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Investigation of acute toxicity and LC_{50} value of Cu for a fish *Oreochromis niloticus*

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Abstract
The present investigation is about to determine the acute toxicity and 96-Hr LC_{50} value of Cu for the fish *Oreochromis niloticus.* 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. With the increase of the concentration of metal the response of the fish mortality were increased gradually. The result showed that LC_{50} of copper sulphate for the fish, *Oreochromis niloticus* is 30 mg/l. The mortality of the fish is directly proportional to the concentration of the exposed metals.

Keywords: Acute toxicity, 96-Hr LC_{50}, *Oreochromis niloticus*

Introduction
Acute toxicity is the discernible adverse effect induced in an organism within a short time of exposure to a substance. In the present test, 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 which must be stated. Fishes have direct economic importance and are quite sensitive to the wide array of pollutants discharged in the aquatic ecosystems. Fishes are widely used to assess water quality of aquatic ecosystems because they serve as pollution bioindicators [1-2]. Fish may concentrate large quantities of toxic metals from polluted aquatic environments [3]. The heavy metal concentration in the body of fish depends upon feeding habits, trophic status, and food availability, physico-chemical properties of water, and metabolic rate of animal and toxicity of heavy metals [4-5].
Human activities are major responsible for water pollution. Water polluted due to pollution is looked upon with disdain. Water pollution affects the fish severely and proves lethal to them. Water pollution imposes this adverse effect on all kinds of aquatic flora and fauna. Fishes are mainly affected from the human nuisance. So, it is the need of time to pay adequate attention to this issue and execute necessary corrective measures. [6] Fishes die due to pollution of water from pesticides adjacent the cultivation fields. Pesticides flow off into the water proving fatal for the aquatic life. [7] Metals are the common pollutants of the rivers in Punjab province entering them with industrial and municipal waste waters. The heavy loads of metals in the rivers have adversely affected the original fish fauna, including major carp's viz. *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* that are on the verge of loss in the aquatic habitats. [8] Therefore, heavy metals are considered as chief environmental pollutants and have long been known as settled contaminants of aquatic environments. [9] Metals contamination of the environment results both from natural sources and industrial activities in accumulation an additional contribution from air. [10] The toxic effects of various heavy metals may hinder the physiological and metabolic functions, rate of growth, reproductive efficacy and ultimately causes mortality in fishes. [11] Toxicity tests have been performed on fishes to evaluate the effect of toxicants on various aquatic organisms under laboratory conditions. To assess susceptibility and survival potential of the test organisms 96 hr LC_{50} tests of some particular toxicants have been conducted. The fingerling stage of fish is more reliable to conduct toxicity test of various waterborne toxicants. [12, 13]. The aim of the research work was to find out acute toxicity and LC_{50} value of Cu for a fish *Oreochromis niloticus* is.

Material and Methods

The present investigations were conducted at fish seed hatchery, Department of Fisheries and aquaculture, University of Veterinary and Animal Sciences, Ravi Campus. Individuals of *Oreochromis niloticus* were obtained from grow out ponds kept in holding tanks, supplied with flow through aerated water and acclimated for 2 weeks, in the laboratory before conducting acute toxicity tests. The whole investigation was bifurcated into two segments viz. metals acute toxicity assays and tissue distribution assays.

Metals Acute Toxicity Assays

Laboratory tests were conducted to determine the 96-hr LC_{50} and lethal concentrations of copper (Cu) for *Oreochromis niloticus* at constant temperature (30 ºC) and pH (7.5) of water.

i. Preparation of Stock Solutions

Pure compound of $CuSO_4$ was dissolved in deionized water and the stock solutions (1000 ppm) were prepared.

ii. Experimental Conditions

Acute toxicity tests were performed in glass aquaria attached with aeration system. Glass aquaria were filled with 80-liter metal free 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. The fish of following average weights and lengths were tested for their tolerance limits in terms of 96-hr LC_{50} and lethal concentrations.

Ten individuals of experimental fish were tested against each concentration of metal for the determination of both 96-hr LC_{50} and lethal concentrations. During acute toxicity trials, the fish were subjected to 12-hr photo period. The fish were not fed during acute toxicity trials. The concentration of copper was started from zero up to the onset of 96-hr LC_{50} and lethal concentrations. The aquaria were examined after every 2 hours for fish mortality. The dead fish were weighed individually after being lightly blotted dry at the time of mortality observations.

Probit Analysis of each test dose against fish response (mortality) was performed by adopting standard protocol [14]. The physico-chemistry of the test media viz. water temperature, pH, total ammonia, dissolved oxygen and electrical conductivity were made at 12 hour intervals throughout the test period of each 96-hour experiment by following the methods of [15]. Water temperature was measured with the help of digital thermometer. Dissolved Oxygen and Electrical Conductivity were determined by electronic meter (HI 9147) pH measured by electronic meter (HI1285-5). Total ammonia was calculated as: Rochelle salt solution (Dissolved 5g of potassium sodium tartrate tetra hydrate-K NaC_4 $H_4O_6.4H_2O$ in 100ml distilled water. Remove ammonia by boiling off approximately 30ml of the solution. After cooling, make the volume to 100ml by adding more distilled water).

iii. Tissue Distribution Assays

The dead fish, obtained after 96-hr LC_{50} and lethal toxicity responses, were dissected and their body organs viz. gills, liver, skin and muscle for the determination of their respective exposure metals by following the methods of [16, 17].

Statistical analysis

For acute toxicity tests, the mortality data were statistically analyzed by using Probit Static Bioassay test system. The 96-hr LC_{50} and lethal concentrations were determined along with 95% confidence intervals. The data obtained from present investigation were statistically analyzed by using SPSS 13.00 computer program.

Results and Discussion

Ten individuals of *Oreochromis niloticus* were tested against Copper concentration for the determination of both 96-hr LC_{50} and lethal concentrations at constant temperature (30 ºC) and pH (7.5) of water. During the present investigation the 96-hr LC_{50} of $CuS0_4$
for the fish, *Oreochromis niloticus* was found to be 30 mg/l (table.1). The present investigation of LC_{50} of 96 hours for *O. nilloticus* reveals and are shown in the table no.1 that total individuals of the exposed fish were 10 and the mortality rate increased with the increase of the lethal concentration. Figure 1 reveals the graphical representation of the probit kill against log of concentration by using SPSS 13.00.

Physico-chemical parameters are also very important to count for the survival of the current selected experiment that's why, during the whole experiment some important physio chemical parameters like temperature, pH, Dissolved oxygen and electrical conductivity and ammonia were recorded.

During present investigation, significantly maximum ammonia excretion by the fish was observed at higher concentrations of metal (Copper test medium). At higher concentrations of
metals, the dissolved oxygen contents of the test mediums also decreased significantly. This shows that high concentrations of metallic ions induced stress in the fish that resulted in the influence of the respiration of the fish. More ammonia releases by the fish with the increasing rate of the metal.

Metals have long been recognized as serious pollutants of the aquatic environment. They caused serious impairment in metabolic, physiological and structural system, when present in high concentration.

In present study hyper- excitation and fast jerking movements were noted in fish
before death. Too much behavioral changes (cough and yawn) at higher concentration
might be due to manifestation of the disturbances in the physiological mechanism which
is supposed to initiate, maintain and terminate the behavior [17] stated that
increased cough and yawn is due to the increased secretion of mucous which deposited
on the gills to combat the toxicity produced by heavy metals. It also reduced the gaseous
diffusion causing less supply of oxygen, causing immediate fish death.

Table 1: Relation between concentration of Copper Sulphate and the percentage mortality of the fish

Dose mg/l	Log of concentration	Total individuals	Mortality	Mortality %	Probit kill
0	-	10	0	0	0.000
6	0.7790	10	1	10	0.320
12	1.0791	10	2	20	1.827
18	1.2552	10	3	30	3.629
24	1.3802	10	4	40	5.170
30	1.4771	10	5	50	6.359
36	1.5563	10	7	70	7.247
42	1.6232	10	8	80	7.903
48	1.6812	10	9	90	8.390
54	1.7323	10	10	100	8.752

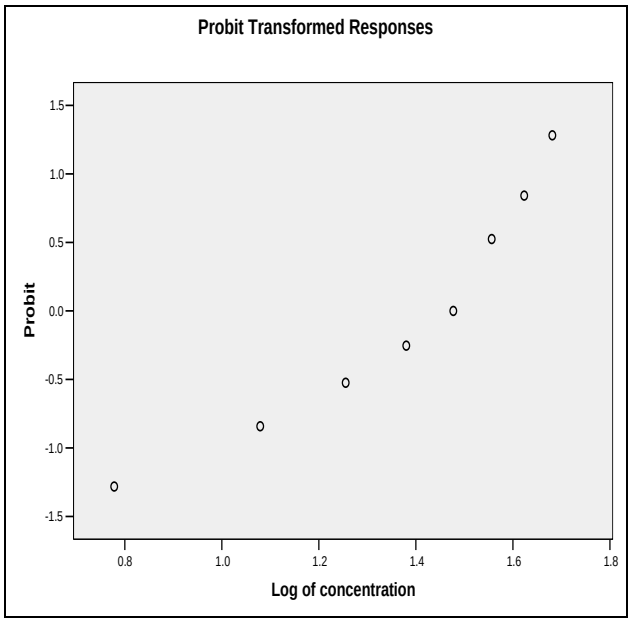

Fig 1: Regression line between the probit kill of *Oreochromis niloticus* and log concentration of Copper Sulphate

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