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DETERMINATION OF ACUTE TOXICITY OF COPPER AND COBALT FOR *TILAPIA NILOTICA*

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ABSTRACT

During the present investigation, the 96-hr LC_{50} and lethal concentrations of copper and cobalt for *Tilapia nilotica* were determined under controlled laboratory conditions at constant pH (7.25), total hardness (255 mgL^{-1}) and temperature (30 °C). During acute toxicity studies, the physico-chemical parameters of water viz. temperature, pH, dissolved oxygen, electrical conductivity, carbon dioxide, total ammonia, calcium, sodium, magnesium, potassium and total hardness were monitored at 12-hr intervals for each test. Fish were exposed to different concentrations of copper and cobalt, separately, starting from zero with an increment of 0.05 and 0.5 mgL^{-1}. After 96-hr exposure of various concentrations of each metal, the fish mortality data were recorded with three replicates for each concentration. The 96-hr LC_{50} and lethal concentrations for each metal was computed by using Probit analyses method at 95% confidence interval. The 96-hr LC_{50} and lethal concentrations of copper for *Tilapia nilotica* were computed as 25.00±0.65 and 47.56±1.18 mg L^{-1}, respectively. However, the tolerance limits of fish for cobalt, in terms of 96-hr LC_{50} and lethal concentrations were calculated as 96.14±0.58 and 178.46±2.04, respectively. The tolerance limits of fish for both copper and cobalt varied significantly in terms of 96-hr LC_{50} and lethal concentrations. However, fish were significantly more tolerant to cobalt than that of copper. With the increase in metallic ion concentration of the test media (water), the level of ammonia and carbon dioxide increased, while that of dissolved oxygen decreased constantly. Total ammonia of the test media showed significantly direct relationship with carbon dioxide while the same remained significantly negative with dissolved oxygen indicating decrease in oxygen consumption by the fish under metallic ion stress, at different concentrations of copper and cobalt that enhanced the ammonia excretion by the fish.

Keywords: Acute toxicity, LC50, Lethal conc., *Tilapia nilotica*, Copper, Cobalt.

INTRODUCTION

In the present era, one of the major environmental concerns is deterioration of natural resources due to controlled or uncontrolled human activities including disposal of industrial and urban wastes, mining and smelting of natural ores, accidental or processed spillage and application of sewage sludge to agricultural land (Ghosh and Singh, 2005). Among natural resources, water is the most valuable for mankind as its quality is directly linked with human welfare (Alexander, 2008, Rasool and Irum, 2014). A wide range of organic and inorganic contaminants are being regularly released into ecosystem including that of explosive and petroleum products, phenol, textile dyes and other hazardous wastes. Among them, heavy metals are the major component as inorganic contaminants (Jadhav *et al.*, 2010). Over the past few decades, these metals have also been recognized as serious threat to aquatic ecosystems due to their increased influx in natural water bodies. Heavy metals are potentially harmful to most of aquatic fauna, flora and also to human health at some level of exposure, because most of them make reactive oxygen species and are carcinogenetic in nature (Yang and Rose 2003; Farombi *et al*., 2007; Sobha *et al.*, 2007).

Fish are regarded as the most inductive factor in different studies for the estimation of heavy metals because they cannot escape from the detrimental effects of heavy metal pollution. In addition to this, often being at top of aquatic food chain, they eat concentrated large amounts of bioaccumulated metals in prey (Rashed, 2001; Olaifa *et al*., 2004). Toxic effects of heavy metals to the fish include respiratory disorders and growth abnormalities (Hashemi *et al.,* 2008). So, it is very important to know at what level these substances are present in nature and when they become toxic, because they can persist for a long period of time, bio-accumulate and even bio-magnify in aquatic ecosystem (Papagiannis *et al*., 2004).

Among heavy metals, copper plays a vital role in normal physiological regulatory functions of cardiovascular and nervous systems. Copper is also an integral part of various enzymes and protects cells against destruction by oxidation (Hogstrand and Haux, 2001). Despite of the fact that copper is an essential trace metal for several fish metabolic functions, it can be highly toxic to life when it reaches its toxic level by causing biochemical and physiological alterations by generating free radicals (McGeer *et al*., 2000; Shariff *et al*., 2001; Shah, 2002; Monteiro *et al*., 2005). Another heavy metal of great concern is cobalt, which doesn’t occur naturally as a base metal but is normally associated with copper and nickel ores (Barceloux, 1999). Mining and smelting processes of cobalt bearing ores, burning of fossil fuels and uses of cobalt containing phosphate fertilizers are the major anthropogenic sources of environmental cobalt (Linna *et al*., 2004). Toxicity of cobalt causes haem oxidation and blockage of inorganic calcium channels in fish gills (Yamatani *et al*., 1998; Bargagli, 2000).

One of the classical approaches in bio-monitoring is acute bioassay that has been regarded as ‘work horse’ in toxicological studies. It is normally conducted for the period of 48- and 96-hrs and takes death as end point. Acute bioassay can be used to calculate the ‘safe level’ of toxic substances in water bodies (Mohapatra and Rengarajan, 1997). In this regard, the present research work is planned to determine the acute toxicity of copper and cobalt for *Tilapia nilotica*. Tilapias are endemic to the African continent, but within the past thirty years, interest has been made in their commercial farming in developing countries. They are being used worldwide as an experimental specimen due to their malleability and rusticity to laboratory conditions.

MATERIALS AND METHODS

The present research work was conducted in wet laboratory at Fisheries Research Farm, Department of Zoology and Fisheries, University of Agriculture, Faisalabad. The acute toxicity of copper and cobalt for *Tilapia nilotica* was determined in terms of 96-hr LC_{50} and lethal concentrations.

Fish Collection and Acclimatization

Tilapia nilotica fingerlings of the 90 days age group were collected from the Government Fish Seed Hatchery, Faisalabad and were acclimatized in the tanks for one week prior to the experiment. During that period fish were fed with the crumbled feed but were not fed during the last 24-hours of adaptations and throughout acute toxicity tests.

Physico-chemical Parameters

The experiment was conducted in glass aquaria with 50 liters water capacity. Prior to the start of the experiment, it was assured that all aquaria were properly washed with distilled water to remove any sort of impurities and dust particles. Experiment was performed in controlled laboratory conditions at pH (7.25), total hardness (255mg/L) and temperature (30 °C). For the maintenance of desired pH, HCl and NaOH were utilized as per requirement. Salt of EDTA was utilized to decrease or increase the total hardness of water. A constant flow of air was assured during the whole experiment through an air pump. All the physico-chemical tests viz. temperature, pH, dissolved oxygen, electrical conductivity, total ammonia, calcium, sodium, magnesium, potassium and total hardness were performed by following A.P.H.A (1998) on daily basis.

Test Media

Analytical grade copper chloride and cobalt nitrate was used for the preparation of stock solutions that was diluted as desired. Fish were exposed for 96 hours, separately, against different concentrations of copper and cobalt starting from zero with an increment of 0.05 and 0.5 mg/L for low and high concentrations, respectively.

Acute Toxicity Test

The experiment was carried out at stocking density of 10 fish/aquarium. Concentration of each test media was increased gradually and within 3.5-hr, level of metal concentration was maintained to 50% of toxicant concentration, while full toxicant concentrations were attained in 7-hr of exposure.

Collection of Mortality Data

Fish mortalities were recorded at 12, 24, 36, 48, 60, 72, 84 and 96-hrof exposure, and dead fish were removed immediately from the test media.

Statistical Analysis

Probit analyses method was used to calculate the 96-hr LC_{50} and lethal value for both copper and cobalt at 95% confidence interval (Hamilton *et al*., 1977). In order to find out the relationships among physico-chemical parameters, correlation and regression analyses were performed. The statistical differences among various parameters, defined for this study, were studied by using analyses of variance and student Newmann-Keuls test.

RESULTS

The sensitivity of *Tilapia nilotica*, in term of LC_{50} and lethal concentrations was determined against two heavy metals viz. copper and cobalt. Laboratory trials were performed at constant pH (7.25), temperature (30°C) and total hardness of water (255 mgL^{-1}).

Copper

During acute toxicity trials, fish were tested against different concentrations of copper metal (0, 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36, 39, 42, 45 mgL^{-1}). Fish mortality data was collected at every 12-hr interval of metal exposure (Figure 1). Mortality data was computed, through probit analyses, to find out the LC_{50} and lethal concentrations of copper for *Tilapia nilotica*. Estimated LC_{50} for copper was 25.00±0.65 mgL^{-1} while, lethal concentration was estimated as 47.56±1.18 mgL^{-1}(Table 1).

Cobalt

During acute toxicity trials, fish were tested against different concentrations of cobalt metal (0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170 mgL^{-1}). Mortality data recorded during acute toxicity of cobalt tests with *Tilapia nilotica* is presented in Figure 2. Mortality data was computed through probit analyses method to determine the 96-hr LC_{50} and lethal concentrations of cobalt to *Tilapia nilotica* which were estimated as 94.16±0.53 and 178.46±2.04, respectively (Table 1).

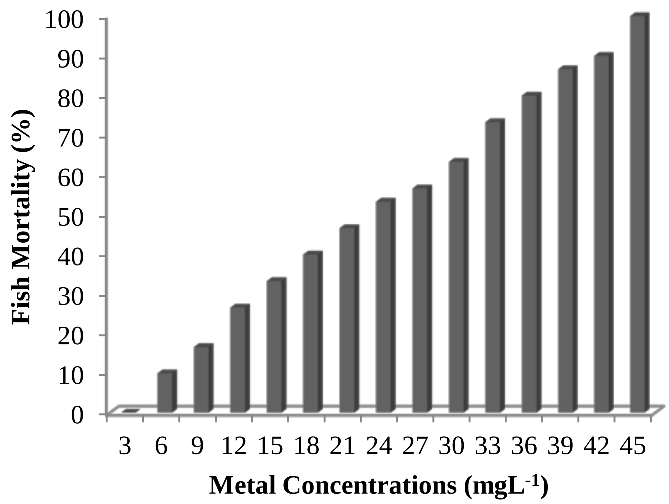

Figure 1: Mean percent mortality of 90 day *Tilapia nilotica* against different concentrations of copper.

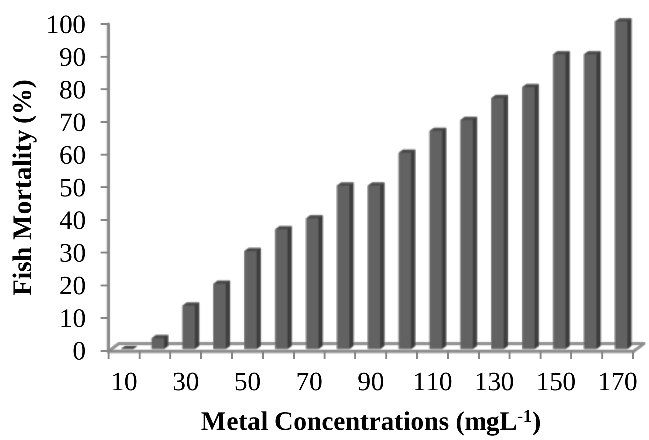

Figure 2: Mean percent mortality of 90 day *Tilapia nilotica* against different concentrations of cobalt.

Table 1: Calculated 96-hr LC_{50} and lethal concentrations of copper and cobalt for *Tilapia nilotica.*

Treatment		Mean 96-hr LC_{50} (mgL^{-1})	95% Confidence interval (mgL^{-1})	Mean 96-hr Lethal conc. (mgL^{-1})	95% Confidence Interval (mgL^{-1})
Cobalt	R_1	24.88±1.71[a]	21.04 - 28.06	49.17±3.35[a]	43.85 - 58.00
	R_2	25.83±1.52[a]	22.50 - 28.67	46.39±2.74[a]	42.00 - 53.42
	R_3	24.25±1.65[a]	20.54 - 27.30	47.12±3.02[a]	42.30 - 55.00
	***Mean±SD**	**25.00±0.65 [b]**		**47.56±1.18 [a]**	
Cobalt	R_1	93.75±5.86[a]	80.77 - 104.64	180.62±11.00[a]	162.90 - 208.63
	R_2	95.00±5.56[a]	82.72 – 105.33	175175.73±9.94[a]	159.53-200.74
	R_3	93.75±5.78[a]	80.96 – 104.50	179.03±10.71[a]	161.70 - 206.25
	***Mean±SD**	**94.16±0.58 [b]**		**178.46±2.04 [a]**	

	Copper	**Cobalt**
LC_{50} conc.	**25.00±0.65 [b]**	**94.16±0.53 [b]**
Lethal conc.	**47.56±1.18 [a]**	**178.46±2.04 [a]**

Single column and* row means with similar letters are statistically non-significant at $p<0.05$.Conc. = Concentration.

DISCUSSION

Acute toxicity tests were performed with *Tilapia nilotica*, to determine the LC_{50} and lethal concentrations of copper and cobalt to the fish. During the study period it was observed that fish showed more sensitivity toward copper than cobalt. Subathra *et al*. (2007) reported 96-hr LC_{50} of copper for *Mystus vittatus* as 18.62 mgL^{-1}, while, Chinni and Yallapragda (2000) reported 96-hr LC_{50} of copper as 2.53 $mgL^{-1}$for *Penaeus indicus*. They reported Cu, the most toxic to the fish, followed by Cd, Zn and Pb. Shariff *et al*. (2001) conducted study to determine LC_{50} of copper for *Puntius gonionotus*. Fish were exposed for 24, 48, 72, 96 and 120-hr and LC_{50} values were estimated at 2.17, 0.91, 0.57, 0.53 and 0.42 mgL^{-1}, respectively.

During the present investigation, 96-hr LC_{50} and lethal values of copper and cobalt for *Tilapia nilotica* were 25±0.53 and 95.00±1.48 mgL^{-1}, respectively. However, lethal concentrations of cobalt and copper for *Tilapia nilotica*, were estimated as 178.00±1.31 and 48.00±0.25 mgL^{-1}, respectively. A number of studies have been conducted to test the tolerance limit of *Tilapia nilotica* against heavy metals. However, very little data is available on acute toxicity of cobalt to *Tilapia nilotica*.

Numerous studies had been performed to assess toxicity of copper and cobalt upon different fish species. A study upon *Mugil cephalus* by Rajkumar (2011) suggested that copper was more toxic to fish than the other metals used in that study (viz. Cd, Pb & Zn). In separate studies with *Cyprinus carpio*, Naji *et al.* (2007) reported 96-hr LC_{50} of cobalt as 327.5 mgL^{-1}. Yaqub and Javed (2011) reported that Indian major carps viz. *Labeo rohita, Cirrhinus mrigala and Catla catla* showed more sensitivity toward cobalt than cadmium metal. Subathra *et al.* (2007) tested acute toxicity of copper to *Mystus vittatus* at two different life stages, juveniles and adults, which were exposed to different concentrations of $CuSO_4$. They concluded that copper was more toxic to juveniles than that of adult fish.

Gundogdu (2008) investigated the tolerance limit of *Onchorhyncus mykiss*, in terms of 96-hr LC_{50}, for copper and zinc ions by preparing the stock solutions of pure salts of zinc chloride and copper sulphates. Copper was found to be a more toxic metal than zinc for *Onchorynchus mykiss* (Rainbow trout). A study conducted by Ebrahimpour *et al.* (2010) on the acute toxicity of copper and zinc to *Capoeta fusca* with reference to soft, hard and very hard water environment, concluded that copper is more toxic to the fish than that of the zinc.

It is necessary to study the relationship and effects of heavy metal concentrations upon physicochemical parameters of water. It was observed during the entire study period that with increases in test metal concentrations, the level of total ammonia and carbon dioxide also increased, while, dissolved oxygen showed an inverse relationship with test metal concentrations. This showed that at high concentrations of heavy metals, oxygen consumption by fish increased significantly. Moreover, high contents of carbon dioxide and total ammonia, with increase of test metal concentrations, added stress upon fish. Significant variations in physico-chemical parameters viz. dissolved oxygen, electrical conductivity, Carbon dioxide, total ammonia, calcium, sodium, magnesium and potassium.

It was observed that dissolved oxygen contents and consumption by fish decreased with increase in metal concentrations viz copper and cobalt. Rafia and Devi (1995) also reported that oxygen consumption by the fish (*Mystlxs gulio)* altered due to exposure to different concentrations of copper and zinc. Copper was found to be a more potent respiratory inhibitor than zinc. Shereena and Logaswamy (2008) studied the impact of heavy metals (copper sulphate, cadmium carbonate, zinc sulphate and lead nitrate) on the oxygen consumption of *Tilapia mossambica*. They reported decreases in oxygen consumption by the fish under metal stressed conditions. At higher metal concentrations, the carbon-dioxide of test media had also increased. Abdullah and Javed (2006) reported that ammonia excretion by the fish increased significantly at a higher concentration of metals.

Toxicity of metals was also greatly influenced by hardness of water during the present investigation. Ebrahimpour *et al.* (2010) studied the acute toxicity of copper and zinc to *Capoeta fusca* with reference to soft, hard and very hard water environment.

Their results showed that toxicity of copper and zinc metals decrease significantly with increasing water hardness. A study conducted by Straus (2003) using copper exposed fingerling of *Oreochromis aureus* revealed that toxicity increases with a decrease in total alkalinity. Rathore and Khangrot (2002) reported that there was a significant (P=0.05) positive relationship between water hardness and toxicity of metal concentrations. Witeska and Jeezierska (2003) found that environmental conditions such as oxygen concentration, temperature, hardness, salinity and presences of other metals modify metals' toxicity to the fish. Boqomazov *et al.*(1991) observed an inverse relationship between water pH and concentrations of mobile iron, zinc and cobalt. Increases in water temperature can enhance the uptake of metals by the aquatic organisms.

Kallanagoudar and Patil (1997) studied the influences of water hardness on copper, zinc and nickel toxicity to *Gambusia affinis* and found copper to be more toxic to the fish than nickel and zinc at the different water hardness values. Effects of water hardness on the toxicity of nickel ($NiCl_2$) and cobalt ($CoCl_2$) in *Capoeta fusca* was studied by Pourkhabbaz *et al*. (2011). Their results showed that water hardness had considerable influence on 96-hour LC_{50} values of both nickel and cobalt. Whereas, significant increase in LC_{50} values for both of the metals was observed with very hard water, which were 204.8 and 127.2 mg/L for both copper and nickel, respectively.

Total ammonia showed significant positive correlation with carbon dioxide while, significantly negative relationship was observed with dissolved oxygen. Total ammonia showed non-significant correlations with pH, temperature and total hardness. Electrical conductivity showed non-significant relationships with all physic-chemical parameters viz. temperature, pH, dissolved oxygen, total hardness, carbon dioxide, total ammonia, calcium, potassium, sodium and magnesium for both metals (copper and cobalt).

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