

LETHAL AND SUB-LETHAL EFFECT OF COPPER CHLORIDE TO CLARIAS BATRACHUS (LINN) UTILIZE STATIC ACUTE BIOASSAY

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Abstract

Acute toxicity bioassay of copper (Cu) for the freshwater catfish, *Clarias batrachus* was conducted for evaluation of the toxicity. The 96th LC₅₀ values recorded for Cu was 15 ppm, 29 ppm and 35 ppm respectively. The result of this study indicates that the order of toxicity of this heavy metal is Cu in short duration acute toxicity experiments. The toxicity was found to be dependent on dose and duration of experiment for heavy metal.

Key words : Acute toxicity, Copper chloride. *Clarias batrachus*.

Introduction:

Over the last twenty-five years, the publication of water quality standards for heavy metals has rapidly increased worldwide. Evaluation of the data indicates that some previously accepted generalizations about toxicity of metals to aquatic life are invalid. Field studies are also reviewed and the results of these are combined with the laboratory data in order to identify safe concentration.

Metals are a unique class of toxicants that occur and persist in nature but their chemical form may be changed because of physiochemical, biological or anthropogenic activities. Their toxicity may be altered as they assume different chemical forms.

Metals differ from the toxic substances in that they are neither created nor destroyed by humans. Metals are probably the oldest known toxicants to humans. Lead uses may have begun prior to 2000 B.C. Hippocrates is credited in 370 B.C. with the first description of these metals' activities on the earth.

Copper is an essential nutrient that is incorporated into a number of metallo enzymes involved in haemoglobin formation, drug/xenobiotic metabolism, carbohydrate metabolism,

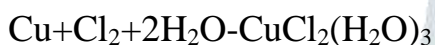
catecholamine biosynthesis, the cross linking of collagen, elastin, hair keratin and the antioxidant defence mechanism. Copper is readily absorbed from the stomach and small intestine.

Although copper homeostatis plays an important role in the prevention of copper, exposure to excessive levels of copper can result in a number of adverse health effects including liver and kidney damage, anaemia, immune toxicity and developmental toxicity and developmental toxicity.

Copper though essential in diet, but it can be harmful when large single or daily intake occurs. Anthropogenic activities such as mining, preparation of toxicants, electroplating, paint, pigment industries, hydrated copper chloride ($\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$) is extensively used.

The test chemical copper (II) chloride is the chemical compound with the chemical formula CuCl_2 . This is light brown solid, which slowly absorbs moisture to form a blue green hydrates.

Copper (II) chloride is prepared commercially by the action of chlorination of copper:



Once prepared, a solution CuCl_2 may be purified by crystallization.

Fish is an opportunistic feeder, behaving as scavenger and it can survive for months together during drought without food. It feeds on large aquatic insects, clams, crustaceans, earthworm, small fish, larvae, aquatic plants and debris.

Economically highly valued fish for food, *Clarias batrachus* (Linn) belongs to the class : osteichthys, order: cypriniformes, family: claridae, genus: clarias and species: batrachus. The present work is an endeavour to assess the effect of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ on the haematology, histology and cell bio-chemistry of the experimental fish *Clarias batrachus*.

Material & Methods:

Study material : 100 healthy fish weighing about 40-50 gm procured from natural unpolluted sources reservoir and brought to the laboratory, examined for any pathological symptoms, treated with 0.1% $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ solution to avoid any dermal infection and acclimatized in dechlorinated tap water for 15 days.

The test fishes were selected and maintained in separate aquaria before actual experimentation.

Preparation of meal solution: the heavy metal copper chloride ($\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$) was selected for toxicological experiments. Dechlorinated tap water which was stored in a large overhead tank for about ten days was used for conducting the toxicity experiments. The physic-chemical characteristics of the dechlorinated tap water were determined as per standard methods (APHA, 2012) and the following readings were recorded.

Ph : 7.2-7.4 (range)

Temperature ($^{\circ}\text{C}$) 24 – 28 (range)

Dissolved oxygen (DO) mg/L : 6.9 7.1 (range)

Alkalinity (CaCO_3) (mg/L) : 160 – 170 (range)

Total hardness (CuCO_3) mg/L : 160 – 180 (range)

Stock solution of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ were prepared following standard guidelines (OECD, 1981 APHA, 2012) and dilution upto sublethal dose of 3 ppm for treatment for 20 days and 40 days.

Experimental design and toxicity bioassay :

In order to determine the LC_{50} of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ and their combination, method of Lutchfield and Wilcoxon (1949) was followed. Successive batches of 10 fishes were exposed to different concentrations of heavy metals under standard laboratory conditions after 24, 48, 72 and 96 hrs duration. After specified time interval, the number of fish dead and alive were noted. The experiments were carried out in glass aquaria of 100 L capacity in static laboratory conditions.

TABLE

LC_{50} 95% confidence limits and slope function values of Cu to *Clarias batrachus* in acute toxicity bioassay

Exposure Period (hrs.)	Parameter	Copper Value
24	LC_{50} mg/L 95%	25
	Confidence level, slope	20.40 – 30.60
	Function	$Y=15.35 x 14.29$
	Regression (R^2)	0.979
48	LC_{50} mg/L 95%	20
	Confidence level, slope	16.39 - 24.40

	Function Regression (R ²)	Y= 15.35 x 14.28 0.979
72	LC50 mg/L 95% Confidence level, slope Function Regression (R ²)	17.50 14.30 – 21.35 Y = 13.21 x 2.85 0.610
96	LC50 mg/L 95% Confidence level, slope Function Regression (R ²)	1.5 59 – 20.50 Y = 15.35 x 0.00 0.932

Results and Discussion :

Fishes are the simple and reliable biomarker of copper pollution of aquatic bodies (Taylor et al., 2000; Lodhi et., 2006). The metallic ion present in water enters the fish body and gets accumulated in various organs like liver and kidney.

In the present study, exposure of fish *Clarias batrachus* at 2 ppm concentration of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ for 20 days and 40 days caused significant alterations in haematological parameters. The alterations recorded in various physic-chemical parameters such as temperature, Ph, DO, hardness, alkalinity, chloride etc. under control conditions in the present finding the RBC, total count, Hb% and PCV% was recorded decreased significantly however, the decrease was more significant with the increase of duration i.e. from 20 days to 40 days. WBC count, ECR on the contrary was recorded significantly from 20 to 40 days (table) as compared to control.

The exposure of *C. batrachus* to sublethal concentration of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ significantly decreased Hb% RBC count and PCV% values leading to anaemia. The present finding also shows conformity with the findings of Effer et al. (1980) while working on whole lake response to low level copper sulphate treatment.

Histological biomarker of toxicity in fish organs are useful indicators of environmental pollution (Peebua, 2008). The significant decrease observed in the total protein value may be due to the toxic effect of copper chloride hydrate on the immune system and the haemodilution

effect. The present findings shows conformity with the findings of Hussain et al. (1996) and Muckkaway et al. (1996) who reported decrease in total protein in atrazine exposed with tilapia and catfish.

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