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Estimation of median lethal concentration (LC_{50}) and behavioral alterations of amur carp (*Cyprinus carpio haematopterus*) in response to copper sulfate

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Abstract
The present investigation was carried out to determine the acute toxicity and behavioural responses of Amur carp (*Cyprinus carpio haematopterus)* fingerlings towards copper sulphate ($CuSO_4$). The fish were exposed in aquaria to different concentration of metal and the concentration of metal was increased gradually for 96 hours to investigate the response of the fish. Experimental fishes of average length of 7.92±0.46cm and weight of 8.20±0.28g were exposed to seven different concentrations of $CuSO_4$ with triplicates of each concentration. The median lethal concentration (LC_{50}) of copper to Amur carp for 96 h was found to be 1.811. With the increase of the concentration of metal the response of the fish mortality was also increased gradually. In addition, the behavioral changes of Amur carp at different metal concentrations were determined. Physiological responses like rapid opercular movement and frequent gulping of air was observed during the initial stages of exposure after which it became occasional. All these behavioral observations can be considered to monitor the quality of aquatic ecosystem and severity of pollution.

Keywords: Acute toxicity, amur carp, copper sulphate, LC_{50}

1. Introduction

Aquatic environment forms the major part of our environment and resources. The contamination of aquatic ecosystems has caused major environmental and health concerns worldwide because the aquatic environment is the ultimate sink of pollutants produced by natural and anthropogenic sources [1]. With increase in urbanization and industrialization, the industrial effluents containing heavy metals get enters into aquatic environment and causes physiological and biochemical disturbances in aquatic organisms. Heavy metals may affect organisms directly by accumulating in their bodies or indirectly by transferring to the next trophic level of the food chain [2]. Heavy metals become toxic when they are not metabolized by the body and accumulate in the soft tissues [3].

Acute toxicity is expressed as the median lethal concentration (LC_{50}) that is the concentration in water which kills 50% of a test batch of fish within a continuous period of exposure. Fishes are widely used to assess water quality of aquatic ecosystems and are quite sensitive to the wide array of pollutants discharged in the aquatic ecosystems as they serve as bioindicators of pollution [4]. There is a strong correlation between physiological changes with behavioural disruption, thus it provides ecological relevance to physiological measures of toxicity [5]. The concentration of heavy metals present in the body of fish depends upon food and feeding habits, trophic status, physico-chemical properties of water, metabolic rate of animal and the degree of toxicity of heavy metals [6]. Moreover, fish is a good bioaccumulator as it has the optimum size for analysis and a long lifespan and is easily obtained in large quantity to be sampled for accumulated metals [7, 8]. Bioaccumulation is a process where chemicals infiltrate an organism either through exposure to a contaminated medium or through consumption of food containing the chemicals [9].

2. Materials and Methods

The present investigation was conducted in the aquaria at the Wet laboratory of College of Fisheries, GBPUA&T, Pantnagar, Uttarakhand, India.

Amur carp fingerlings were collected from the hatchery of College of Fisheries, GBPUA&T, Pantnagar, Uttarakhand, India. Fingerlings were acclimatized for 15 days in the laboratory before conducting the acute toxicity tests. The experiment was conducted to examine LC_{50} and behavioral responses of Amur Carp with respect to copper concentration.

2.1 Preparation of stock solution: The stock solution of 1000ppm was prepared by dissolving 3.921g of $CuSO_4.5H_2O$ in 1000 ml of double distilled water. The test concentrations were prepared by diluting appropriate aliquots of the stock solution with distilled water.

2.2 Experimental Conditions: Acute toxicity tests were performed in glass aquaria with well aeration system. Glass aquaria were filled with 50 liter of tap water and stock solution was added on metallic ion basis to get the desired concentration of metal. To avoid stress to the fish, the desired metal concentration in each aquarium was attained within 7 hours of the start of the experiment.

2.3 Water Quality Parameters: All the physico-chemical parameters like temperature, total dissolved solids (TDS), pH, dissolved oxygen (Wrinkler's method), free CO_2 and alkalinity (titration method) were recorded individually in each test container at exposure times of 24, 48, 72 and 96 hr following APHA [10].

2.4 Fish Collection and Acclimatization: Fingerlings of *Cyprinus carpio haematopterus* of average length of 7.92±0.46cm and weight of 8.20±0.28g were procured from the Fish Seed Hatchery, Pantnagar and were acclimatized in the tank for one week prior to the experiment. Acclimated fish were not fed 24-hr prior to the start of the experiment and throughout acute toxicity tests.

2.5 Acute Toxicity Tests: The experiment was carried out at stocking density of 5 fish per aquarium. Fishes were exposed for 96 hours against 7 different concentrations of copper starting from 1ppm to 4ppm with an increment of 0.5ppm i.e. 1ppm, 1.5ppm, 2ppm, 2.5ppm, 3ppm, 3.5ppm and 4ppm.

2.6 Behavioural Responses and Mortality Data: Behavioural changes and physiological responses of the fish were observed after the exposure of various copper concentrations. Fish mortalities were recorded at an interval of 24, 48, 72 and 96-hr of exposure, and dead fish were removed immediately from the test media.

2.7 Statistical Analysis: Finney's Probit[11] analysis method was used to calculate 96-hrs LC_{50}. The 96-hr LC_{50} and lethal concentrations were determined at 95% confidence level

3. Results and Discussion

The present study aimed to find the lethal concentration of Amur carp to potentially hazardous heavy metal copper and to observe their behavioural and physiological responses at sublethal concentration.

3.1 Water Quality Analysis: Physico-chemical parameters play a very important aspect to use water for drinking, domestic, agricultural or industrial purpose [12]. To assess the quality of water, accurate and timely information on physico-chemical parameters is necessary[13]. So, during the whole experiment some important physico-chemical parameters like temperature, TDS, pH, dissolved oxygen, free CO_2 and alkalinity were recorded. It was observed that environmental parameters like temperature, dissolved oxygen, TDS and free CO_2 influence toxicity level of pollutant to fish [14]. The physico-chemical parameters of test water during the experimental period are shown in the table 1.

Table 1: Physico-chemical parameters of water during exposure of Amur carp to $CuSO_4$

S. No.	Parameters	Unit	Value±S.D.
1	Temperature	°C	24.48±0.38
2	Total Dissolved Solids	mg/L	264.00±0.58
3	pH	-	7.58±0.46
4	Dissolved oxygen	mg/L	6.10±0.52
5	Free CO_2	mg/L	0.80±0.30
6	Alkalinity	mg/L	316.00±32.00

3.2 Behavioural alterations: Behavioral alterations in animals are indicative of internal disturbances of the body functions. Behavior is a selective response that is constantly adapting through direct interaction with physical, chemical, social and physiological aspects of the environment [15]. Behavioral response of an organism links physiological function with ecological processes, so it seems ideal method for assessing the effect of aquatic pollutants on fish populations. There is a strong correlation between physiological changes with behavioural disruption, thus it provides ecological relevance to physiological measures of toxicity [5]. The intensity of change in observed behavioral patterns increased with increase in concentration of $CuSO_4$ in the treated water. The exposed fish were hyperactive and shows abnormal behavioural patterns like erratic swimming, occasional mouth openings, jerky movements and surfacing behavior. During sublethal concentration, the fish shifts from silvery to pale coloration with erratic swimming movement thereafter as the concentration approaches to lethal dose, fish becomes lethargic and settle at the bottom with bulged and hemorrhagic eyes. The behavioral patterns of Amur carp at 24hrs, 48hrs, 72hrs and 96hrs for different concentrations were presented in table 2. Behavioral changes at higher concentration might be due to manifestation of the disturbances in the physiological mechanism which is supposed to initiate, maintain and terminate the behaviour [16]. Nekoubin [17] also observed rapid opercular movement and frequent gulping of air during the initial stages of copper exposure to fish. Behavioral observations were recorded for air gulps, startle response, mode of swimming, schooling, equilibrium and general activity of fish during the experiment. Al-Tamimi and Al-Azzawi [18] observed the behavioral changes and morphological abnormalities of the control fish and the fish exposed to various concentrations of copper. The behavioral observations recorded were air gulps, altered mode of swimming and schooling, loss in equilibrium and general activity of fish during the experiment.

Table 2: Behavioral Patterns observed in Exposed Amur carp of experimental treatments

Doses of treatment	Behavioral Observations			
	24 hrs	48hrs	72hrs	96 hrs
Control	Silvery white with normal movement	Silvery white with normal movement	Silvery white with normal movement	Silvery white with normal movement
1 ppm	Pale silvery with normal movement	Pale silvery with slow movement	Erratic swimming movement	Sluggishness with sinking at bottom
1.5 ppm	Pale silvery with normal movement	Yellowish coloration with slow movement	Pale coloration, open mouth occasionally	Rapid opercular movement
2 ppm	Pale silvery with rapid movement	Dull coloration with gathering of fishes at the corners	Jerky movements with high stress	Mucous secretion, Rolling the body
2.5 ppm	Dull and pale silvery with slow movement	Dull coloration with gathering of fishes at the corners	Surfacing for gulping of air, mucous secretion from mouth and gills	Jerky movements with loss in equilibrium
3 ppm	Dull and pale coloration with slow movement	Pale silvery with slow movement	Erratic swimming movement	Jerky movements with loss in equilibrium
3.5 ppm	Dull coloration with gathering of fishes at the corners	Jerky movements with loss in equilibrium	Excessive mucous secretion, lesions on ventral side	Lethargy settle at the bottom with bulged and hemorrhagic eyes
4 ppm	Slow movement with gathering of fishes at the corners	Surfacing with rapid operculum movement, mucus deposition	Lethargy settle at the bottom with rapid opercular movement	Total mortality

3.3 Acute Toxicity Test: Mortality count was observed for 96 hrs and calculated at an interval of 24 hrs basis at different concentrations of $CuSO_4$ as shown in the Table 3. For estimation of LC_{50} different concentrations of $CuSO_4$ was taken from 1ppm to 4ppm, at time interval of 0.5 ppm respectively. Mortality count was found to be directly proportional to concentration of dose and exposure time. The LC_{50} value for $CuSO_4$ was calculated by Finney's Probit analysis method at 96 hours of exposure was found to be 1.811 using Microsoft Excel. The regression equation of mortality percentage of fish and $CuSO_4$ concentration showed a positive relationship. The log of concentration of dose corresponding to the mortality probit value represents the LC_{50} value. Microsoft Excel was used to find regression equation of the type Y=aX+b, where Y is mortality percentage and X is log of concentrations. The LC_{50} value was derived from the best fit line obtained. Figure 1 reveals the graphical representation of the probit kill against log of concentration. The results are in support of the findings that the rate of mortality for any fixed time increases with increase in concentration of dose, duration of exposure and regular mode of entrance of toxicant [19]. The wide variation in sensitivity of different species on different heavy metals depends on various factors like age, sex, weight and physical stage of animal. It was found that the mortality rate of fish increased with the increase in the lethal concentration [16].

Table 3: 96-hrs LC_{50} value of $CuSO_4$ calculated by Finney's Probit Analysis method

S. No.	Dose (ppm)	Log 10	Total no. of fishes	Dead fishes	Mortality (%)	Probit kill
1	1	0	15	3	20.00	4.16
2	1.5	0.176091259	15	4	26.67	4.39
3	2	0.301029996	15	7	46.67	4.92
4	2.5	0.397940009	15	10	66.67	5.44
5	3	0.477121255	15	11	73.33	5.61
6	3.5	0.544068044	15	13	86.67	6.13
7	4	0.602059991	15	15	100.00	8.09

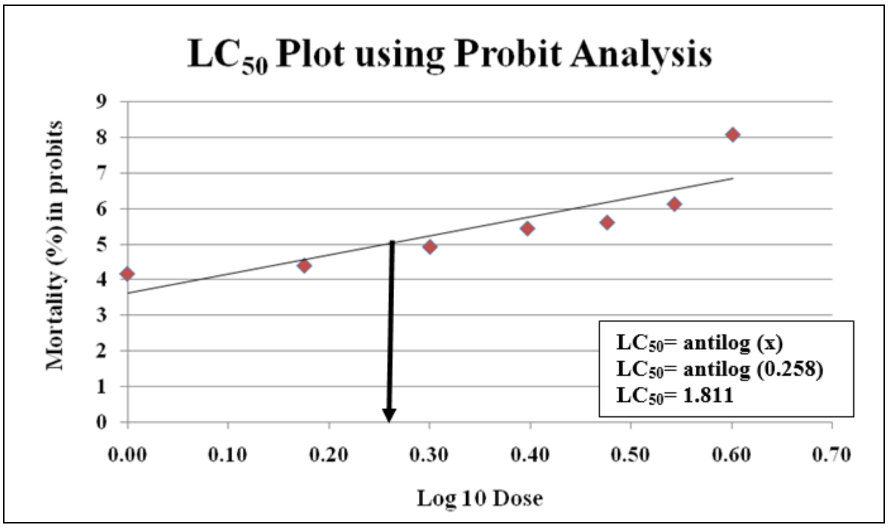

Fig 1: Plot of log concentration of $CuSO_4$ vs Probit Mortality values using Probit Analysis

4. Conclusion
In the present investigation, the 96 hr LC_{50} value for copper was found to be 1.811 ppm by Finney's probit analysis method of data evaluation for acute toxicity bioassay. According to the results of the present study, it was found that there was a positive relationship between the concentration levels and rate of mortality. When the concentration level increased, the mortality rate increased as well. Mortality was also related to the retention time of $CuSO_4$ in water, the more the retention time of the $CuSo_4$ in the water, the more the mortality rate of the fish. The current study also showed that the excessive copper concentration has profound effect on Amur carp, which is represented by noticeable changes in behaviour such as erratic swimming, rapid opercular movement, mucous secretion, rolling the body and jerky movements with loss in equilibrium. These behavioral alterations may caused due to manifestation of the disturbances in the physiological mechanism due to pollutant activity, which is supposed to maintain balance of the body.

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