreading of excess of mucus over the body surface. The fish occupied twice the area than that of 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 operculum wide open. A change in color 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 behavior 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 copper sulphate 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.

3.4. Oxygen consumption

The rate of whole oxygen consumption of control and copper sulphate treated fishes are presented in table 2. The data indicates that in fish exposed to lethal and sub lethal concentrations of copper sulphate , oxygen consumption was reduced .The whole oxygen consumption was reduced by about 22.64 per cent on day one and reached maximum reduction of 70.13 percent 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 1 to day 2, from then it was gradual reduction. However in sub lethal concentrations, the day 1 showed high rate of decrease of 25.10 percent, which reached 19.22 by day 7. Subsequently, there was an improvement in oxygen consumption as by day 14, it was reduced to 12.92 percent and day 21 showed minimal decrease which was recorded at 2.19 percent. All the results are significant at 0.05, levels.

Table 2:Oxygen consumption of *Cyprinus carpio var.communis* of Mansbal lake exposed to Copper sulphate

Me-	Control	1	2	3	4	1	7	14	21
-a- -n- -%-	0.8234	0.6534	0.5557	0.4433	0.2459	0.6167	0.6651	0.7170	0.8053
- change S-		- 20.6440	- 32.5123	=46.1677	- 70.1309	- 25.1067	- 19.2232	- 12.9288	-2.197
-D	0.0030	0.0011	0.0018	0.0011	0.0012	0.0017	0,0020	0,0010	0.0042

4. DISCUSSION:

In the field of aquatic toxicology, fishes have an important role in toxicity testing and hazardous evaluation (Annon,1972).The assessment of toxicity of copper sulphate with reference to aquatic biota , especially fish is crucial in establishing the toxicity evaluation. The LC_{50} 96 hrs value of copper sulphate to *Cyprinus carpio var. communis* was found to be 33µg/L(Table.1). This data clearly indicates that copper sulphate is toxic to *Cyprinus carpio var. communis* as to other fish species, copper sulphate 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). Various symptoms of poisoning can be observed from studies involving the determination of LC_{50}.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 sulphate they became very 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 reported .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 the loss of equilibrium and rolling of body, they also exhibited convulsions prior to death. The fish very often come to the surface in order to avoid toxic environment .Moreover, examination of gill of dead fish revealed that the gill lamellae color was changed from red to brown.

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 spiraled 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 .The mean LC50 value of copper sulphate estimated in the present investigation was 33µg/L(Table 1).The unusual behavior of the fish , *Cyprinus carpio var.communis* in stress condition may be due to obstructed functions of neurotransmitters. The gill opercular movements increased considerably 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 in 1995 and Muniyan and Veeraraggahavan ,1999,Chaudhary*et al.,2001*.The excessive secretion of mucus over the gills was found to inhibit the diffusion of oxygen during the process of gaseous exchange. It suggests that the copper sulphate is not safe to non-target organisms like fishes. In order to protect whole aquatic ecosystem, awareness must be provided to the farmers so as to control the agricultural pests by natural biological methods.

The behavioral changes are the manifestation of motivational, biochemical, physiological and environmentally influenced state of the organism. The migration of the fish to the bottom of the tank following the addition of the copper sulphate clearly indicates the avoidance behavior of the fish, which was reported by Murthy(1978) in trout. The opercular movement of the fish was found to ceases immediately following exposure to copper. 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 copper through gill chamber (Santhakumar and Balaji;2000;Prashanth &Patil,2006).The increased ventilation rate by rapid , repeated opening & closing of mouth & opercular coverings accompanied by partially extended fins (coughing) was observed in the present study. This could be due to clearance of accumulated mucus debris in gill region for proper breathing, which was suggested by Prashanth *et al., 2005.*The erratic swimming of treated fish indicates loss of equilibrium.

It is likely that the region in the brain, which is associated with the maintenance of equilibrium, might 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 severity of these movements varied with pesticide concentration. The surfacing phenomenon of fish observed under copper sulphate exposure might 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 behavior such as nudge and nip were found to increase following exposure to the toxic material. Orientation and locomotor patterns were found to be involved in most aspects of fish behavior 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 hyper excitability of the fish invariably in the lethal exposure to free copper/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 copper 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 copper is time dependent. Since most fish breathe water in which they live, changes in the chemical properties thereof 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 copper sulphate affects the oxygen consumption of the fish *Cyprinus carpio var.communis* 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). In addition to gill damage decrease in hemoglobin 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 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 disturbance in 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 sulphate. 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 sulphate 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 sulphate. If so, the lowering of the oxidative metabolism in *Cyprinus carpio var.communis* might have been compensated at least by some degree of glycolysis. Behavioral characteristics are obviously sensitive indicators of toxicant effect. It is necessary, however, to select behavioural indices of monitoring that relate to the organisms behavior 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 underestimation. The inhibition of oxygen consumption is explained by this manner in which copper sulphate is incorporated into this fish system for energy. There is an indispensable need to evaluate more toxicity data for wide range of animal groups of the aquatic eco-web in order to understand the broad spectrum of copper sulphate 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.

5. CONCLUSION:

From the above discussion it is concluded that copper sulphate causes deleterious effects on fishes and alters their behavioral and 0_2 consumption characteristics .In sub lethal concentration it may not be fatal for an individual organism but it does affect the growth rate and reproduction resulting in disturbance to whole community and tropic levels of food chains, ultimately the ecosystem.

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