Fishery Technology 54 (2017) : 237 - 243

Determination of LC_{50} of Copper in *Cirrhinus mrigala* (Hamilton, 1822) and *Ctenopharyngodon idella* (Steindachner, 1866)

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Abstract

Static bioassays were conducted on *Cirrhinus mrigala* (8±0.5g) and *Ctenopharyngodon idella* (8.5±1g) fingerlings, to determine the acute toxicity of copper. The experiments were designed as three replicates with five exposure groups arranged exponentially(0.25, 0.75, 1.62, 3.25 and 6.62 mgl^{-1}) for *C. mrigala* and five concentrations (0.5, 0.75, 1.62, 2.5 and 3.25 mgl^{-1}) for *C. idella*, besides the controls. Each exposure group comprised of 10 fish each. The median lethal concentration (96-h LC_{50}) of copper was determined as 0.74 mgl^{-1} Cu for *C. mrigala* and 3.07 mgl^{-1} Cu for *C. idella* respectively. Copper proved to be more toxic to *C. mrigala* than *C. idella.* The mortality rates increased with increasing copper concentrations, in both species.

Keywords: *Cirrhinus mrigala, Ctenopharyngodon idella,* copper, acute toxicity

Introduction

Heavy metal contamination has devastating effects on the ecological balance of the recipient environment by altering the diversity of aquatic organisms (Farombi et al., 2007; Vosyliene & Jankaite, 2006; Ashraf, 2005; Javed, 2005) especially to the fish community (Olaifaet al., 2004). Heavy metals, like all toxicants, when present in high concentrations in aquatic ecosystems, are capable of severely interfering with the biological systems, producing damage to the structure and function of a particular organism (Spacie & Hamelink, 1985).

Although heavy metals are often referred to as a common group of pollutants, individual metals pose different problems in freshwater environment and therefore they have to be considered separately (Loyd, 1992). The toxic effects of heavy metals on fish are multidirectional and manifested by numerous changes in physiological and chemical processes of their body systems (Dimitrova et al., 1994). The capacity to counter uptakeby excretory, metabolic, storage and detoxification mechanisms varies between different species and different metals (Heath, 1987; Langston, 1990; Eaton et al., 1995).

Copper is an essential trace element required in small amounts by fish for metabolism of carbohydrates and is needed for synthesis of haemoglobin. However, concentrations that exceed 20 micrograms per gram (μgg^{-1}) can be toxic (Wright & Welbourn, 2002). Fish are 10 to 100 times more sensitive to the toxic effects of copper than mammals (Forstner & Wittman, 1979). Copper releases to the biosphere come mostly from anthropogenic activities such as mining and smelting, industrial emissions and effluents, and municipal wastes and sewage sludge (Wright & Welbourn, 2002). Copper compounds are widely used as biocides to control algae, macrophytes and ectoparasites of fish. Copper compounds are also used in agricultural fertilizers (Eisler, 1998), aqua feeds, as impurities in fertilizers (Boyd & Massaut, 1999) wood preservatives (Edwin & Sreeja, 2011)and as active principles of pesticides used in the activity (Tacon & Forster, 2003).

Copper is generally more toxic to organisms in freshwater than in saltwater (Brooks et al., 2007). Copper toxicity in fishes causes impairment of

Received 21 July 2016; Revised 03 October 2017; Accepted 06 October 2017

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osmoregulation and ion regulation in the gill (Blanchard & Grosell, 2005) and inhibition of ATP-driven pumps and ion channels (Katranitsas et al., 2003). Sub-lethal copper levels result in the loss of chemosensory function (McIntyre et al., 2008).

The acute toxicity tests are conducted to measure the susceptibility and survival potential of organisms to toxic substances such as heavy metals (Eaton et al., 1995). Acute Copper toxicity experiments were conducted on freshwater fishes such as *Cyprinus carpio* (Panawon, 2000), *Labeo rohita* (Adhikari, 2003) and rainbow trout (Ayse, 2008). Acute copper toxicity studies in *C. mrigala* and *C.idella* is lacking. Hence to measure the susceptibility and survival of the two species, 96 h LC_{50} tests were conducted with copper.

Materials and methods

Male and female fingerlings of *C. mrigala* and *C. idella* ranging in length from 3.5 to 4 inches and weight of 8-8.5 g were procured from a private fish farmer in Kaikaluru, Andhra Pradesh. They were acclimated at a temperature of 28±2°C and fed with rice bran and oil-cake. The copper in water and feed used for the experiments was below detectable level.

As the approximate toxicity of the test material was unknown, a range-finding test was conducted to determine the concentrations that should be used in the definitive test as per APHA, 1989. The experiments were designed as three replicates in tubs containing 100 l of water. Cupric chloride was used asthe copper agent. Double-distilled water was used wherever necessary and the copper agent was of extra pure grade.

Each replicate had five exposure groups for copper as well as one control for *C. mrigala* and five exposure groups for copper (as well as one control for *C. idella*. Each exposure group comprised 10 fish. Feeding was terminated 24 h prior to initiating the tests. Mortality, if any, was only less but not more than 5% during the 48 h immediately before conducting the test. Temperature has not varied by more than ± 2°C during the 96 h test and ± 1°C during any 48 h. Mortality, if any, in a control system was not more than 10%.

To determine LC_{50}, a 96 h test with five toxicant concentrations arranged exponentially and a control were used, according to the results of the range-finding test, adopting the static bioassay method. The experiments were designed as three replicates in tubs containing 100 l of water. Each replicate had five exposure groups for copper as well as one control for *C. mrigala* and the same for *C. idella*. The water quality parameters are the same as those of the range–finding tests. Feeding was terminated 24 h prior to initiating the tests. The variations in temperature was similar to the range finding test. Mortality, if any, in a control system, was not more than 10%.

The experiment contained five copper concentrations (0.25, 0.75, 1.62, 3.25 and 6.62 mgl^{-1}) besides the control (not containing copper) for *C. mrigala* and five copper concentrations (0.5, 0.75, 1.62, 2.5 and 3.25 mgl^{-1}) besides the control (not containing copper) for *C. idella.*

The tubs were checked daily for temperature and dissolved oxygen as they are important factors to be monitored in toxicity experiments. Fish were not fed for one day prior to startingthe experiments to the end of the 96 h experiment period. Aeration was also avoided to make sure that there is no loss of toxicant during that period. Thus build-up of metabolic products and occurrence of high concentrations of carbondioxide and ammonia were avoided. Death was diagnosed by lack of swimming behaviour.

The term LC_{50} is in accordance with APHA (1989), which is the concentration at which 50% of test organisms survive for a specified exposure time. This term has been superseded by median lethal concentration (LC_{50}).

The LC_{50} concentration values were analysed by Probit Analysis (Finney, 1971). Data on mortalities recorded in the three replicates for each concentration were pooled. Regression analysis based on probit (transformed percentage mortality) against log-dose was calculated for each metal independently and considering these calculations for the lethal concentrations (LC_{50}), fiducial limits were determined. Statistical analysis was carried out using computer program, BIOSTAT™ package.

Results and Discussion

The mortality rates increased with increasing copper concentrations, in both *C. mrigala* as well as *C. idella* as shown in Fig. 1 and Fig. 2 respectively. No mortality occurred in the control groups.

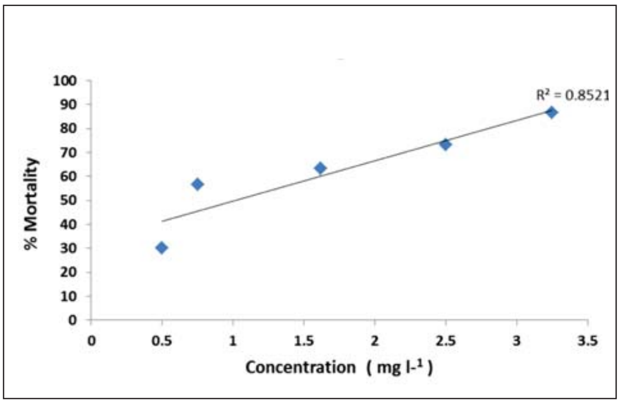

Fig. 1. Mortality (%) in different cupric ion concentrations at the end of 96 hr. exposure experiment with *C. mrigala*

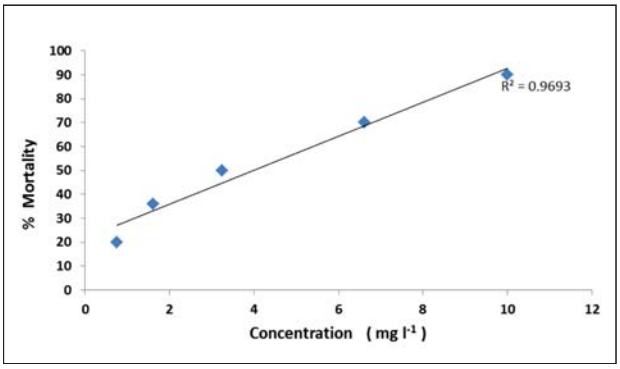

Fig. 2. Mortality (%) in different cupric ion concentrations at the end of 96 hr. exposure experiment with *C. idella*

The concentration values causing 50% mortality at the end of 96 h period, were analysed. LC_{50} was observed at concentrations of 0.74 mg l^{-1} Cu for *C. mrigala* and 3.07 mg l^{-1} Cu for *C. idella* respectively, after 96 h exposure. The concentration values were analysed by Probit Analysis (Finney method) and LC_{50} values were calculated as 0.84 mg l^{-1} Cu, for *C. mrigala* and 2.66 mg l^{-1} Cu for *C. idella* respectively (Table 2 and Table 3). There were no significant differences (p=>0.05) between the observed and calculated mortality. Fig. 3 and 4 show dose response relationship in differentcupric ion concentrations at the end of 96 h exposure experiment with *C. mrigala* and *C. idella.* 95% confidence limits have been calculated for both *C. mrigala* and *C. idella* with copper. The calculated 96 h. LC_{50} values with 95% fiducial limits, their upper and lower limits, and the probit regression equations for toxicity of copper to *C. mrigala* and *C. idella* are given in Table 1.

Similarly, the concentration values were also analysed for LC_5, LC_{10}, LC_{25} , LC_{75} and LC_{90} by Probit Analysis. The LC_5, LC_{10} , LC_{25}, LC_{75} and LC_{90} for *C. mrigala* were calculated as 0.08 mgl^{-1} Cu, 0.14 mgl^{-1} Cu, 0.32 mgl^{-1} Cu, 2.15 mgl^{-1} Cu and 5.01 mgl^{-1} Cu and the LC_5, LC_{10}, LC_{25}, LC_{75} and LC_{90} for *C. idella* with copper were calculated as 0.29 mgl^{-1} Cu, 0.47 mgl^{-1} Cu, 1.05 mgl^{-1} Cu, 6.27 mgl^{-1} Cu and 14 mgl^{-1} Cu respectively. No mortality was observed at concentration of 0.04 mgl^{-1} Cu for *C. mrigala* and 0.25 mgl^{-1} Cu for *C. idella*. It indicates that *C. mrigala* and *C. idella* can be safely consumed if the concentration of copper in water is below 0.04 mgl^{-1} Cu and 0.25 mgl^{-1} Cu respectively. Regression statistics for concentration values of copper and percentage mortality of *C. mrigala and C. idella* in different cupric ion concentrations at the end of 96 h. Exposure experiment is given in Tables 2 and 3.

The results of this study indicated that mortalityrate was influenced by the concentration levels of the heavy metals, as well as the kind of metal used. Besides it was found that there was a positive relationship between the mortality and concentration levels. When the concentration level increased, the mortality rate increased as well. Copper proved to be more toxic to *C. mrigala* than *C. idella.* The susceptibility of fish to a particular heavy metal is a very important factor determing the LC_{50} values. The fish that is highly susceptible to toxicity of one metal, may be less or non-susceptible to the toxicity of another metal at the same concentration (Sajid & Muhammad, 2006).

Table 1. 96 h LC_{50} values with 95% confidence limits and probit regression equations for toxicity of copper to *Cirrhinus mrigala* and *Ctenopharyngodon idella*.

Name of the fish	Lower limit	96 h. $LC_{50}(mgl^{-1})$	Upper limit	Probit Regression Equation
Cirrhinus mrigala	0.5138	0.8477	1.1537	Y = 5.1158 + 1.6143 X
Ctenopharyngodon idella	1.9066	2.6619	3.6045	Y = 4.2801 + 1.693 X

Y = Predicted Probit 5.1158 & 4.2801 = Intercept values 1.6143 & 1.693 = Beta values
X = Log_{10} value [Concentration (Stimulus)

Table 2. Regression statistics for concentration values of copper and % mortality of *Cirrhinus mrigala* in different cupric ion concentrations at the end of 96 h exposure experiment.

LD50	2.6619	LD50 Standard Error	0.4344
LD50 LCL	1.9066	*LD50 UCL*	3.6045
Log10[LD50]	0.4252	*Standard Error*	0.0706
Beta	1.693	*Intercept*	4.2801
Beta Standard Error	0.2896		

Table 3. Regression statistics for concentration values of copper and % mortality of *Ctenopharyngodon idella* in different cupric ion concentrations at the end of 96 h exposure experiment.

LD50	0.8477	LD50 Standard Error	0.1762
LD50 LCL	0.5138	*LD50 UCL*	1.1537
Log10[LD50]	-0.0718	*Standard Error*	0.0896
Beta	1.6143	*Intercept*	5.1158
Beta Standard Error	0.3582		

Similar experiments were also conducted by Panawon (2000), Adhikari (2003), and Ayse (2008) using different fish species, *viz.,* common carp, *Labeo rohita, Catla catla* and rainbow trout respectively. In the above mentioned studies, copper sulphate was used as the copper agent. The values obtained by toxicity testing (eg., LC_{50}) are very dependent on the conditions under which tests were performed, so that interpretation of LC_{50} values needs to be done with caution (Walker et al., 1996).

The findings in the present study are in agreement with the results of Adhikari (2003) and Ayse (2008), in the toxicity levels of copper, though a different fish species and copper salt were used, probably because of the same life-history stage and similarity in the appropriate weight of the fish used.

A 96 h LC_{50} value of 0.56 mgl^{-1} with copper sulphate was determined for *Labeo rohita* fingerlings, by Adhikari (2003). In the present study, 96 h LC_{50} values of 2.57 mgl^{-1} Cu and 0.84 mgl^{-1} Cu with cupric chloride, were determined for *C. mrigala* (8 ± 0.5 g) and *C. idella* (8.5 ± 1g) fingerlings respectively.

Although metals like copper, work as enzyme co-factors as part of a metabolic system, and are essential elements for normal cellular functioning, its higher quantities are toxic for the cell (Cohen et al.,

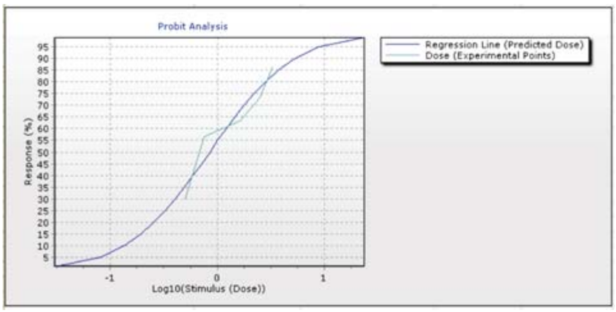

Fig. 3. Probit graph showing dose (experimental points and regression line) response (% mortality) relationship in different cupric ion concentrations at the end of 96 h exposure experiment with *Cirrhinus mrigala*

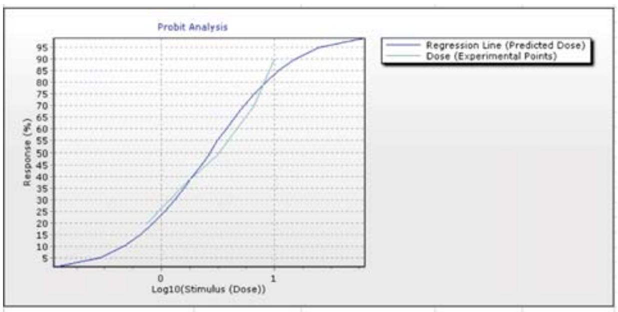

Fig. 4. Probit graph showing dose (experimental points and regression line) response (% mortality) relationship in different cupric ion concentrations at the end of 96 h Exposure experiment with *Ctenopharyngodon idella*

2001). The body has the ability to tolerate excesses of the trace elements but this ability is limited. If the dietary level of a trace element is greater than the body's ability to cope with it, toxicity symptoms will develop (Shinn et al., 2009) Metal accumulation is affected by some of the same parameters that affect toxicity and is potentially one of the most valuable tools for identifying and quantifying the impact of metals in aquatic environments (Borgmann & Norwood, 1994). Absorption of heavy metals can occur via. two pathways, as discussed by Bryan (1976) and demonstrated in a comparative study by Alquezar et al., (2008). The first is absorption from solution. Ion transfer through the gills serves as a good example. Metals may, however, also diffuse passively through skin and gills as a soluble complex down gradients created by adsorption at the surface. The second pathway is absorption from food or particles. After these trace elements are absorbed, it is transferred from the gills and intestine to the blood and distributed to other parts of the body (Hogstrand & Haux, 1991).

Copper has been used effectively for many years to control algae and fish parasites in freshwater and marine systems. Water chemistry and other environmental factors will determine how much copper will be biologically available and for how long. However, the copper concentrations required for effective treatment may be acutely toxic for some species of finfish (Cardeilhac & Whitaker, 1988). Chronic copper exposure will also adversely affect fish health. Copper may cause toxic effects even at low levels under certain conditions. Some species of fish are highly sensitive to copper and will die even at concentrations below therapeutic levels. Factors that affect survival include, the amount of free copper (Cu^{2+}) in the water, the sensitivity of the fish exposed, the age of the fish (Furata et al., 2008), the acclimation time to target concentration (Sellin et al., 2005), presence of dissolved substances that may bind with copper and reduce its activity, including carbonates, the presence of "live foods" that may absorb and bio accumulate copper in their bodies; water pH (Cardeilhac & Whitaker, 1988).

Copper will damage a number of organs and systems, including the gills, liver, kidney, immune system, and nervous system (Cardeilhac & Whitaker, 1988). Gills are the most affected organs during acute toxicity and will become blunt and thickened and lose ability to regulate body fluid ion concentrations (Pickering & Lazorchak, 1995). Copper also suppresses immune system function, and can affect the lateral line of fish. Prolonged copper exposure also may result in reduced growth (Wong et al., 1999). During toxicity, in addition to general signs of distress (e.g., increased respiration), fish may display darkening and behavioral abnormalities like, lethargy, incoordination, problems with posture and balance, and eventually, death (Cardeilhac & Whitaker, 1988).

The results of this study clearly illustrated that the toxic effect of copper to fish, ie., the 96 h LC_{50} value varied according to the species and it's susceptibility to the metal. These findings helped to understand the species-specific dose-response relationship. The dose-response relationship can be compared with the effect of these particular heavy metals on the particular species in the field.

Fish occupies the highest trophic level in aquatic system (APHA, 1981) and humans can be exposed to metals through food web. In predators at top of the food chain, the levels of such pollutants may reach toxic concentrations with deadly results (Shuhaimi et al., 2010), thus implying a need for analytical monitoring of copper jn aquaculture ponds. Hence, assessment of toxicity on a particular organism exposed to a particular toxicant will reveal facts regarding the health of a given ecosystem and would eventually help us to propose policies to protect the ecosystem.

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