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ACUTE TOXICITY AND BIOACCUMULATION OF HEAVY METALS IN RED TILAPIA FISH

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ABSTRACT

Juvenile hybrid tilapia (*Oreochromis* sp.) was tested with different concentration of heavy metals under varying exposure time to examine acute toxicity effect on their survival rate and bioaccumulation in fish tissues. Copper (Cu), cadmium (Cd) and zinc (Zn) was used at rates 0.0, 0.50, 1.0, 3.0 and 5.0 mg L^{-1}. The medial lethal concentration of Cu, Cd and Zn (96h LC_{50}) was determined to be 0.45, 0.7 and 2.1 mgL^{-1}, respectively in a Probit transformed concentration - response curves. Fish tissues were digested in Nitric acid (65%) and Hydrogen peroxide (35%) under microwave oven and analyzed by inductive coupled plasma optical emission spectrometry (ICP–OES, Model Perkin Elmer Optima 5300DV, USA). Tilapia fish mortality was significantly higher with higher concentration of toxic metals. The fish toxicity by heavy metals was in the following order: Cu > Cd > Zn. The liver tissues obtained the highest accumulation of Zn (423 mg kg^{-1}) followed by Cu (136 mg kg^{-1}) with the highest concentration of each toxic metal. The gill tissues recorded the highest accumulation of Cd (121.0 mg kg^{-1}) with the highest concentration of Cd while muscle tissues accumulated the highest Zn concentration (31.0 mg kg^{-1}) with the highest concentration of Zn. Accumulation of heavy metals in fish tissues of different organs was in the following order: liver > gills > muscles for Cu and Zn while gills > liver > muscles for Cd. Regardless of tissue organs accumulation of toxic metals increased with higher concentrations of heavy metals and exposure period.

Key words: Acute toxicity, exposure, bioaccumulation, LC_{50}, LT_{50}.

INTRODUCTION

Contamination of heavy metals in aquatic environment is increasing globally and describes one of the most critical environmental risks (Nriagu *et al*., 1998). Certain heavy metals are common in our environment and trace amounts are required for human wellbeing such as iodine (I), iron (Fe), selenium (Se), molybdenum (Mo), cobalt (Co) Cu, Mn, and Zn. These elements are commonly found naturally in food stuffs, fruits and vegetables (WHO, 1996). Some of them are essential nutrient required for life but are poisonous substances to aquatic organisms in extreme concentrations (USEPA, 1985). Heavy metals are also common in industrial applications such as in the manufacture of pesticides, batteries, alloys, electroplated metal parts, textile dyes, steel, and so forth (IOSHIC, 1999). Heavy metals become toxic when they are not metabolized by the body and accumulate in the soft tissues. Generally, acute toxicity is usually from a sudden or unexpected exposure to a relatively high concentration of chemicals in a short period of exposure, consequently, acute effects symptoms can appears after exposure (Ahmed *et al*., 2013). Acute toxicity of heavy metals can damage to blood composition, affect on gastrointestinal system including the liver and other vital organs (Naughton *et al*., 2011) as well as impact on neurological system (Pohl *et al*., 2011). Sometimes, changes in growth, behavior and reproduction or may be conducing to death of fresh water organisms (Rand *et al*., 2003).

In bioaccumulation process the tissues of a living organism can absorb toxic metals if their availability is very high in environment or food. In addition their tendency to move up the food chain as one species consumes another, becoming increase in concentration of a substance than as they go, is called biomagnification (Rana, 2006). The level of heavy metal in fish tissues is influenced by biotic, abiotic and environmental factors such as fish species, habitat, fish age, concentration of metal in water, exposure period, water temperature, pH in water, dissolved oxygen (DO) concentration, water salinity and other physiological conditions (Scott *et al*., 2004; Tsai and Liao, 2006; Has-Schon *et al*., 2006; Uysal *et al*., 2008; Vinodhini and Narayanan, 2008; Ling *et al*., 2009; Rema and Philip, 2012). Tilapia is the third group of the most important farmed fish in the world, after carps and salmonids. It's culture is also one of the fastest aqua cultural growth with an average annual growth rate of 13.4% (FAO, 2004). Target samples are hybrid tilapia, which has become popular through collaborative program with world fish centre on Genetic Improvement of

Farmed Tilapias as the GIFT. The hybrid tilapia has been selected due to the following reasons: high productivity, significant improvements in growth rate in successive generations, as well as remarkable survival rates in the Malaysian aquacultures, which became an important food source for human beings (Ponzoni *et al.*, 2010; Ponzoni *et al.*, 2005;).

Tilapia and other cichlids along with top ten species groups for 91.0 % of the total aquaculture contribution to fisheries food supply because of faster production rate, tolerance with salinity and meets the market needs (FAO, 2007). A number of local studies have been focused on the toxicity and bioaccumulation of heavy metals on tilapia and other fish species (Mokhtar *et al*., 2009; Taweel *et al*., 2011; Low *et al*., 2011; Ashraf *et al*., 2012) but no information is available on hybrid tilapia fish in Malaysia. Therefore, this study has been undertaken on hybrid tilapia fish because it appears to have great economic and ecological importance in Malaysia. The objectives of this study were to investigate the acute toxicity effect of different concentrations of Cd, Cu, and Zn on survival of hybrid tilapia and to quantify the accumulation levels of toxic metals in the fish tissues among muscles, gills and liver with varying exposure period.

MATERIALS AND METHODS

Acute toxicity assay: The study was carried out with hybrid tilapia (*Oreochromis* sp.) under different concentrations of heavy metals. The commencement and termination date of experiment was Nov 2013 – Jan 2014. About 500 healthy fingerlings (7.0±1 g body weight and 7.5±2 cm total length) were collected from a commercial aquaculture in Perak, Kampar, Malaysia; then transported in oxygenated water proof plastic bags to the laboratory and handled properly to minimize injury and stress physiology in order to reduce the number of dead organisms. Acclimatization was done in a 50-L glass aquarium for 48h. A dry commercial food pellets with 25% of crude protein was provide to feed fish during this period. Thereafter fingerlings were transferred to 5-L (20 x 20 x 40 cm) test containers/glass aquarium for toxicity assay. Air pumps and individual air stone diffusers were provided for well aeration. The stock solution (1000 mg L^{-1}) of Cd, Cu, and Zn was freshly prepared by dissolving of analar grade chemically pure salts of $CdSO_4$, $CuSO_4$, and $ZnCl_2$ obtained from Merck (Germany) with deionized water. All toxicity test concentrations were made from the stock solution using appropriate calibrated analytical pipettes and graduated cylinders. Cadmium, Cu and Zn was used at rates of 0.0, 0.5, 1.0, 3.0 and 5.0 mg L^{-1} range determined based on results of preliminary tests; and test was carried out with three simultaneous replicates per treatment. Each metal were prepared by adding a calculated volume from the stock solution into test containers considering an equivalent of respective heavy metals. Experiments were exposed at light: dark regime of 16:8h and 26±2ºC for 24, 48, 72 and 96h. Each metal with different concentrations and exposure period was considered as an individual experiment. A stocking density of 10 fish per aquarium/container was used against each metal. The experiment was carried out under a completely randomized design with three replications. No food was supplied for fish during experimental period. Test solutions were replaced by fresh ones of the same respective concentration at every 24h interval until 96h exposure (APHA *et al*., 1999). During each replication, the fish were exposed to a control group (no metal added) and four concentrations of each element Fish mortalities were recorded at 6, 12, 24, 48, 72, and 96h exposure, and dead organisms were removed regularly from the test solutions. The aim of the test was to determine the median lethal concentration (LC_{50}) which was estimated by the probit transformed concentration - response curves (USEPA, 2002).

Bioaccumulation test: Juvenile hybrid tilapia fish was exposed at various concentrations of Cu, Cd and Zn. The median lethal time (LT_{50}) was determined from higher concentration of each toxic metal with different exposure at 15, 18, 53, 48 and 96 h, respectively (Fig. 1). The active fish was collected and dissected into gills, liver and muscles (dorsal surface of the fish) by using stainless steel knife (scalpels). The tissues of fish organs were then dried in an oven at 105ºC for 24 h until they reached a constant weight. The dry samples of each organ was grounded using a porcelain mortar and pestle. From each sample, muscles, gills and liver tissues were digested by using closed vessel (Nguyena *et al.*, 2005, Uysal *et al*., 2008) in a microwave oven (Milestone model Start D, Italy) for analysis. The samples were digested by adding 6 ml nitric acid (65 %) and 1ml H_2O_2 (35%). A ramped temperature control program was applied at 150ºC during 15 minutes followed by 15 minutes at 150ºC and 10 minutes cooling down in the microwave until they reached at room temperature. The residues were then dissolved and diluted to 50 ml for muscle and gill and 25 ml for liver sample in deionized water. Then the samples were filtered using Whatman filter paper (0.45 µm). The concentration of heavy metals in fish samples were determined by ICP–OES (Perkin Elmer AA Analyst). All glassware were soaked in nitric acid for 3 days and rinsed with deionized water before being used (Csuros and Csuros, 2002). The instrument was calibrated with chemicals standard solution prepared from commercially available chemicals. Standard stock solutions of Cd, Cu and Zn were prepared from titrasol (1000 mg/L) of each element to make the calibration, all reagents used were of analytical reagent grade (Merck, Germany). The working solution was freshly prepared by diluting an appropriate aliquot of the stock solutions. In order to check the validity of the measurements for accuracy and precision, certified reference materials (Dogfish muscle: DORM-2, National Research Council, Canada) were analyzed for each element. The detection limit is defined as the concentration corresponding to 3 times the standard deviation of 10 blanks

Data analysis: LC_{50} at 24, 48, 72, 96h exposure values were calculated by probit analysis (USEPA, 2002). Data were analyzed through analysis of variance (ANOVA) using SPSS software. The differences among means were evaluated through LSD test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Median lethal time and median lethal concentration: The higher LC_{50} values were recorded with Zn at rates of >5 and 5 mg L^{-1} under 24, 48, 72 and 96 h exposure, respectively. The lower LC_{50} values were recorded with Cu at rates of 1.85, 0.90, 0.55 and 0.45 mg L^{-1} 24, 48, 72 and 96 h exposure, respectively (Fig. 2). The LT_{50} and LC_{50} values decreased with higher levels of toxic metal concentrations and exposure respectively (Fig. 1 and 2). The results of LC_{50} values at 48 h exposure coincided with the findings of Subathra and Karuppasamy (2008). They reported that heavy metals toxicity of *Mystus vittatus* fingerlings LC_{50} for Cu was 18.6 ppm under 96-h exposure whereas Othman *et al*., (2010) found that *Rasbora sumatrana* (cyprinidae) was (0.005ppm) for Cu and (0.10 ppm) for Cd. The fish toxicity in these studies was in the following order: Cu > Cd > Zn. The toxicity of heavy metal differed among test organisms, which was attributed to several factors such as the mechanism action of the different metals, chemical characteristics of the test solution and sensitivity or tolerance of test organism (Otitoloju and Don-Pedrok, 2002; Straus *et al*., 2003). The LC_{50} values indicated that Cu ranked most hazardous among tested heavy metals and caused significant mortality followed by Cd. Similar results were reported by Grosell *et al*., (2002) and they reported that acute toxicity effect of Cu on rainbow trout gills inhibited branchial Na^+ and Cl^- uptake that lead to mortality. Gundogdu (2008) found that Cu ion concentrations were more toxic than Zn for rainbow trout fish. Tilapia fish had a higher sensitivity to Zn during 96-h (LC_{50} at 2.1 ppm). These results are consistent with study of Rema and Philip (2012) and they found 4.2 ppm in *Oreochromis mossambicus* while *Oreochromis niloticus* was more tolerant to Zn under 96-h at LC_{50} with 60 ppm (Firat and Kargin, 2010).

Bioaccumulation of toxic metals: Accumulation of toxic metals in fish tissues were significantly affected by treatment variations (Table 1). Regardless of fish organ the highest accumulation was recorded from higher concentration of toxic metal. Fish exposed with heavy metals concentrations, liver recorded higher concentration of metals than gill followed by muscle. Among different organs liver obtained the highest accumulation regardless of toxic metals. Gill tissues recorded higher levels of Cd with the highest concentration (5 mg L^{-1}) of Cd. The metal accumulation among fish organs were in the following order: liver > gills > muscles for Cu and Zn while gills > liver > muscles for Cd. The maximum level of toxic metals accumulation was observed in liver (72 mg kg^{-1} for Cd 136 mg kg^{-1} for Cu and 423 mg kg^{-1} for Zn) with higher concentration of each toxic metal (Table 1). The levels of toxic metals increased with higher exposure among all heavy metals. Tilapia fish has greater capacity for metal bioaccumulation due to low sensitivity of some heavy metals (Mokhtar *et al*., 2009). The toxic metal accumulation in fish tissues depends on concentration and exposure as well as other factors, such as interaction with other metals, water chemistry, and metabolic activity of fish (Heath, 1995). The results of the present research demonstrated that exposure with heavy metals were affected on bioaccumulation levels. Among tested metals, Zn had a lower impact on fish survival and it was accumulated in higher level. Toxic metals accumulation levels were in the following order: Zn > Cu > Cd in all organs except gill with Cd at the rate of 3.0 mg L^{-1}. Our results showed that concentrations of most metallic ions accumulated in lipid tissues especially in liver. Similar studies were reported by Wong *et al*., (1981) and they found that accumulation of tetramethyl lead by rainbow trout which could be due to the lipophilic properties of metallic compounds and were likely to be found partitioned into fish especially in the lipid tissue. The results of the present study are similar to the findings of Subathra and Karuppasam (2008). They reported that accumulation of Cu in liver of control and tested fish were 12.4 and 82.1 mg/kg, respectively. Liver appears to be one of the most important sites for Zn accumulation in channel punctuates and principal site which represent storage of metal in the fish while the metal levels in the gills reflect concentrations of element in the ambient water (Senthil *et al*., 2008). The high levels of accumulated heavy metals in liver may be attributed to the sequestering and binding of this metal by metallothionein (MT) (Montaser *et al*., 2010). Some of essential elements are found in fish under homeostatic regulatory control such as Cu, and usually Cu concentration have adjustable below 50.00 mg kg^{-1} dry weight (Couture and Rajotte, 2003). But any impact or loss to mechanism of homeostatic control will be over loaded; hence, Cu concentration in liver can increase (Subathra and Karuppasamy, 2008). Furthermore, the higher accumulation in liver may alter the level of various biochemical parameters and may also cause severe liver damage (Abdel-Warith *et al.,* 2011).

Table 1. Bioaccumulation (mg kg^{-1} dry wt.) in muscles, gill and liver of hybrid tilapia under different concentrations of heavy metals and exposure

Toxic metals	Fish organs	Concentration (mg L^{-1})				
		0.0	**0.5**	**1.0**	**3.0**	**5.0**
Cd	muscle	0.1e	0.3e	0.91e	1.4e	2.4e
	gill	0.3e	2.8e	22.1c	120.8a	32.6c
	liver	0.5e	20.6d	21.0d	35.7c	71.8b
Cu	muscle	1.4f	4.6f	2.5f	4.0f	--
	gill	6.3f	10.5e	6.4f	38.4d	--
	liver	19.4e	78.3c	117.5b	136.0a	--
Zn	muscle	11.5g	16.1g	18.1g	16.9g	30.8f
	gill	49.5f	40.3f	72.2e	105.4d	98.1d
	liver	93.9d	177.3c	185.0c	217.6b	422.8a

Values with same letter differ non-significantly (P>0.05)

Table 2. Fish mortality in percent with heavy metals concentrations and exposure.

Exposure (h)	Cd Concentration (mg L^{-1})				
	0.0	**0.5**	**1.0**	**3.0**	**5.0**
24	0.0	7.0g	20.0f	40.0d	60.0c
48	0.0	20.0f	33.0e	73.0b	100.0a
72	0.0	20.0f	53.0c	100.0a	-
96	0.0	30.0e	70.0b	-	-
	Cu Concentration (mg L^{-1})				
Exposure (h)	**0.0**	**0.5**	**1.0**	**3.0**	**5.0**
24	0.0	20.0f	27.0e	70.0b	100.0a
48	0.0	30.0d	53.0c	80.0b	-
72	0.0	40.0d	73.0b	100.0a	
96	0.0	57.0c	93.0a		
	Zn Concentration (mg L^{-1})				
Exposure (h)	**0**	**0.5**	**1.0**	**3.0**	**5.0**
24	0.0	0.0	20.0d	20.0d	40.0bc
48	0.0	7.0e	27.0cd	27.0cd	43.0b
72	0.0	20.0d	30.0cd	40.0bc	50.0b
96	0.0	27.0cd	37.0c	50.0b	73.0a

Values with same letter differ non-significantly (P>0.05)

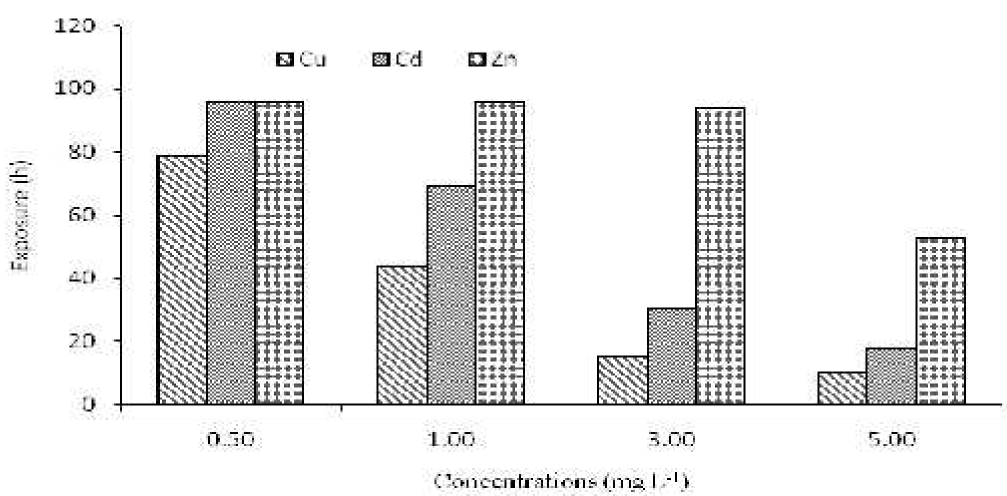

Fig. 1. Median lethal time (LT50) of heavy metals in tilapia fish under different concentrations and exposure

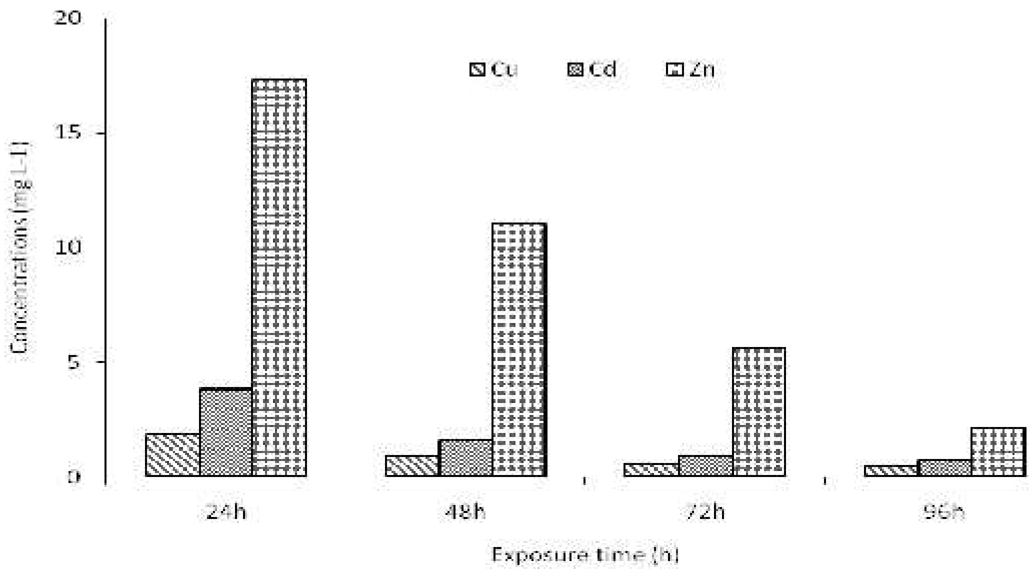

Fig. 2. Median lethal concentrations (LC50) of heavy metals in tilapia fish under different exposure

Fish mortality increased with higher concentration and exposure of heavy metals (Table 2). This was possibly due to these toxic metals directly influenced on respiration process of tilapia fish. Similar results were demonstrated by Chen *et al*., (2012). The heavy metal toxicity is ascribed to the fall in the diffusing capacity of the gill, the decrease of oxygen tension and consumption, the physiological imbalance, restlessness, the fall in blood pH, the increased gill ventilation, the opercular movement, the breathing rate and the concentration of metabolic products. Furthermore, smaller sized species are more sensitive to acute toxicity of heavy metals than the larger ones (Grosell *et al.*, 2002). When the fish were exposed to heavy metals toxicants, the effects of chemical exposure disturb the homeostasis and changes in physiological processes of fish that may influence on survival such as the metals concentrate in the cell membranes and causing lysis as well as biotic concentrations activate certain enzymes which participate in metabolic synthesis of the organic compounds in fishes (Tan *et al*., 2007; Fidan *et al*., 2008). The gill seems to be a chosen site next to the liver organ. The present results supports the view of Karuppasamy (2004) who suggested that the accumulation of heavy metal in gills may be attributed rapidly to the large amount of water that passes through the gills to supply oxygen under stress of toxicity.

The present study showed that Cd at the rate of 3.0 mg L^{-1} accumulated more in gill tissues than liver tissues. The gill accumulated more concentration of heavy metal than liver followed by muscle for short time exposure, nevertheless, the heavy metal level was in liver > gills for long term exposure (Bervoets *et al.,* 2001). Firat and Kargin (2010) reported that Zn and Cd ions caused an increase in alanine aminotransferase and aspartate amino transferase activities in blood which used in the diagnosis of damage in liver, muscle, and gills, as well as, increased in concentrations of serum, albumin, and transferring which are responsible for transport of heavy metals from the gills to other organ throw blood of *Oreochromis niloticus*. The results in the present study revealed that the fish muscles contained lower concentration of metals compared to other organs. The toxic metal concentration in control fish muscles were within the approved limits for human consumption and lower maximum level limit which has been reported by Malaysian Food Act 1983 and Food Regulations 1985 (MHM 2012) whereas the metal content in muscles tissues of treated fish was varied significantly ($p<0.05$) higher than in the control groups. These results are similar to Vinodhini and Narayanan (2008). They reported that heavy metals were spread uniformly over the body muscles in lower ratios and this information can be used to estimate the biochemical measurements alteration in fish metabolism.

Conclusions: The results revealed that tilapia fish had a higher sensitivity to Cu and considered the most hazardous among tested toxic metals followed by Cd and Zn. The hybrid tilapia fish showed poor response to Zn. The juvenile hybrid tilapia fish is capable of accumulates heavy metals in their tissues from an aquatic environment and the ability of fish is another important factor to consider for future study. In addition these data constitute an important reference to assess the hazard of metal accumulation in fish tissues in the ecotoxicological testing scheme.

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