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Sub-lethal effect of copper sulphate on certain haematological parameters of *Clarias batrachus* (Linn.)

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ABSTRACT

In the present study the sub-lethal effect of copper on various haematological parameters of *Clarias batrachus* were studied. Copper (as copper sulphate) was used to prepare the stock solution from which different standard concentrations were prepared. A total of 16 specimens of *Clarias batrachus* (mean weight and length 100gm and 18-20 cm, respectively). were used for this purpose. In exposed fishes various haematological changes were noticed. The RBC counts and haemoglobin percentage was decreased significantly from the normal values.

Key words: Copper sulphate, Haematological parameters, *Clarias batrachus*.

INTRODUCTION

The effects of heavy metals on aquatic organisms is currently attracting wide spread attention particularly in studies related to industrial pollution. High toxicity of industrial pollutants have been known since long time, but their harzardous nature as pollution of aquatic environment has been a matter of concern only after a large number of deaths of fishes occurred in different areas due to different metals.

A survey of literature on heavy metal toxicity clearly shows that the heavy metals cause several haematological, biochemical and reproductive disorders both in the laboratory animals as well as on the aquatic organisms. The toxicity of copper and other heavy metals has been studied since mid 1920, (Dilling *et al,*1926), Carpenter 1927, Ellis 1937, Lauric *et al.,* 1938, Davidson,1949, Ray *et al.,* 1964).

Copper is a common pollutant in surface water and its toxicity is largely atttibutable to its cupric (Cu^{2+}) form, which is readily complexed by inorganic and organic substances and adsorbed onto particulate matter. Complexed copper is biologically unavailable but plants and animals may absorb some copper in the environment.

Elevated levels of copper may become acutely or chronically toxic to aquatic lives. Acute effects may be death, chronic effects could be reduced growth, shorter life span, reproductive problems, reduced fertility, behavioural changes and haematalogical changes. The toxicity of copper to aquatic life varies with the physical and chemical conditions of the water. Factors like water hardness, Alkalinity, pH, dissolved oxygen and temperature effect the toxicity of copper.

Clarias batrachus is used for this experiment because of its hardy nature and, commercial importance in India.

MATERIALS AND METHODS

Procurement of test fishes

Alive, healthy and disease free fishes (*Clarias batrachus*, weight 80-100 gm and length 18-22 cm) were collected from the local fish market, Bhopal and brought to the laboratory, the fishes were kept in the glass aquarium to observe any visible pathological symptoms. Before introducing in the aquarium fishes were treated with 0.1% KmnO4 solution to obviate any dermal infection.

Acclimatization of test fishes

Fishes were acclimatized to laboratory conditions for a period of one week. No mortality was recorded during this period. The fishes were fed with chopped meat daily. After acclimatization, fishes were kept in different concentration of copper sulphate in different aquaria.

Source of heavy metals

Copper sulphate were used for the preparation of various toxic concentrations (stock solution) by adopting the dilution techniques. Adequate Quantity of distilled water was used to get the required concentration for investigations. Sub-lethal levels of the above metal was determined on the above said fish species by the probit analysis method. Unchlorinated water was analysed for temperature, hydrogen ion –concentration (pH), Dissolved oxygen, total alkalinity and total hardness. Sixteen fishes were exposed to sub-lethal concentration for 8,16 and 24 hours under acute studies. Sixteen fishes were exposed to sub-lethal

Table-1:Sub-lethal effect of Copper Sulphate on RBC count and Haemoglobin percentage of *Clarias batrachus* (Linn) exposed with (5mg/l)

Condition of fish	No. of fish exposed	Duration of exposure	No. of fish tested	RBC (10^{-6}/cu mm)	HB% (g/100ml)
Control(6)	16	-	-	3.26 ± 0.0081	13.10 ± 0.0084
1	-	8 hours	3	3.00 ± 0.0083	13.20 ± 0.0083
2	-	16 hours	3	2.60 ± 0.0082	12.06 ± 0.0081
3	-	24 hours	3	2.70 ± 0.0085	12.32 ± 0.0085

Values expressed in Mean ± SD .Anova have been performed between four groups i.e. control, 1, 2, 3 for RBC and Haemoglobin percentage. The four groups were found to be significantly different (P< 0.01).

Table-2:Sub-lethal effect of Copper Sulphate on RBC count and Haemoglobin percentage of *Clarias batrachus* (Linn) exposed with (12 mg/l)

Condition of fish	No. of fish exposed	Duration of exposure	No. of fish tested	RBC (10^{-6}/cu mm)	HB% (g/100ml)
Control(6)	16	-	-	3.00 ± 0.0081	13.03 ± 0.0084
1	-	8 hours	3	1.60 ± 0.0083	13.25 ± 0.0081
2	-	16 hours	3	1.64 ± 0.0085	10.98 ± 0.0083
3	-	24 hours	3	2.06 ± 0.0082	11.02 ± 0.0085

Values expressed in Mean ± SD .Anova have been performed between four groups i.e. control, 1, 2, 3 for RBC and Haemoglobin percentage. The four groups were found to be significantly different (P< 0.01).

concentration for 45 days under chronic studies. Haematological studies were made after 15, 30 and 45 days of exposure. In acute and chronic studies feeding was stopped one day before the experiment started and under chronic studies refeeding was done after one day of exposure.

Large size glass aquaria were chosen to avoid space problem to fish, water temperature, hydrogen ion – concentration (pH), dissolved oxygen, carbon dioxide, total alkalinity, calcium hardness and total hardness was determined before sacrifice of fish.

Fish behaviour was observed and recorded accordingly. Control groups were maintained for all the above experiments, after exposure the fish was sacrificed for haematological examination.

RESULTS AND DISCUSSION

In the present study, attempts have been made to investigate the effect of sub-lethal concentrations of copper sulphate on various haematological parameters of *Clarias batrachus* (Linn) on comparative approach from 8 to 24 hours for acute studies (Table 1 and 2) and 15 to 45 days for chronic studies(Table -3).

Copper occurs naturally within the environment. At low concentrations, it is an essential element both for plants and animals. However, large doses can be harmful. Long-term exposure to copper can cause liver and kidney damage and effects on blood. In fish, copper is a classical limiting factor as it is both essential and toxic. As a micronutrient, it is necessary for haemoglobin synthesis and a component of cytochrome oxidase.

Table-3: Sub-lethal effect of Copper Sulphate on RBC count and Haemoglobin percentage of *Clarias batrachus* (Linn) exposed with (5 mg/l)

Condition of fish	No. of fish exposed	Duration of exposure	No. of fish tested	RBC (10^{-6}/cu mm)	HB% (g/100ml)
Control(6)	16	-	-	3.26 ± 0.0083	13.1 ± 0.0816
1	-	15 days	3	1.89 ± 0.0081	12.72 ± 0.0081
2	-	30 days	3	2.06 ± 0.0082	11.89 ± 0.0084
3	-	45 days	3	2.90 ± 0.0085	12.89 ± 0.0082

Values expressed in Mean ± SD .Anova have been performed between four groups i.e. control, 1, 2, 3 for RBC and Haemoglobin percentage. The four groups were found to be significantly different ($P < 0.01$).

In this study, the fish exposed to copper were observed to be highly irritable and displayed frenzied swimming when approached, they swam upside down, their bodies were covered with thick mucus. These observations were similar to those of OLAIFA and ONWUDE (2002) who worked with *Clarias gariepinus* exposed to copper in soft water.

The red blood cells decreased below the control. This suggests that at higher concentrations, there could have been destruction of red blood cells. Similar results were also observed for haemoglobin(HB) values, which suggested Haemodilution which could lead to aneamia.

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