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METAL MIXTURES TOXICITY AND BIOACCUMULATION IN *TILAPIA NILOTICA* AT 96-HR LC₅₀ EXPOSURE

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ABSTRACT

Laboratory tests were conducted on fingerlings of *Tilapia nilotica* to check the bioaccumulation patterns of lead (Pb), cadmium (Cd) and cobalt (Co) during acute toxicity at constant water temperature, pH and total hardness. Metal mixtures viz. Lead + Cadmium, Cobalt-Lead and Cadmium-Cobalt were selected to assess the acute toxicity in terms of 96-hr LC₅₀ along with bioaccumulation patterns in selected organs of *Tilapia nilotica* (skin, muscles and liver). Sensitivity of *Tilapia nilotica* in terms of 96-hr LC₅₀ for all mixtures varied significantly. The 96-hr LC₅₀ of binary metal mixtures agreed in that order: cadmium-cobalt > lead-cadmium > cobalt-lead with statistically significant differences at $p < 0.05$. Regarding overall tendencies of organs to accumulate metals, the liver exhibited a significantly higher tendency to amass metals, followed by skin, while muscles showed the least metallic ion load. Among mixtures, the lead-cadmium mixture exposure caused significantly higher (107.60 $\mu\text{g/g}$) accumulation in fish, while cadmium-cobalt showed a significantly lower (94.22 $\mu\text{g/g}$) tendency to cause amassing of these metals.

Keywords: Metal Mixtures, Toxicity, Bioaccumulation, *Tilapia nilotica*, Liver

INTRODUCTION

Among different pollutants of aquatic environments, heavy metals have been recognized as serious pollutants because of their non-biodegradable nature, long half-life and potential to accumulate in different tissues of organisms. Compared to the other types of aquatic pollution, heavy metals pollution is less visible but its effects on the ecosystems and humans can be severe (Edem *et al.*, 2008; Yirgu, 2011). These inorganic chemicals (heavy metals) are emitted to the environment through natural and anthropogenic activities like urban and industrial discharges, agriculture, mining and combustion processes (Barone *et al.*, 2013; Ambreen *et al.*, 2015). Metals such as lead, cadmium, chromium, arsenic and mercury are among the non-essential elements which exhibit toxic effects even at very low concentrations, while cobalt, zinc, manganese, nickel and copper are

biologically essential elements which exhibit toxic effects at high concentrations (Couture and Rajotte, 2003). In an aquatic environment, metal toxicity can be influenced by different abiotic environmental factors such as water hardness, oxygen, pH and temperature (Kotze *et al.*, 1999), and by biotic factors like age, weight, species and sex. Elevated levels of heavy metals concentrations may lead to toxic effects or bioaccumulation. Bioaccumulation is the general term used to describe the net uptake of chemicals from the environment through inhalation, ingestion and dermal routes, and from any source in the aquatic environment where chemicals are present (Tulonen *et al.*, 2006). Fish have the ability to accumulate heavy metals in their different tissues through absorption and humans can be exposed to heavy metals through the food chain. This can cause acute and chronic effects in humans (Dogan and Yilmaz,

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2007) because fish play an important role in the human diet. The bioaccumulation of heavy metals in living organisms and their possible bio-magnification is actually the pathway of pollutants transfer from one trophic level to another level (Ghannam *et al.*, 2015). Various metals can accumulate in the fish's body in different amounts. These differences may be due to differential affinity of metals in fish tissues, differences in uptake, and deposition and excretion rates (Akan, 2012).

Individual effects of lead, cadmium and cobalt on different fish species have been extensively studied in the past. In their natural environment, fish are usually exposed simultaneously to a mixture of essential and non-essential metals where different interactions among metals are possible. There are very few studies about the acute toxicity and bioaccumulation of metals in the form of mixtures (Kazlauskiene *et al.*, 1996). Therefore, the present research endeavors to quantify the accumulation of binary metals mixtures, viz. Lead-Cadmium, Cobalt-Lead and Cadmium-Cobalt in selected organs of *Tilapia nilotica* at 96-hr LC₅₀ exposure.

MATERIALS AND METHODS

Acute Toxicity Tests (96-hr)

Binary metal mixtures toxicity tests were conducted in the laboratories at the Fisheries Research Farms, University of Agriculture, Faisalabad, Pakistan. Fingerlings (180-age) of *Tilapia nilotica* were purchased from a local market and brought in the hatchery where they acclimatized in cemented tanks prior to the start of the experiment. They were fed with crumbled feed (30% Digestible Protein and 3.00 Kcal/g Digestible Energy) twice a day. However, the fish were not fed during the acute toxicity tests. Prior to the start of the experiment, glassware and aquaria were thoroughly washed with water and stocked with ten

individuals of *Tilapia nilotica* having similar weights. They were exposed separately to different concentrations of each mixture for 96 hours. The acute toxicity assay was conducted to determine the 96-hr LC₅₀ of binary metal mixtures viz. Lead-Cadmium, Cobalt-Lead and Cadmium-Cobalt for *Tilapia nilotica*. Three replications of each test were used for the determination of 96-hr LC₅₀. Appropriate quantities of chemically pure chloride compounds of lead, cadmium and cobalt were separately dissolved in deionized water for stock solutions preparation on a molar basis while, the required binary metal mixtures preparations of individual solutions of each metal was diluted to the ion equivalence basis (1:1 ratio). For the determination of 96-hr LC₅₀ values, the concentration of each mixture was started from zero with an increment of 0.05 and 5 mgL⁻¹ (as total concentration) for low and high concentrations, respectively. To avoid sudden stress, concentrations of each mixture in aquaria was increased gradually and a 50% test concentration was maintained within three hours with full toxicant concentration in seven hours. Water pH, temperature and total hardness were maintained at 7.75, 30°C and 225mgL⁻¹, respectively, during the whole study period (96-hr). Temperature was kept constant by using electric heaters while chemicals, i.e. CaSO₄ and EDTA, were used to increase and decrease the water hardness respectively. Constant air was supplied to each aquaria with an air pump connected to a capillary system during the whole experimental period. Observations on fish mortality were made after 12-hr intervals, dead fish were collected and their mortality data were compiled.

Metals Mixtures Bio-accumulation Experiments

At the end of 96-hr LC₅₀ toxicity trials, dead fish were dissected and their

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selected tissues, viz. skin, muscles and liver, were separated for heavy metals analyses, rinsed with distilled water and blotted with blotting paper. These tissues were digested in HNO₃ and HClO₄ (3:1 V/V) by placing flasks on a hot plate until a clear solution was obtained (S.M.E.W.W. 1989). Digested samples were cooled, diluted, filtered and then checked for respective metal concentration (lead, cadmium and cobalt) by using an Atomic Absorption Spectrophotometer (AAnalyst-400 Perkin Elmer, USA). All these metals were measured in air-acetylene flame. Calibration standards for each metal were made by serially diluting stock solutions with reagent grade water and checked standards were run along with samples. Analysis of each sample was made in triplicate. A calibration curve was repeated after every five samples.

Data Analyses

Mean values of the 96-hr LC₅₀ were calculated for each mixture treatment at 95% confidence intervals by using the Probit Analyses method (Ezeonyejiaku and Obiakora, 2011) with the help of a MINITAB computer package. Data were statistically analyzed by using MSTATC software by using the methods of Steel *et al.*, (1996). Data were analyzed in terms of metal accumulation in different tissues of fish using the analysis of variance followed by Duncan's Multiple Range test. All tests were accepted as statistically significant when $p < 0.05$.

RESULTS

Acute Toxicity Tests (96-hr)

The 180-age *Tilapia nilotica* was tested for sensitivity (in terms of 96-hr LC₅₀) against waterborne binary mixtures of lead, cadmium and cobalt separately, at constant laboratory conditions. The mean 96-hr LC₅₀ values with 95% confidence interval limits of each mixture for *Tilapia nilotica* obtained after Probit analyses are given in Table 1. Fish showed differential

sensitivity toward all binary metals mixtures. Regarding toxicities of metals mixtures, *T. nilotica* exhibited significantly more tolerance against cobalt-lead, followed by that of the lead-cadmium mixture as evident from their 96-hr LC₅₀ values of 62.68 ± 0.12 and 49.16 ± 0.07 mgL⁻¹ respectively. However, *T. nilotica* exhibited higher sensitivity against the cadmium-cobalt mixture (37.93 ± 0.10 mgL⁻¹). The 95% confidence interval limits for this mixture ranged from 31.80 – 42.15mgL⁻¹. Toxicities of mixtures to *T. nilotica* were increased in that following sequence: cobalt-lead < lead-cadmium < cadmium-cobalt with statistically significant differences at $p < 0.05$.

Metals Mixtures Bio-accumulation

Table 2 shows the mean values of metals accumulation in different tissues of *Tilapia nilotica* at 96-hr LC₅₀ exposure. There were significant differences for the accumulation of metals (Pb, Cd and Co) in different tissues viz. skin, muscles and liver of fish.

Lead-Cadmium

The 96-hr LC₅₀ exposure of the lead-cadmium mixture to *Tilapia nilotica* caused significant amassing of both these metals in the liver as evident from their mean value of 87.39 ± 1.29 ug g⁻¹, while muscles exhibited the least tendency to accumulate these metals. However, the residues of cadmium were found higher in fish tissues as compared to lead.

Cobalt-Lead

The binary mixture of cobalt and lead caused significantly higher accumulations of this mixture in fish livers, followed by that of skin and muscles, while the overall means showed that contents of cobalt were higher in tissues as compared to lead.

Table 1: Mean 96-hr LC₅₀ values (mgL⁻¹±SD) of binary metals mixtures.

Species	Metal Mixtures		
	Lead-Cadmium	Cobalt-Lead	Cadmium-Cobalt
<i>Tilapia nilotica</i>	49.16±0.07 b (CI = 44.25 – 53.03)	62.68±0.12 a (CI = 52.46 – 71.16)	37.93±0.10 c (CI = 31.80 – 42.15)

CI = Confidence Interval; Means with similar letters in single row are non-significant at p<0.05.

Table 2: Tissue specific metals accumulation (µgg⁻¹±SD) in fish at 96-hr LC₅₀ exposure.

	Tissues/Organs				
	Skin	Muscles	Liver	*Overall Means	
<i>Tilapia nilotica</i>					
Lead-Cadmium					
Lead	53.24±0.13	14.47±0.07	86.47±0.06	51.39±36.04 b	107.60
Cadmium	62.54±0.26	17.78±0.03	88.30±0.14	56.21±35.68 a	
Means±SD	57.89±6.58 b	16.13±2.34 c	87.39±1.29 a		
Cobalt-Lead					
Cobalt	54.74±0.17	22.53±0.03	89.24±0.19	55.50±33.36 a	104.68
Lead	44.37±0.12	17.12±0.07	86.05±0.10	49.18±34.72 b	
Means±SD	49.56±7.33 b	19.83±3.83 c	87.65±2.26 a		
Cadmium-Cobalt					
Cadmium	58.44±0.05	10.77±0.07	81.12±0.11	50.11±35.91 a	94.22
Cobalt	47.21±0.09	9.20±0.05	75.93±0.14	44.11±33.34 b	
Means±SD	52.83±7.94 b	9.99±1.11 c	78.53±3.67 a		

Means with similar letters in single row and overall column are non-significant at p<0.05.

Cadmium-Cobalt

Regarding the tissue specific bio-accumulation of the cadmium-cobalt mixture, the liver of *Tilapia nilotica* exhibited a higher ability to accumulate both these metals as obvious from their mean values of 81.12±0.11 and 75.93±0.14 µgg⁻¹ respectively. The load of cadmium and cobalt in selected tissues of *Tilapia nilotica* were in the order: liver > skin > muscles. Results revealed that the uptake of cadmium was higher in fish tissues as compared to cobalt.

Regarding overall tendencies of fish organs, the liver amassed significantly higher amounts of metals, viz. lead, cadmium and cobalt, at 96-hr LC₅₀ exposure of binary mixtures, while muscles contained the least metals

concentrations. However, the overall amassing pattern of mixtures followed the order: lead-cadmium > cobalt-lead > cadmium-cobalt.

DISCUSSION

The acute toxicities of waterborne metals mixtures, viz. Pb-Cd, Co-Cd and Cd-Co, to *Tilapia nilotica* were evaluated in terms of 96-hr LC₅₀ at constant laboratory conditions. *Tilapia nilotica* showed resistance to Co-Cd while it exhibited significantly more sensitivity towards the Co-Cd mixture. Metals such as cobalt, chromium, copper, iron, nickel, selenium and zinc are essential components for metabolism. Therefore, certain concentrations of these metals are required for the normal physiological functioning of fish (Mahboob *et al.*, 2014),

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while lead and cadmium are non-essential metals and are very toxic even in less concentrations (Fernandes *et al.*, 2008). The strongest interactions often occur in binary mixtures and the interactive effects may become minor with an increased number of mixture components (Lydy *et al.*, 2004; Liu *et al.*, 2015). Toxicity of metal mixtures depends on the relative affinity and potency of toxicants, dissolved metal concentrations, ratios and the background solution composition (Balistrieri and Mebane, 2014). Mixtures of metals are more toxic than that of individual metals. Acute toxicity of metals and their mixtures followed the order: Cu+Hg > Hg > Cu > Cd+Hg > Hg for *Oncorhynchus mykiss* (Jeziarska and Sarnowski, 2002). Similarly, Munshi *et al.* (2005) observed the acute toxicity (LC₅₀) as Cd > Cu > Cd+Cu for *Oreochromis mossambicus*. Fikirdesici *et al.* (2012) investigated the acute toxicity of Cd, As and a Cd+As mixture to *Daphnia magna* by calculating the toxic unit approach. Cadmium was found to be more toxic than As, while the mixture of Cd+As showed more toxicity than Cd alone. Sindhe and Kulkarni (2004) also conducted the toxicity tests for Hg, Cd and their mixture (Hg+Cd). The order of toxicity in terms of 96-hr LC₅₀ was: Hg > Hg+Cd > Cd. Similarly, Rashed (2001) also reported that *Oreochromis niloticus* exhibited a higher sensitivity against mixtures of metals rather than an individual metal.

Amassing of metals in fish can be considered an indicator of pollution in the aquatic environment because it is the ability of organisms to concentrate an element from food and water to a level higher than that of its environment. The difference in metal accumulation in different organs might be a result of their capacity to induce metal binding proteins such as metallothioneins (Canli and Atli, 2003). Following entering the fish body, heavy metals accumulate in the kidney, liver, skin, gills, fins, muscles, heart,

scales, gut and brain (Kousar and Javed, 2014; Ambreen *et al.*, 2015). At 96-hr LC₅₀ exposure, the overall amassing pattern of mixtures followed the order: lead-cadmium > cobalt-lead > cadmium-cobalt during the present study. It was observed that the liver accumulated a higher quantity of all selected metals, followed by skin, while muscles showed the least tendency for such accumulations. In general, the uptake and bio-accumulation of metals in fish followed the order: liver > skin > muscles. Liver is the main centre for metabolism and detoxification (Iqbal *et al.*, 2005). Rugmony *et al.* (2005) observed that salmonids were more sensitive to higher levels of Cd. However, when fish were exposed to the mixture of Cd and Pb (Cd+Pb), the accumulation of both these metals was significantly increased. Similarly, Arain *et al.* (2008) also reported significant concentrations of cadmium ($9.30 \pm 1.44 \mu\text{g g}^{-1}$) and lead ($8.40 \pm 0.60 \mu\text{g g}^{-1}$) in *Oreochromis mossambicus*. Similarly, exposure to the Cu+Cd+Zn+Ni+Co mixture caused significantly higher accumulations of all these metals in fish livers followed by the kidney and gills (Javed and Abdullah, 2004). Skin has maximum surface area and direct contact with the exposure medium. Therefore, the rate of accumulation was less when it was compared with other tissues like the liver, kidney and gills. Skin is also consumed by humans, along with muscles, and therefore, skin is very important for the accumulation point of view (Yousafzai and Shakoori, 2009). Studies have shown that muscle is not an active tissue in accumulating heavy metals, perhaps due to low levels of metallothionein in these tissues (Yilmaz *et al.*, 2007). In the present study, muscle accumulated the least metal burden as compared to other organs, which is in accordance with the findings of Gbem *et al.* (2001), Azmat *et al.* (2006) and Al-Kahtani (2009). Muscle tissues appeared to be the least preferred site for the

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accumulation of metals (Eneji *et al.*, 2011). However, for the routine monitoring of environmental contaminants, muscle is the major tissue of interest because it is consumed by humans.

CONCLUSION

Acute toxicity tests are among the first step in determining the water quality requirements for fish because these studies revealed the toxicant concentrations (LC₅₀) that cause fish mortality even at short exposure durations. Therefore, studies representing the adverse effects of metals on aquatic organisms, especially in fish, are needed. In aquatic environments, metals are actually present in the form of mixtures or different combinations. Therefore, it was a dire need to evaluate the effects of metals mixtures. The results obtained from this research clearly exposed that it is very necessary to control the discharge of heavy metals.

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