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RESEARCH ARTICLE

Behavioral and Respiratory Responses of the Freshwater Fish, *Cyprinus carpio* (*Linnaeus*) exposed to Cadmium Chloride

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ABSTRACT

Discharge of heavy metals into aquatic environment from various sources even below permissible levels creates health hazards in aquatic organisms. Short term definitive test by static renewal bioassay method was conducted to determine the acute toxicity (LC_{50}) of commercial grade heavy metal, Cadmium Chloride on the freshwater fish, *Cyprinus carpio*. Common carp fingerlings were exposed to different concentrations (9 mg/L to 14 mg/L) of Cadmium Chloride for 96 h. The acute toxicity value was found to be 14 mg/L and one fifth of LC_{50} (2.8 mg/L) was selected for sub acute studies. Behavioural patterns and Oxygen consumption were studied in lethal (1, 2, 3 and 4 d) and sub lethal concentrations (1, 5, 10 and 15 d). Common carp in toxic media exhibited irregular, erratic and darting swimming movements, hyper excitability, capsizing, attaching to the surface, difficulty in breathing, loss of equilibrium, gathering around the ventilation filter, lack of body coloration, bloody fins and sinking to the bottom which might be due to inactivation of AChE activity which results in excess accumulation of acetylcholine in cholinergic synapses leading to hyper stimulation. Variation in oxygen consumption (-22.2 to +79.6; -39.4 to 8.9%) was observed in both lethal and sub lethal concentrations of Cadmium Chloride, respectively. 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 mortal.

Key words: Cadmium Chloride, Toxicity, Behaviour, Oxygen Consumption, *Cyprinus carpio*

INTRODUCTION

One of the most critical environmental problems of the century was pollution of rivers and streams with chemical contaminants. Some of these chemicals are biodegradable and quickly decay into harmless or less harmful forms, while others are non-biodegradable and remain dangerous for a long time. Cadmium and Zinc concentration were determined in hepatopancreatic tissues of *Mytilus galloprovincialis*, reared from the Gulf of Italy [1] and likewise many investigations were carried with different Biological parameters in the Rainbow Trout *Oncorhynchus mykiss* [21]. It is important to note that Cadmium is a highly toxic element for all mammals and fish. Now, there is a growing concern worldwide over the indiscriminate use of such chemicals, resulting in environmental pollution and toxicity risk to aquatic organisms. Thus, ecological damage of the environment caused by anthropogenic factors as well as the presence of hazardous agents may affect fish. Fish are an important source of protein for millions of people worldwide. People consume about 70% of fish caught, and nearly 30% are used as animal feed that helps to produce other forms of protein. And the part of some experiments in which expressed presently accumulation of heavy metals in kidney and heart tissues of *Epinephelus Microdon* fish from the Arabian Gulf [3]. Fish are widely used to evaluate the health of aquatic ecosystems because pollutants build up in the food chain and are responsible for adverse effects and death in the aquatic systems. The studies carried out on various fishes have shown that heavy metals may alter the physiological activities and biochemical parameters both in tissues and in blood. The toxic

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effects of heavy metals have been reviewed, including bioaccumulation.

Any change in the behaviour and physiology of fish indicates the deterioration of water quality, since; fish are the biological indicators of water quality. Hence, the present study was under taken to evaluate the aquatic toxicity of Cadmium Chloride with special emphasis on behavioural and Oxygen consumption of the freshwater teleost, *C. carpio* exposed to lethal and sub lethal concentrations of commercial grade heavy metal Cadmium Chloride and Cadmium has gained wide interest in the scientific community in recent years due to its potential human health hazards.

MATERIALAS AND METHODS

Young juvenile healthy and active *C. carpio* (2.0 ± 2.5 g; 4-5 cm) fingerlings were procured from the State Department of Fisheries, Tumkur, Karnataka, India. Carps were acclimatized to laboratory conditions for 15 days at 24 ± 1 °C and are held in large glass aquaria containing dechlorinated tap water of the quality used in the test, whose physico-chemical characteristics were analyzed following the methods mentioned in APHA [2] and found as follows, temperature 29 ± 1 °C, pH 8 ± 0.2 at 26.3 °C, dissolved oxygen: 8.7 ± 9.2 mg/L, carbon dioxide: 6.3 ± 0.4 mg/L, total hardness 23.4 ± 3.4 mg as $CaCO_3$/L, phosphate 0.39 ± 0.002 µg/L, Average Salinity was 0.189% on day one and 0.290 % on the last day, specific gravity 1.0030 and conductivity less than 10 μScm^{-1}. Water was renewed every day and 12-12 h of photoperiod was maintained daily during acclimation and test periods. Fish were fed regularly with commercial fish pellets (40% protein content) procured from market. During acclimation, feeding was stopped a day prior to exposure of test medium.

Concentrations of the test compound heavy metal $CdCl_2$ used in short term definitive tests were between the highest concentration at which there was 0% mortality and the lowest concentration at which there was 100% mortality in the range finding tests. Replacement of the water medium was followed by the addition of the desired amount of the test material. Fish were exposed in batches of ten to varying concentrations of cadmium chloride with 20 L of water in six replicates for each concentration along with a control group.

Commercial grade heavy metal ($CdCl_2$) was procured from the local market of Bangalore, Karnataka, India, under the trade name Thermo Fisher Scientific India Pvt. Ltd., Supplied by Vasa Scientific Co., Bangalore, Karnataka, India. The expiry date of the test substance checked prior to initiation of the treatment was found suitable for the exposure. Required quantity of Cadmium Chloride was pinched directly from stock concentrated solution by using variable micropipette.

For LC_{50} calculation, mortality was recorded every 24 h and dead fish were removed when observed, every time noting the number of fish deaths at each concentration up to 96 h. Duncan's Multiple Range Test (DMRT) was employed for comparing mean mortality values after estimating the residual variance by repeated measures ANOVA [23] for arc sine transformed mortality data (dead individuals/initial number of individuals). Time of exposure was the repeated measure factor while treatment (concentration and control) was the second factor. In addition, LC_{50} were compared by the method of APHA [2]. The LC_{50} with 95% confidence limit for cadmium chloride were determined/estimated for 96 h by probit analysis [9].

One fifth of the LC_{50} (2.8 mg/L) was selected as sub lethal concentration for chronic study (1, 5, 10 and 15 d). The control and Cadmium Chloride exposed fishes were kept under continuous observation during experimental periods. The whole animal Oxygen consumption was measured for lethal and sub lethal concentrations besides control by following the method of [22] as described by [18].

Each experiment was repeated four times and the mean value was calculated. The data obtained were analyzed statistically by following DMRT [6].

RESULTS

There is no evidence that cadmium at any concentration is essential or beneficial to living organisms, but this metal accumulates in the tissues of a wide variety of marine organisms [7, 4]. A number of studies have reported the toxicity of small amounts of this metal to marine animals [13, 20]. Acute toxicity of Cadmium Chloride for the freshwater fish, *C. carpio* was found to be 14 mg/L. The upper and lower 95% confidence limits were found to be 12.87 mg/L and 16.55 mg/L respectively (**Table 1**). It is evident from the results that the Cadmium Chloride can be rated has highly toxic to fish.

In the present study the control fish behaved in natural manner i.e., they were active with their well-coordinated movements. They were alert at slightest disturbance, but in the toxic environment fishes exhibited irregular, erratic and darting swimming movements, hyper excitability, capsizing, attaching to the surface, difficulty in breathing, loss of equilibrium, gathering around the ventilation filter, lack of body coloration, bloody fins and sinking to the bottom which is due to inhibition of AChE activity leading to accumulation of acetylcholine in cholinergic synapses leading to hyperstimulation [14]. They slowly became lethargic, hyper excited, restless and secreted excess mucus all over the body. Mucus secretion in fish forms a barrier between body and toxic media thereby probably reduces contact of toxicant so as to minimize its irritating effect, or to eliminate it through epidermal mucus. Similar observations were made by [17] and [16]. Opercular movements increased initially in all exposure periods but decreased further steadily in lethal exposure compared to sub lethal exposure periods. Increased gill opercula movements observed initially may possibly compensate the increased physiological activities under stressful conditions [19].

DISCUSSIONS

Moreover the effect of $CdCl_2$ the fact that heavy metals cannot be destroyed through biological degradation and have the ability to accumulate in the environment make these toxicants deleterious to the aquatic environment and consequently to humans who depend on aquatic products as source of food and likewise biomarkers of Oxidative stress and Heavy metal levels as Indicators of environmental pollution in African cat fish *Clarias gariepinus* from Nigeria Ogun River [8]. Gulping of air may helps to avoid contact of toxic medium. Surfacing phenomenon i.e., significant preference of upper layers in exposed group might be a demand of higher oxygen level during the exposure period [11]. Finally fish sunk to the bottom with their least opercular movements and died with their mouth opened.

In sub lethal exposure fish body became lean towards abdomen position compared to control fish and was found under stress, but that was not fatal. Leaning of fish indicate reduced amount of dietary protein consumed by the fish at heavy metal stress which was immediately utilized and was not stored in the body weight [10].

Variation in oxygen consumption is the indicator of stress, which is frequently used to evaluate the changes in metabolism under environmental corrosion. It is clearly evident from the studies (**Table 2**), that Cadmium Chloride affected oxygen consumption of *C. carpio* under lethal and sub lethal concentrations. Fish exposed to lethal concentration depicted increased Oxygen consumption on day 1 to day 3 and decreased on day 4. In sub lethal exposure oxygen consumption increased on day 1 and decreased on day 5 and 10 but day 15 witnessed least decrement as compared to control (**Figure 1**). The rate of Oxygen consumption in lethal and sub lethal medium were found in the order of 1>2>3>4 and 1>2>3<4 respectively.

Since most fish breathe in water in which they live, changes in the chemical properties thereof may be reflected in the animal's ventilatory activity, particularly if the environment factors affect respiratory gas exchanges [15]. Gills are the major respiratory organs and all metabolic pathways depend upon the efficiency of the gills for their energy supply and damage to these vital organs causes a chain of destructive events, which ultimately lead to respiratory distress [12]. Pronounced secretion of mucus layer over the gill lamellae has been observed during cadmium chloride stress. Secretion of mucus over the gill curtails the diffusion of oxygen [5], which may ultimately reduce the oxygen uptake by the animal.

Table 1: Median lethal concentration (LC_{50}), slope and 95% confidence limits of Cadmium Chloride to the freshwater exotic carp, *C. carpio*

Heavy metal	96 h LC_{50} value (mg/L)	Slope	95% Confidence limits	
			Upper limit	Lower limit
Cadmium Chloride	12.87 ± 16.55	5.54	12.87	16.55

Table 2: Oxygen consumption (ml of oxygen consumed/g wet weight of fish/h) of the fish, *C. carpio* following exposure to lethal (14 mg/L) and sub lethal (2.8 mg/L) concentrations of Cadmium Chloride

Estimations	Control	Exposure periods in days							
		Lethal				Sub lethal			
		1	2	3	4	1	5	10	15
Oxygen consumption	0.26^f	0.47^a	0.42^b	0.27^e	0.21^h	0.28^d	0.24^g	0.16^i	0.29^c
± SD	0.012	0.010	0.006	0.014	0.011	0.008	0.015	0.010	0.007
% Change	-	79.6	59.3	3.5	-22.2	5.9	-10.1	-39.4	-8.9

Values are means ± SD (n = 6) for oxygen consumption in a column followed by a letter are significant (P ≤ 0.05) according to Duncan's Multiple Range Test

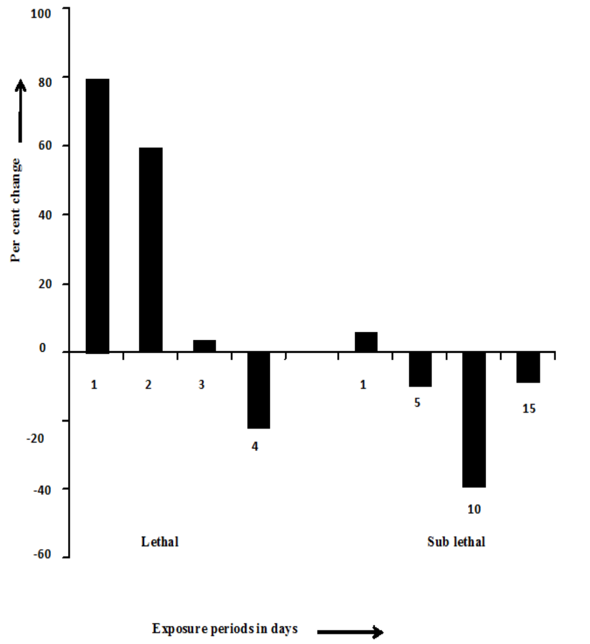

Figure 1: Oxygen consumption (ml of oxygen consumed/g wet weight of fish/h) of the fish, *C. carpio* following exposure to lethal (14 mg/L) and sub lethal (2.8 mg/L) concentrations of Cadmium Chloride

CONCLUSION

The results of the study evidenced that the analysis of data from the present investigation evidenced that Cadmium Chloride is highly toxic and had profound impact on behaviour and respiration in *C. carpio* in both lethal and sub lethal concentrations. Thus, it has led to the altered fish physiology. Variation in the oxygen consumption in Cadmium Chloride exposed fish is probably due to impaired oxidative metabolism and heavy metal induced stress. However the exact mechanism through which this is achieved needs to be studied further.

CONFLICT OF INTEREST STATEMENT

We declare that we don't have any sort of conflict of Interest.

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