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MEDIAN LETHAL CONCENTRATION OF COPPER TO GOLDFISH *CARASSIUS AURATUS* LINNAEUS

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This study was undertaken to determine acute toxicity level (median lethal concentration) of copper to goldfish *Carassius auratus* Linnaeus, a freshwater cyprinid. The experiment was conducted in filtered (mesh size 1 μm) freshwater having total hardness 34.2 (as $CaCO_3$) in a static condition. Copper sulphate ($CuSO_4$ $5H_2O$) was used as a source of copper. The LC_{50} values of copper were determined from probit values of cumulative mortality percentage against exposure concentrations (dose response) using simple linear regression model $Y = a + bX$. The LC_{50} values of copper to goldfish (10.5 ± 1.43 g) were determined as 0.44 ± 0.06, 0.36 ± 0.04, 0.20 ± 0.03, 0.19 ± 0.02, 0.15 ± 0.02 and 0.14 ± 0.02 ppm for 12, 24,48,72, 96 and 120 hr. respectively.

Key words: Goldfish, Copper, Toxicity.

Introduction

Median lethal concentration (LC_{50}) is one of the simplest form of toxicity test, defined as the concentration of any toxic substance cause mortality of 50% test organisms [1]. It is a relatively short-term acute toxicity test for fish, where lethal effects (mortality) occur usually within 96 hr. To develop water quality criteria it is necessary to determine sublethal level of any pollutant/toxicant in a specific environmental condition, which brings (a) various physiological and biological changes in the aquatic ecosystem, (b) maximum allowable toxicant concentration (MATC), (c) effect of environmental factors on the toxicity of such toxicants, (d) toxi city of a toxicant to a test species and (e) relative sensitivity of an aquatic organism to a toxicant [2,3].

The 96 hr. LC_{50} value of copper was determined for coho salmon *Oncorhynchus kisutch* [4], goldfish *Carassius auratus* [5], blue gill *Lepomis macrochirus* [6], for longfin dace *Agosia chrysogaster* [7] and for chinook salmon *O. tshawytcha* [8]. The 96 hr. LC_{50} values of copper to various fish determined by the different researchers appeared to be quite different from each other. There was no information available on the acute toxicity (96 hr. LC_{50}) level of copper to goldfish *Carassius auratus* for relatively soft water under tropical conditions. This experiment was designed to determine acute copper toxicity to goldfish *Carassius auratus* Linnaeus in a freshwater static condition to generate novel data further research.

Materials and Methods

Goldfish (*Carassius auratus*) was selected as the test fish on the basis of the general guidelines described in Standard Methods for Examination of Water and Waste Water [2]. It is a widely used fish in toxicity studies and is susceptible to copper (Cu). Required number of Cu unexposed goldfish were purchased from a commercial farm in Kuala Lumpur. The test fish were treated with 50 ppm formalin for 36 hr. to remove common ectoparasites prior to experimentation. Then the fish were acclimatizated in the fresh water static condition for 20 days. The test fish were fed @ 2% body weight, once daily during acclimatization. The feeding of test fish were ceased three days before transferred to the test aquaria, to avoid effect of feces on Cu adsorption during the experiment. Seasoned (7 days) and filtered (mesh 1 μm) tap water was used for this experiment to avoid suspended solids. The presence of Cu content in tap water as trace metal was below detectable limit of flame atomic absorption spectrophotometer (AAS) (Shimadzu AA-670/G V-6, Japan). Glass aquaria (150 L) were cleaned with 10% HNO_3 and sundried before stocking with the test fish. Ten acclimatized goldfish (each 10.5 ± 1.43 g) were randomly distributed in four glass aquaria containing 100 L solution of 0.0, 0.10, 0.30, 0.50, 0.70 and 1ppm Cu for 120 hr. to determine the preliminary toxicity range. Analytical grade copper sulphate ($CuSO_4$ $5H_2O$, MERCK No. 2790) was used as a source of Cu. All the aquaria were kept under artificial aeration continuously during 120 hr. LC_{50} test. Fresh stock solution 1000 ppm Cu was used to prepare test solution. On the basis of preliminary toxicity range, test fish were exposed to nominal (expected or selected) 0.05, 0.10, 0.15, 0.20, 0.30 and 0.5 ppm Cu for 120 hr. LC_{50} test. The cumulative mortality of test fish were recorded after 6, 12, 24, 48, 72, 96 and 120 hr. The experiment was done in triplicate.

The LC_{50} values of copper to goldfish for 12,24, 48, 72, 96 and 120 hr. were determined from probit values of cumulative mortality percent against Cu exposure concentrations,

using simple linear regression model Y = a + bX [2]. Total hardness ($CaCO_3$), diurnal temperature, dissolved oxygen, pH etc. were recorded daily.

Actual concentration of copper in the test solutions were measured daily using flame AAS (Shimadzu AA-670/G V-6) to record soluble residual copper. Flame AAS was equipped with computerized microprocessor, automatic background correction system and automatic setting of analytical conditions by elements key only. The standard stock solution (1000 ppm) was prepared using ampules containing 1 g Cu (Merck, Titrisol No.15055). The standard stock solution was acidified with 1 ml of concentrated nitric acid and kept at 4°C. The working standard solutions were prepared from preserved standard stock solution (1000 ppm) by serial dilution. The standard samples were aspirated to the AAS as per instruction of the manufacturer to obtain standard curve and subsequently determined the strength of the test solutions. The blank was distilled and deionized water.

Results and Discussion

The LC_{50} values of copper to goldfish (*C. auratus*) were determined as 0.44, 0.36, 0.20, 0.19, 0.15 and 0.14 ppm for 12, 24, 48,72, 96 and 120 hr. respectively. The fitted regression lines of determined LC_{50} values were shown in Fig.1. The determined LC_{50} values of copper to goldfish were plotted against exposure period to obtain the standard LC_{50} curve (Fig. 2). The cumulative mortality (%) of goldfish were plotted against Cu exposure concentration and exposure time to obtain mortality curves (Fig.3).

The initial nominal concentration of Cu did not differ significantly ($P<0.05$) to the actual exposure concentrations of Cu. The actual Cu exposure concentrations were 0.053±0.004, 0.101±0.003, 0.151±0.002, 0.201±0.003, 0.299±0.003, 0.499±0.006 in place of initial nominal (expected) 0.05, 0.10, 0.15, 0.20, 0.30 and 0.50 ppm, when measured within 0.5 hr. of solution preparation. There were no mortality in the control tanks during the 120 hr. LC_{50} test. Physicochemical parameters of test solutions varies slightly during 120 hr. LC_{50} test. The temperature of test solutions ranged from minimum 24 to maximum 30°C, DO minimum 7.5 to maximum 8.6 mg/l, pH minimum 6.5 to maximum 7.2 and total hardness 34.2 mg/l as calcium carbonate during 120 hr. LC_{50} test.

The determined LC_{50} values of copper to goldfish comply with the straight line (except at 48 hr.) indicating the degree of goodness of fit as a standard curve. In general, the pattern of curve including the determined value of 48 hr. is curvilinear. This pattern is similar to the standard LC_{50} curve

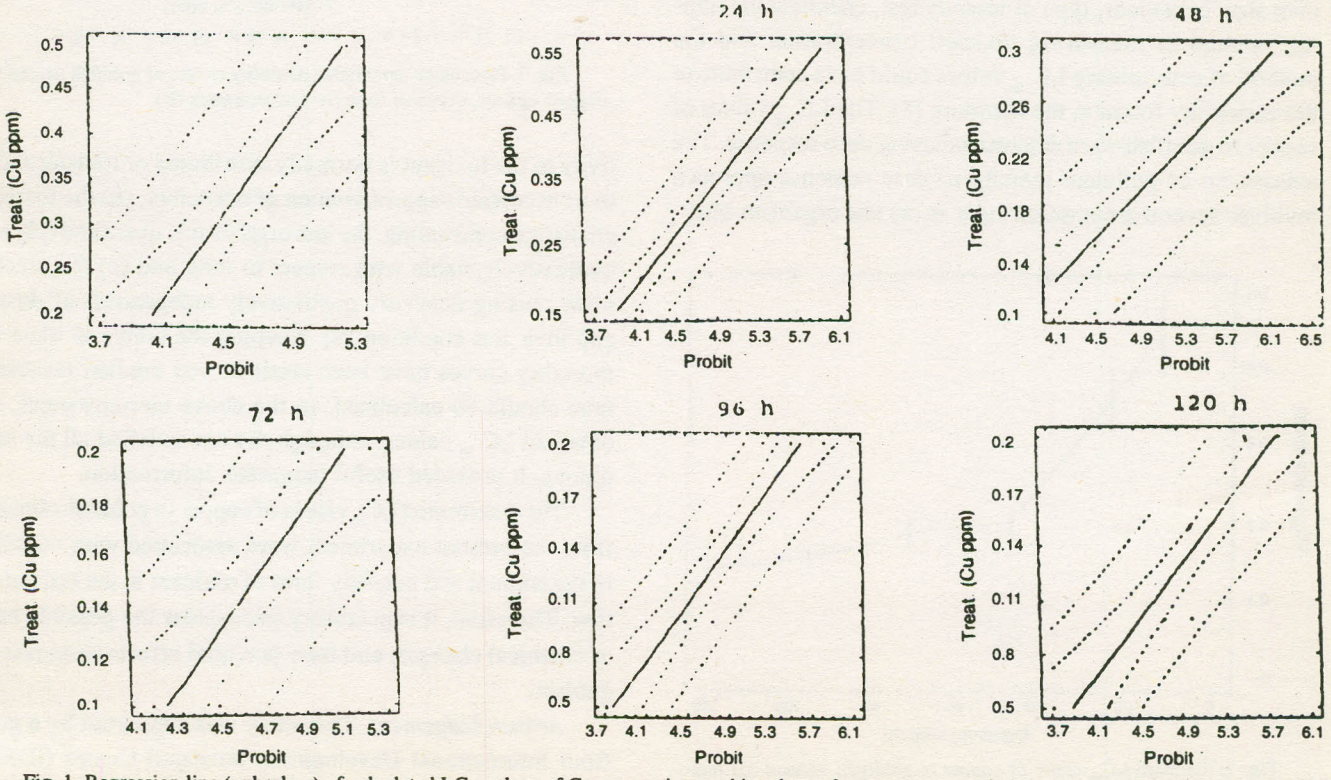

Fig. 1. Regression line (unbroken) of calculated LC_{50} values of Copper against probit values of percentage cumulative mortality of Goldfish exposed for different durations: Range of 1st and 2nd pairs of broken lines represent 99% and 95% confidence limits respectively.

of copper to goldfish *Carassius auratus* [5] cadmium to goldfish *Carassius auratus* [9] and copper to longfin dace *Agosia chrysogaster* [7].

The 96 hr. LC_{50} values of copper obtained from the present study were not similar to those reported by other researcher even for same duration in fresh water static conditions [5]. The differences amongst the studies could have been probably due to variation in, total hardness, temperature of test solutions, age and variety of test fish. They used fish of standard length ranged from 3.1 to 6.0 cm (weight not mentioned). Their experimental temperature ranged from 21 to 22.5°C, dissolved oxygen 5.05 to 4.4 mg/l, pH 7.1 to 9.3 and total hardness was 45 to 96 mg/l as $CaCO_3$' which are different from the present study. Moreover the effective toxicity level of metal normally increased in high temperature and low total hardness of test solution [10].

The result of the present study for 96 hr. LC_{50} value was also different from the values reported for coho salmon *Oncorhynchus kisutch*, 60-70 µg/l [4], longfin dace *Agosia chrysogoster*, 210 µg/l [7], for blue gill *Lepomis microchirus*, 100 µg/l [6] and for chinook slamon *O. tshawatscha*, 32±4 µg/1 [4]. These variation in results could have been due to the differences in genera and size, total hardness, temperature, pH etc. of test solution and bioassay test system.

No absolute LC_{50} values of metal could be obtained, even in similar condition [6]. However, genetic variability, specimen size, behaviour, type of toxicity test, chemical variability, method of measuring toxicant concentration and the method of determining LC_{50} values could have contribute to the variability found in the literature [6]. The LC_{50} values of copper to goldfish were determined using dose response. The assessment of pollutant toxicity to dose response approach involves several assumption such as (a) test organism sensitivity to the toxicant is normally distributed or transformable to an acceptable approximation of normality, (b) the toxicant challenge confronting the test organism is quantitatively and qualitatively stable with respect to time and (c) the mechanism causing death are qualitatively independent of dosage and their test condition [9]. Keeping the above in view the mortality curves have been rectified and median resistance time should be calculated. In the above circumstances, the obtained LC_{50} values, although did not satisfied all the conditions, it provided useful pragmatic information.

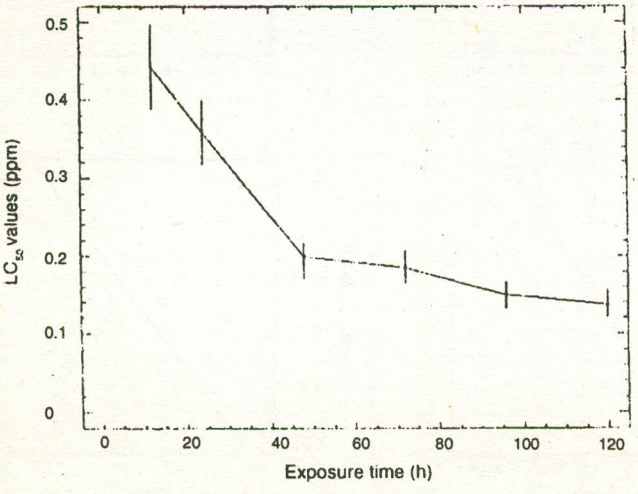

Fig. 2. Standard LC_{50} curve of copper to goldfish: vertical bar represents ± standard error.

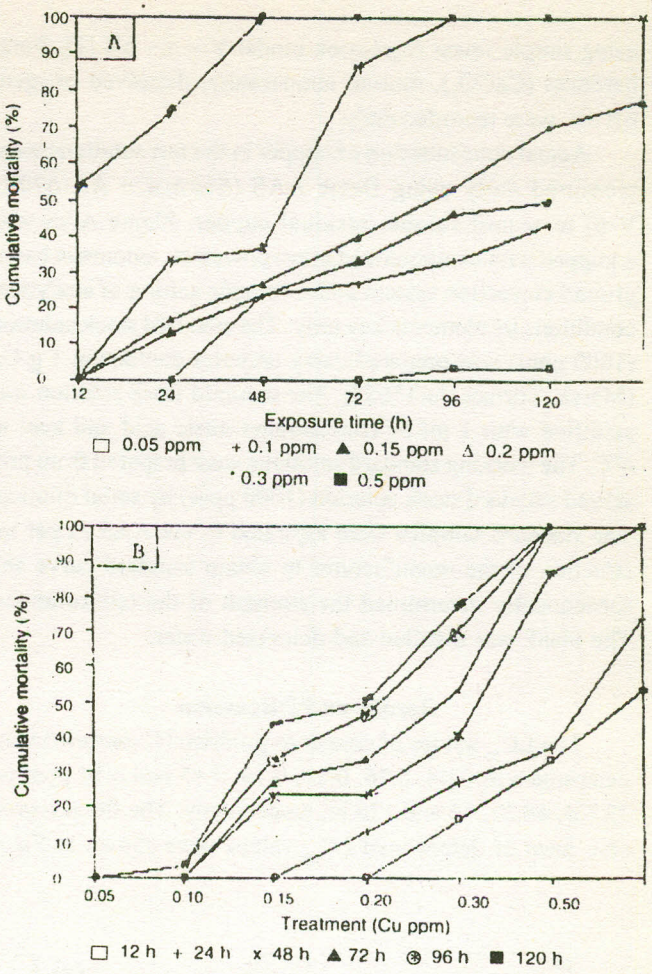

Fig. 3. Percentage cumulative mortality curves of goldfish treated with copper: against, exposure time (A) and treatment (B).

The determined LC_{50} values of copper to goldfish obtained from the present experiment were associated with variation in the amount and possibly form of toxicant in the test condition. Therefore, it is necessary to consider the possible basis of chemical changes, and their potential effects upon test organism.

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