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EVALUATION OF MEDIAN LETHAL CONCENTRATION (LC₅₀) OF *MYSTUS VITTATUS* (BLOCH) FOR COPPER SULPHATE

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ABSTRACT

The toxic effect of Copper on freshwater catfish *Mystus vittatus* was studied. The LC₅₀ values of copper in *Mystus vittatus* for 24, 48, 72 and 96 hours were 20.589, 16.497, 11.815 and 8.665 mg/L respectively. The cat fish was silvery grey in body in the control group throughout the experiment. The body colour changed from original silvery grey to dark colour in heavy metal treated fish. The fish maintained in freshwater behaved normal as usual. But when the fish was exposed to copper, erratic swimming, abnormal posture, dis-balance, sluggishness, imbalance in posture, increase in surface activity, opercular beating, and gradual loss of equilibrium and spreading of excess of mucus all over the surface of the body were observed.

Keywords: Acute toxicity, Behavioural changes, LC₅₀, Copper.

INTRODUCTION

Aquatic environment is plagued with various kinds of aquatic pollutants. The aquatic flora and fauna are affected by the toxic substances which eventually enter into their system or bring about external damage. Alterations in the chemical composition of the natural aquatic environment usually affect behavioral and physiological systems of inhabitants, particularly fish. Fish are very sensitive to a change in their environments and can play a significant role in assessing potential risks associated with contamination in aquatic environment of new chemicals. Several species of fish are susceptible to deleterious effects when exposed to heavy metals, pesticides and other environmental stressors (Stalin *et al.*, 2008). The damage caused by any toxicant or pollutant in an organism is called toxicity. Toxicity tests are experiments designed to predict the concentrations or doses of toxicant and its duration of exposure required to produce an effect (Cope *et al.*, 2004). Toxicity is species-specific because individuals have different levels of response to the same dose of a toxic substance (Smith and Stratton, 1986).

The toxicity bioassays are used to detect and to calculate the potential toxicological effects of chemicals on organisms. These tests provide a data base that can be used to assess the risk associated with a situation in which the organisms live. A variety of methods have been developed to evaluate the hazard and potential toxicity of chemicals to organisms, such as acute toxicity test, sub-acute toxicity test or chronic toxicity test. Acute toxicity is the severe effect suffered by an organism from short term exposure to toxic chemicals (Koprucu *et al.*, 2006). Arsenic toxicity in certain fishes was studied in detail by Prakash and Verma (2019a, 2019b, 2020a, 2020b, 2020c) and Verma and Prakash (2019a, 2019b & 2020).

Metals are well known inducer of oxidative stress, and assessment of oxidative damage and antioxidant defenses in fish can reflect metal contamination of the aquatic environment (Thangam *et al.*, 2014). Heavy metals can combined with organic molecules and accumulate in the various body's tissues of organism and food chain. Heavy metals accumulate in the tissue of living organisms and move it along the food chain creates risks in terms of food

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security; also exposure of fish with these metals can lead to toxic effect, such as tissue damage and death. So, the toxic metals generally exhibits chronic toxicity.

Metals especially heavy metals, like copper are important contaminants of aquatic environments worldwide. Metal pollution has increased with the technological process of human society, industry, mining, advanced agriculture, household waste, and motor traffic are all among the activities considered to be major sources of metal pollution. Metals are well known inducer of oxidative stress, and assessment of oxidative damage and antioxidant defenses in fish can reflect metal contamination of the aquatic environment. The ions of heavy metals exert toxicity by generating reactive oxygen species (ROS), causing oxidative stress to aquatic life (Singh, 2019). Metals can accumulate in aquatic organisms including fish, and persist in water and sediments. Speciation of metals, their solubility and complexation, are important factors that influence the toxicity of metals in aquatic environment. The amount of dissolved metal strongly depends on water pH. The interaction of metals can alter toxic effects on aquatic organisms both positively and negatively. Different mode of exposure to metals also play a role in metal toxicity. Fish take up metals through gills, digestive tract and body surface (Thangam *et al.*, 2014). Copper are absorbed through gills and gastrointestinal track by fish organisms, free of body uptake way they are chiefly accumulated in metabolically active tissues such as liver and kidney. Gills are indirect contact with the outer environment with the pollutants. Liver act in the transformation of basic nutrients and in detoxification on storage of toxic materials (Thangam *et al.*, 2014).

Median Lethal Concentration (LC_{50}) is the particular concentration of a toxicant or heavy metals in the experimental water at which just 50% of the test organisms are able to survive for a specific period of exposure. Many investigators had accepted this method for the determination of acute toxicity, even in recent years for scientific, economic and ethical reasons the LC_{50} test have been prescribed as one of the toxicity tests. Experimentally, this is done by administering a chemical at different doses to a group of organisms and then observing the resulting mortalities in a set time periods like 24, 48, 72 and 96 h. The acute toxicity data are important and beneficial in the fixation of sub lethal concentrations for chronic toxicity tests. Probit analysis is a type of regression used to analyze binomial response variables. Probit method is widely accepted and most accurate method for calculating LC_{50} . Therefore the present investigation aimed to evaluate the acute toxicity bioassay of heavy metal, copper against fresh water fish, *Mystus vittatus*, by using SPSS.

MATERIALS AND METHODS

The freshwater catfish, *Mystus vittatus* (7.0 – 7.5 cm in length and 8.0 -8.5 gm in weight) were collected from local fresh water bodies, washed with 1% solution of $KMnO_4$ for 3-4 minutes to remove any dermal infection and then transferred to the plastic jar containing 50L dechlorinated tap water for acclimatization up to 10 days. During acclimatization the fishes were fed with commercial fish feed. The feeding of fishes was stopped 24 hours prior to the toxicity test, to minimize the contamination from metabolic wastes. The mortality was recorded after a period of 24, 48, 72 and 96 h and dead fishes were removed from aquarium. Stock solution was prepared by dissolving 3.92 g of Copper sulphate in 1000 ml of distilled water than various concentrations of copper sulphate were prepared from this stock solution. Eight sets of ten healthy fishes were subjected to serial dilutions of the stock solution of $CuSO_4$ (from 3-30 mg/L). One set of control group (containing ten fishes) was also set up. Mortality of fishes were monitored at regular intervals throughout the test and dead fishes were immediately removed which did not responded even to strong tactile stimuli. The LC_{50} values for metal copper was obtained employing probit analysis. In probit analysis, the concentrations were converted in to log concentrations and percentage mortality values were converted in to probit values. Regression lines of probit against logarithmic transformations of concentrations were made. Confidential limits (upper and lower) of the regression line with chi-square test was applied to compare the observed Y values and expected Y values test were calculated by SPSS software 26 version.

RESULT AND DISCUSSION

The percent mortality observed for each concentration was calculated and converted to probit by means of SPSS software. Acute toxicity test *i.e.* LC_{50} values show susceptibility of fish to particular toxicant and reflect their survival potential. In the present investigation, the LC_{50} values and the 95% confidence limits of heavy metals, copper sulphate for fresh water catfish, *Mystus vittatus* for 24, 48, 72 and 96 hours are presented in table 1 and Fig. 1-4. In the present investigation the LC_{50} values of copper in *Mystus vittatus* for 24, 48, 72 and 96 hours were 20.589, 16.497, 11.815 and 8.665 mg/L respectively. However, fishes exposed to dechlorinated water were observed to be healthy and normal. In the present study the result revealed that the mortality rate increased with increasing concentration of distillery effluent and for a particular concentration the mortality rate increase with increasing period of exposure.

Table 1: LC₅₀ values with 95% confidence limits for *Mystus vittatus* exposed to CuSO₄.

Exposure Period (hr)	LC ₅₀ values of CuSO ₄ (mg/l)	95% Confidence limits (mg/l)		Regression Equation	Chi-Square value	Coefficient of determination (R ² Linear)
		Lower Limit	Upper Limit			
24	20.589	18.326	23.221	Y=-7.53+2.46X	3.159	0.950
48	16.497	14.295	18.852	Y=- 6.59+2.35X	3.194	0.918
72	11.815	9.696	13.850	Y=-5.00+2.03 X	1.430	0.977
96	8.665	6.457	10.848	Y=-2.96+1.37 X	2.570	0.944

Fig. 1: Regression line (based on Probit analysis) of Log concentration of CuSO₄Vs % mortality of *Mystus vittatus* (24 hours). **Fig. 2: Regression line (based on Probit analysis) of Log concentration of CuSO₄Vs % mortality of *Mystus vittatus* (48 hours).** **Fig. 3: Regression line (based on Probit analysis) of Log concentration of CuSO₄Vs % mortality of *Mystus vittatus* (72 hours).** **Fig. 4: Regression line (based on Probit analysis) of Log concentration of CuSO₄Vs % mortality of *Mystus vittatus* (96 hours).**

The death of fish could be due to the lethal action of pollutant like heavy metal that causes alterations in physiological and biochemical process related to cellular metabolic pathway. The toxicity of any toxicant for fish depends on species, sex, age, weight, dose or concentration, exposure period, organic or inorganic form etc. (Tiwari and Prakash, 2021). However, sublethal

concentrations of heavy metals also induce substantial changes in the biological organization of fish (Srivastava and Prakash, 2018; Kumar *et al.*, 2019).

It is well known that toxicity will depend upon (a) the chemical form of the metal (b) the presence of other metals (c) the physiological status of the organisms and (d) the

environmental, physico-chemical parameters like temperature, dissolved oxygen and the pH of the water. The toxicity of the metal is also dependent upon the residence time of the metal concerned. Generally most metals have long residence times and hence, exert their toxic effects over long periods (Vasanthi *et al.*, 2019).

CONCLUSION

The LC₅₀ can be used as a relative measure to study the impact of the heavy metal concentration on test fishes at different intervals. This toxicity test on the effect of copper on *Mystus vittatus* offers a rapid method for assessing the heavy metal impact on this fish. This type of preliminary investigations can be useful for deriving the safe level of heavy metal concentration (especially copper) that can be released into the aquatic environments.

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