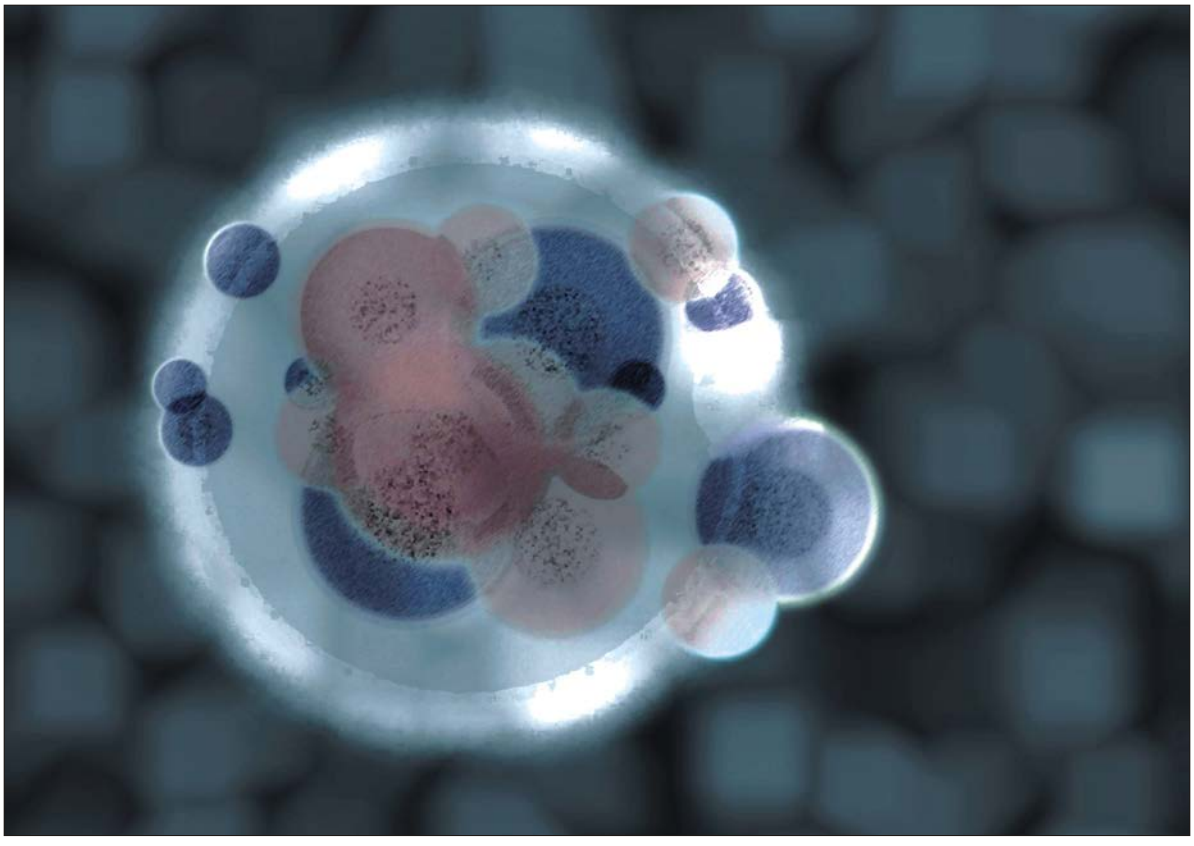

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In vivo Acute Toxicity Tests of Some Heavy Metals to Tilapia Fish (*Oreochromis niloticus*)

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Abstract: Fingerlings tilapia fish (*Oreochromis niloticus*) were exposed in laboratory conditions to a range of Copper (Cu), Cadmium (Cd), Lead (Pb) and Zinc (Zn) concentrations separately and the mortality rate were registered after 24, 48, 72 and 96 h. Median Lethal Concentrations (LC_{50s}) and Median Lethal Times (LT_{50s}) values were calculated by static bioassays for 24, 48, 72 and 96 h with the 95% fiducial limits through Probit analysis. LC_{50} and LT_{50} increased with the decrease in mean exposure times and concentrations, respectively. The LC_{50}s for 96 h for Cu, Cd, Zn and Pb were 1093, 3751, 16177 and 1494 μg L^{-1}, respectively and the LT_{50}s for maximum concentration used for Cu, Cd, Zn and Pb were 20.25, 11.48, 20.70 and 24.66 h, respectively. Metals bioconcentration in tilapia fish increases with exposure to increasing concentrations and Cu was the most toxic to tilapia fish, The toxicity ranking of the four heavy metals was Cu>Pb>Cd>Zn. Comparison of LC_{50} values for studied heavy metals for this species with those for other fishes reveals that tilapia fish (*Oreochromis niloticus*) is equally or less sensitive to heavy metals than most other tested fishes.

Key words: Acute toxicity, bioaccumulation, heavy metals, ICP-MS, LC_{50}, LT_{50}, tilapia fish

INTRODUCTION

Aquatic organisms have been considered to concentrate metals several times greater than environmental levels. Fishes have been used for many decades to evaluate the pollution status of water and are thus considered as excellent biological indicator of heavy metals in aquatic environments (Hedayati *et al*., 2010). Fishes are extensively distributed creatures in the marine and freshwater environments (Taweel *et al*., 2012). Tilapia fish Oreochromis niloticus have some features such as the ability to persist in poor water quality and quick growth (Ayotunde and Ofem, 2008). Trace metals occur naturally in the environment and found in varying levels and are considered the main ecosystem pollutants to the aquatic life (Azmat *et al*., 2012). Anthropogenic activities cause an increased discharge of these metals into the aquatic ecosystem; fish are relatively sensitive to these changes in their environment (Abdel-Warith *et al*., 2011). Toxicity tests are conducted to assess the effects of single or complex mixture of contaminants on the organisms. In freshwater, the existence or absence of fish has been extensively used as a biological indicator of the level of pollution. In acute tests experiments, one of the commonly used measures is the LC_{50}s. In other words, the lethal median concentration that causes mortality in 50% of the test organisms (Boateng *et al*., 2006).

The main purpose of ecotoxicologyy is the estimation of risk for an ecosystem exposed to environmental pressure, including contamination. Although physical-chemical parameters are essential for risk evaluation, the results of biological response to chemical stress have been used as reference to determine the expected biological damage (Ferrer *et al*., 2006). Lead is postulated as one of the most vital pollutants in the environment which builds up in the body of the human due to its low of elimination; cadmium is very toxic to the aquatic organisms, commonly found in industrial area and ninety percent of cadmium derived from human activities (Omer *et al*., 2012). The test fish chosen for the current study, tilapia is available throughout the year, cheaper and protein rich food. Tilapia are presently known as aquatic chicken due to their quick growth, adaptability to a broad range of environmental stresses, disease confrontation, high flesh quality. Thus, tilapia becomes a chosen fish for aquaculture, mainly in tropical and semitropical countries (Nguyen, 2008). The tilapia fish was selected as an experimental organism on the basis of being representative of an ecologically significant animal, widely edible, moreover, amenable to laboratory testing (Al-Attar, 2005). Undertaking the acute tests experiments of heavy metals using aquatic organisms such as fish will assist to improve the environmental observing process. Some heavy metals (Cu and Zn) are essential and some

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nonessential (Pb and Cd). They intensively spread due to anthropogenic activities and natural sources, however, metals accumulate in fish and transfer through food chain to human body (Kamaruzzaman *et al.* 2010; Kamaruzzaman *et al*., 2008).

The results of toxicity tests for copper, cadmium, zinc and lead will contribute data to environmental quality standards in Malaysia. In addition, many laboratory experiments have been carried out to assess the toxicity of heavy metals on different test organisms (Verma *et al*., 2012; Mebane *et al*., 2012; Benjamin and Thatheyus, 2012; Shuhaimi-Othman *et al*., 2011; Bhamre *et al*., 2010; Shuhaimi-Othman *et al*., 2010; Ferrer *et al*., 2006; Straus, 2003; Zyadah and Abdel-Baky, 2000). In the present study, the effect of median lethal concentrations, time and bioconcentration of four selected heavy metals (Cu, Cd, Zn and Pb) on the tilapia fish *Oreochromis niloticus* has been investigated.

MATERIALS AND METHODS

Apparatus: An Oven for drying the samples, Electronic balance-AB 204-S METTLER TOLEDO, Block thermostat-SASTEC model No ST-DBMK2004, Malaysia, Water parameter meter-Quanta and Inductively-coupled plasma Mass spectrometry (ICP-MS) (Model ELAN 9000 Perkin Elmer ICP-MS, USA).

Reagents and quality assurance and quality control: All reagents were of analytical reagent grade. Ultra high purity deionised water was used for all dilutions throughout the whole experimental work done in this study including: preparation of working standards, dilution of digested samples and cleaning of digestion vessels and other equipment that was used. Nitric acid, HNO_3 (65%) and hydrogen peroxide, H_2O_2 (30%) were of ultrapure quality (Merck, Darmstadt, Germany). The element standard solutions from Merck Company to make the calibration were prepared by diluting the stock solutions of 1000 mg L^{-1} of each element. For validation of the analytical method, a recovery study for each run, duplicate samples and blanks were carried out through the digestion reaction. The certified standard reference materials of marine biota sample (SRM2976, freeze-dried mussel tissue, National Institute of Standards and Technology, USA) was used, the results of measurements of CRMs are summarized in Table 1. All the glassware and plastics were soaked overnight in 10% (v/v) nitric acid and rinsed with distilled and deionised water and dried before being used (Khansari *et al*., 2005).

Blank and drift standards were run after fifty determinations to preserve instrument calibration. The concentrations of heavy metal were expressed on a dry weight basis (μg g^{-1} dry wt.). The operational parameter settings were used in ELAN 9000 Perkin Elmer (Table 2).

Table 1: Trace metal concentrations in certified reference material with microwave digestion method, n = 6

Metals	Measured value	Certificate value	Recovery (%)
Cd	0.76	0.82±0.16	92
Cu	4.00	4.04±0.33	97
Pb	1.16	1.19±0.18	97
Zn	120.05	137.00±13.0	87

Table 2: Operational parameter settings were used in ELAN 9000 Perkin Elmer

Characteristics	Instruments condition
RF generator (MHZ)	40
RF power (W)	1000
Spray chamber	Ryton Scott
Nebulizer	Cross-flow
Plasma gas flow (L min^{-1})	15.0
Auxiliary gas flow (L min^{-1})	1.0
Nebulizer gas flow (L min^{-1})	0.60
Sampler and Skimmer cone	Nickel

Studied fish review: Fingerlings of tilapia fish *Oreochromis niloticus* (mean total length, TL, 3.3±0.3 cm; mean weight, 1.5±0.2 g). Fingerlings of tilapia were purchased from an aquarium commercial shop in Bangi, Selangor, Malaysia. Young organisms are often more sensitive to toxicants than is adults viz, the early stages are more sensitive than the later stages (Dung *et al*., 2005). For this reason, the use of early life stages, such as fish fingerlings is required for all tests. In a given test, all organisms should be approximately the same age and should be taken from the same source in order to minimize the diversity of response to experimental materials (US EPA, 2002). Fish fingerlings were transported in oxygenated bags to the laboratory and handled properly to minimize injury and stress physiology in order to reduce the number of dead organisms.

Sample preparation procedures: The purpose of the acute test with fish species is to facilitate in the measurement of possible risk to similar species in natural ecosystems. Acute testing must be performed for a minimum of 96 h (USEPA, 1999). Fish were acclimatized in aquarium under laboratory conditions for two weeks in the laboratory at a temperature of 27-30°C, Light: dark cycle was maintained at 12 h light: 12 h darkness, using fluorescent lights (334-376 Lux) in 150 L stocking tanks using dechlorinated tap water with adequate filtration system (USEPA, 1999). Throughout the acclimatization process, chemical water parameters such as pH, dissolved oxygen concentration and temperature of water in the tanks were monitored daily. In addition, the fish were

fed with commercial fish food Aquadene® four times a day. The standard stock solutions (100 mg L^{-1}) for studied metals were freshly prepared by dissolving of copper sulfate $CuSO_4.5H_2O$, cadmium chloride $CdCl_2.2.5H_2O$, lead nitrate (Pb $(NO_3)^2$ and zinc sulfate $ZnSO_4.7H_2O$ obtained from Merck (Germany) with deionized water . A control with dechlorinated tap water only also was used (Shuhaimi-Othman *et al*., 2010). All toxicity test concentrations were made from the stock solution using appropriate calibrated analytical pipettes and graduated cylinders. Preliminary tests were conducted prior to the definitive test to establish the actual five concentrations for each studied heavy metal. Following a range finding test, five Cu (100,560, 1000, 1800 and 3200 μg L^{-1}), Cd (1000, 5600, 10000, 14000 and 18000 μg L^{-1}), Pb (100, 870, 3200, 5600 and 10000 μg L^{-1}), Zn (5600, 10000, 18000, 32000 and 56000 μg L^{-1}) concentrations were chosen. In the definitive test, the concentrations used in each test were chosen to give from 0 to 100% mortality of test animals within 96 h based on the results of preliminary studies. Tilapia fingerlings were exposed for a four-day period with a range of copper, cadmium, lead and zinc concentrations. The tests were carried out under static conditions with the renewal of the exposure solution every two days by siphoning out the old solution and adding the freshly prepared solutions with the designated studied metal levels. (APHA, 1998). Two replicates of control group with five fish for each group and also for each metal-treated groups consisted of five replicates of ten randomly allocated fish in a 1000 mL glass beaker (Schott Duran®) containing 800 mL of the appropriate solution. Based on the progressive bisection of intervals on a logarithmic scale, log concentrations were selected as the experimental concentrations (APHA, 1975). A total of 10 animals per treatment were used in the experiment and a total of 210 animals were employed in the investigation (APHA, 1992). Samples of water for metal analysis taken before and immediately after each solution renewal were acidified to 1% with ARISTAR® nitric acid (65%) before metal analysis using inductively-coupled plasma mass spectrometry (ICP-MS) (model ELAN 9000 Perkin Elmer ICP-MS, USA) depending on the concentrations.

Analysis of the water physico-chemical variables: For each concentration of the prepared solution, water parameters such as temperature, pH, conductivity, total dissolved solids and dissolved oxygen readings were measured before the tests carried out and every two days by using a Multiparameter hydrolab Quanta® which was recalibrated before each measurement. (The pH must be monitored in low, medium and high test concentrations and must remain >6.0 and <8.0 for freshwater testing (USEPA, 1999). The mean values for tested water quality parameters were presented in Table 3. Freshwater samples are adjusted to pH 7.0, by adding 1N NaOH or 1N HCl dropwise, as required, being careful to avoid over adjustment (US EPA, 2002, 1998).

Table 3: Water quality parameters for acute toxicity bioassay of heavy metals on fingerlings of the Tilapia fish, (*Oreochromis niloticus*)

Parameters	Results	(OECD, 2006)
Hydrogen ion concentration (pH)	7.08±0.01	6.8-8.4
Dissolved oxygen (DO) mg L^{-1}	8.24± 0.07 mg L^{-1}	Above 80%
Temperature°C	27.01	28 ±1°C
Turbidity	0.0	--
Conductivity	245.1±0.6 μS/cm	--
Hardness (Mg and Ca)	16.03±2.04 mg L^{-1}	--

The experiment was monitored from the beginning and mortality was recorded at the time intervals (each 3 h onward till 96 h). The criteria used to determine the death the absence of motion of the tail and gills. The fish were considered dead when there was a lack of response to gentle prodding with a glass rod. The dead fish were removed immediately from exposure beakers. At the end of exposure for four days (96 h), a live fish used to determine the bioconcentration of metals in fish tissues.

Chemical analysis: Samples of water from the control and test metal beakers were taken at intervals, when the experiment was commenced at the beginning, before and after 48 h and at 96 h when the experiment finished for metal analysis. Samples were acidified to 1% with ultra pure nitric acid (70%) and saved for metal analysis by inductively coupled plasma-mass spectrometry (ICP-MS Model Perkin Elmer Elan 9000).

Accumulation-fish tissue digestion: A fish tissue digestion process was conducted to measure the concentrations of studied heavy metals. After the acute test experiment has been finished the rest of tested fish which still alive and fish from the control were taken to perform accumulation tests for all studied metals. Fish were rinsed with deionized water and left for dead before admission to the oven. Each sample comprised two replicates in acid-washed Petri dishes and dried at 80°C for 48 h. Fish were crushed into a fine powder by using a porcelain mortar and pestle. The fish were placed in test tubes in replicates. Each replicate was digested (whole fish) in 2.0 mL nitric acid (Aristar 65%) and left overnight and heated on a block thermostat at a temperature of 100°C for 2 h for the purpose of fish tissue digestion. Heating process was carried out in the fume chamber with a block thermostat-SASTEC model No ST-DBMK 200-4 (Malaysia). Solutions were heated to turn to yellow color and allowed to cool at room temperature. Upon cooling, 0.8 mL of hydrogen peroxide (30%) was added to the solutions. The test tubes were put back on the block

thermostat for an hour until the solutions became clear. The solution was then allowed to cool and put in a 25 mL volumetric flask and added with deionized water, make up to 25 mL. The solutions were then transferred into polyethylene bottles and sent to analyzed metals concentrations in fish tissues by Using An Inductively-Coupled Plasma Mass Spectrometer (ICP-MS) for the determinate the values of Cu, Cd, Pb and Zn (Kamaruzzaman *et al*., 2010). The accuracy was also examined by analyzing, in duplicate standard reference materials (SRM) 2976 Mussel Tissue (Trace Elements and Methylmercury) Freeze-dried tissue, National Institute of Standards and Technology (NIST), (USA) and the results matched with the certified values, (Stephenson and Mackie, 1988).

Statistical analysis: Results were analyzed by one-way ANOVA with Tukey's multiple comparison depending on the experimental design. The significance was reported at p = 0.05 levels. The acute toxic effects of studied heavy metals on Nile tilapia were determined by the use of Probit Analysis LC_{50} and LT_{50} determination method. The computer model (Probit Program Version 1.5 software) was developed by the Environmental Protection Agency (USEPA, 1999).

RESULTS AND DISCUSSION

In all data analyses, the actual, rather than nominal, Cu Pb, Zn and Cd concentrations were used. The LC_{50}s values for tilapia fingerlings obtained for the four metals at different periods of exposure were summarized in Table 4. The data obtained from the acute toxicity test of Cu, Cd, Zn and Pb for Nile tilapia revealed that the fish mortality increased with increasing concentration and/or exposure time. All control results in lower mortality, less than 10% which revealed the acceptability of the tests. The LC_{50}-96 h values for zinc was the highest among all studied metals 16177 μg L^{-1} and copper was the lowest 1093 μg L^{-1}, whereas, for 24 h, it was 64897 μg L^{-1} for Zn and 3286 μg L^{-1} for Cu. Ferrer *et al*. (2006) has reported the LC_{50}-96 h values 1093.40 μg L^{-1} for Pb, 219.20 for Cu and 172.10 μg L^{-1} for Zn in the early life stages of the carb *Chasmagnathus granulata*. Hence, the acute tests of 96, 72, 48 and 24 h showed an opposite relationship between LC_{50}s and exposure time, increase in the concentration reduces the time to kill 50% of the tilapia fish. Straus, (2003) estimated the LC_{50}-96 values for Cu to blue tilapia in different values of alkalinities (43060, 6061, 690 and 180 mg L^{-1} Cu) in water having total alkalinities of 225, 112, 57 and 16 mg L^{-1} CaCO, respectively. Cu was the most toxic to tilapia fish and the

Table 4: Median lethal concentrations (LC_{50}) for tilapia fish fingerlings at different exposure times for Cu, Cd, Pb and Zn

				95% confidence limits	
Metal	Exposure (h)	LC_{50} μg L^{-1}	Slope	Lower	Upper
Cu	24	3286	1.57	1743	49448
	48	1860	1.67	1031	5194
	72	1368	1.75	741	2802
	96	1093	3.52	581	1613
Cd	24	13915	2.22	9099	47562
	48	8364	1.76	4457	16146
	72	4764	1.71	2143	7663
	96	3751	2.27	476	6620
Zn	24	64897	1.73	34276	3911380
	48	37306	1.93	23165	131779
	72	22700	2.16	14626	41022
	96	16177	3.02	4818	25601
Pb	24	12188	1.24	5311	1970765
	48	8939	0.76	2823	9247582
	72	3326	0.95	1208	15446
	96	1494	2.03	133	2948

Table 5: Median lethal times (LT_{50}) for tilapia fish fingerlings exposed to different concentrations of Cu, Cd, Pb and Zn

				95% confidence limits	
Measured metal concentration μg L^{-1}		LT_{50} (h)	Slope	Lower	Upper
Cu	460.95	175.96	3.64	43.69	708.60
	964.07	146.63	6.63	34.63	620.89
	2144.12	46.49	5.31	16.05	134.67
	3509.68	20.25	4.88	7.58	54.08
Cd	1205.95	266.46	4.18	57.02	1245.00
	5205.24	119.99	7.87	28.30	508.83
	9554.71	40.31	3.22	12.53	129.71
	12800.00	22.99	3.97	9.72	54.36
	17612.11	11.48	4.67	4.42	29.82
Zn	5280.20	185.00	2.09	83.24	409.76
	9860.80	182.00	4.09	53.64	619.72
	17600.00	129.00	7.23	28.56	583.88
	30200.21	46.00	3.85	19.68	109.51
	53412.91	25.70	3.42	11.99	55.10
Pb	128.13	177.56	1.95	55.01	573.13
	710.48	144.75	4.45	39.59	529.22
	3365.50	79.99	3.58	34.44	185.78
	5460.12	52.64	2.92	26.95	102.82
	9812.11	24.66	4.19	10.14	59.95

toxicity ranking of the four heavy metals was Cu>Pb>Cd>Zn Shuhaimi-Othman *et al*. (2010) reported that LC_{50}s for 24 and 96 h for Cu were 54.2 and 5.6 μg L^{-1} and for Cd 1440.2 and 101.6 μg L^{-1}, respectively for *R. sumatrana*. For *P. reticulata*, LC_{50}s for 24 and 96 h for Cu were 348.9 and 37.9 μg L^{-1} and for Cd 8205.6 and 168.1 μg L^{-1}, respectively, the Cu was more toxic than Cd to both fishes (Cu>Cd), these result are higher than present study. Zyadah and Abdel-Baky (2000) recorded that the LC_{50}-24 h 15300, 8700 and 102200 μg L^{-1}; LC_{50}-96 h 13100, 4500 and 91200 μg L^{-1} for Cd, Cu and Zn, respectively on *T. Zillii*.

In view of median lethal times (LT_{50}s), the median lethal times (LT_{50}) increased with a decrease in mean exposure concentrations for all studied metals (Table 5) the lowest metals was Cd with 11.48 h in measured

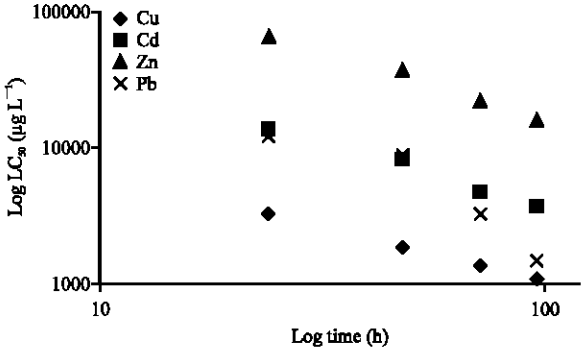

Fig. 1: Relationship between LC_{50} and exposure times for tilapia fish fingerlings

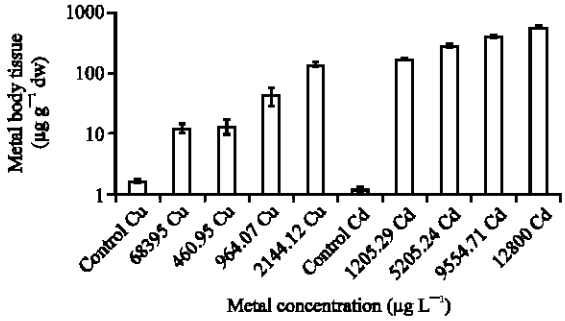

Fig. 2: Bioconcentration of Cu and Cd in tilapia fish fingerlings after four day exposure to different concentrations of Cu and Cd

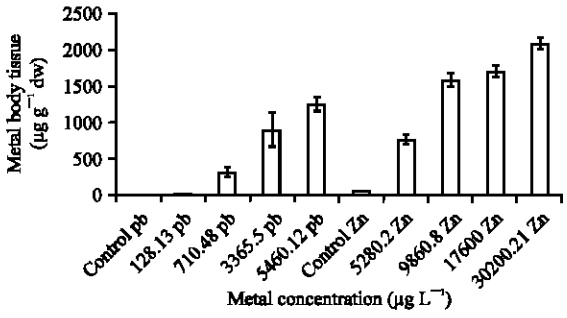

Fig. 3: Bioconcentration of Pb and Zn in tilapia fish fingerlings after four day exposure to different concentrations of Pb and Zn

concentration of 17612.11 µg L^{-1} and with 266.46 h in measured concentration 1205.95 µg L^{-1}. Whereas, the highest metals was Zn with 25.70 h in concentration of 53412.91 µg L^{-1} and with 185 h in measured concentration 5280.20 µg L^{-1}. Shuhaimi-Othman *et al.* (2009) reported that LT_{50} of Cu for freshwater prawn *M. lanchesteri* with concentration of 6.43 µg L^{-1} was 151.1 h and LT_{50} for 97.65 µg L^{-1} was 49.7 h. Results were obtained by Shuhaimi-Othman *et al.* (2011) revealed that LT_{50} for measured Zn concentration 779, 1010, 2456, 3213 and 4822 µg L^{-1} were 260.33, 100.63, 52.52, 17.59 and 13.17 h, respectively. On the other hand, Pb LT_{50} for 475, 1160, 3410, 4829 and 8973 µg L^{-1} were 110.26, 72.39, 55.66, 43.55 and 9.15, respectively.

The relationship between LC_{50}s and exposure times for tilapia fish fingerlings were shown in Fig. 1. It is very clear that the zinc is the highest concentration among all metals, this is reflect the less effect of zinc compared with copper, cadmium and lead, whereas, the strongest metal effect on tilapia is the copper.

Bioconcentration results: Bioconcentration of Cu, Cd, Pb and Zn in surviving tilapia fish were as shown in Fig. 2 and 3. Data for life fish were obtained from four Cu concentrations (68.95, 460.95, 964.07 and 2144.12 µg L^{-1}); four Cd concentrations (1205.29, 5205.24, 9554.71 and 12800 µg L^{-1}); four Pb concentrations (128.13, 710.48, 3365.50 and 5460.12 µg L^{-1}; four Zn concentrations (5280.20, 9860.80, 17600 and 30200.21 µg L^{-1}). Bioconcentration of metals in tilapia fingerlings increases with increasing concentration exposure. Statistical analyses show that there are significant differences (ANOVA, $p<0.05$) in all studied metals bioconcentration compared with control water at the end of four days. The bioconcentrations of heavy metal increase with additions of metal concentrations in water (Rao and Patnaik, 1997).

A comparison of bioaccumulation of heavy metals in tilapia fish showed that among the four metals, Cu was the least accumulated and Zn and Pb were the most accumulated. Shuhaimi-Othman *et al.* (2011) have reported that Cu and Cd were the most accumulated and Ni was the least accumulated to fresh water ostracod *Stenocypris major*. The rate of bioaccumulation of studied metals by the tilapia fish appeared within a wide range, the bioaccumulation factor of Cu, Cd, Pb and Zn by tilapia was 79, 774, 374 and 26 times more than control concentration respectively after 96 h exposure for maximum metals concentration used.

CONCLUSION

Present study indicated that copper highly toxic to tilapia fish. The tested organism was shown to be most sensitive to Cu and most resistant to Cd. The toxicity of the studied metals followed the order: Cu>Pb>Cd>Zn. Large differences appeared in sensitive of tilapia fish fingerlings to Cu, Cd, Zn and Pb, these results indicate

that tilapia is comparatively tolerant to metals in comparison to other fish species. In terms of accumulation, Zn was the least accumulated and Cd was the most accumulated followd by Pb.

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REFERENCES

APHA, 1975. Standard Methods for the Examination of Water and Wastewater. 14th Edn., American Public Health Association, Washington, DC., USA.

APHA, 1992. Standard Methods for the Examination of Water and Wastewater. APHA, Washington, DC.

APHA, 1998. Standard Methods for the Examination of Water and Wastewater. 20th Edn., American Public Health Association, Washington, DC., ISBN: 0-309-09122-5.

Abdel-Warith, A.A., E.M. Younis, N.A. Al-Asgah and O.M. Wahbi, 2011. Effect of zinc toxicity on liver histology of nile tilapia, *Oreochromis niloticus*. Sci. Res. Essays, 6: 3760-3769.

Al-Attar, A.M., 2005. Biochemical effects of short-term cadmium exposure on the freshwater fish, *Oreochromis niloticus*. J. Biological Sci., 5: 260-265.

Ayotunde, E.O. and B.O. Ofem, 2008. Acute and chronic toxicity of pawpaw (*Carica papaya*) seed powder to adult Nile tilapia (*Oreochromis niloticus* Linne 1757). Afr. J. Biotechnol., 7: 2267-2274.

Azmat, H., M. Javed and G. Jabeen, 2012. Acute toxicity of aluminium to the fish (*Catla catla*, *Labeo rohita* and *Cirrhina mrigala*). Pak. Vet. J., 32: 85-87.

Benjamin, D. and A.J. Thatheyus, 2012. Acute toxicity of nickel and cadmium to the cichlid fish, *Oreochromis mossambicus* (Peters). Res. Zool., 2: 19-22.

Bhamre, P.R., S.P. Thorat, A.E. Desai and B.M. Deoray, 2010. Evaluation of acute toxicity of mercury, cadmium and zinc to a freshwater mussel *Lamellidens consobrinus*. Our Nat., 8: 180-184.

Boateng, J.O., F.K. Nunoo, E.H.R. Dankwa and M.H. Ocran, 2006. Acute toxic effects of deltamethrin on tilapia, *Oreochromis niloticus* (Linnaeus, 1758). West Afr. J. Applied Ecol., 9: 1-5.

Dung, L.Q., N.M. Cuong, N.T. Huyen and N.D. Cu, 2005. Acute toxicity test to determine the effects of copper, zinc and cyanide on cobia (*Rachycentron canadum*) resources in North Vietnam. Australasian J. Ecotoxicol., 11: 163-166.

Ferrer, L., S. Andrade, R. Asteasuain and J. Marcovecchio, 2006. Acute toxicities of four metals on the early life stages of the crab *Chasmagnathus granulata* from Bahia Blanca estuary, Argentina. Ecotoxicol. Environ. Saf., 65: 209-217.

Hedayati, A., A. Safahieh, A. Savari and J.G. Marammazi, 2010. Detection of range finding test of mercury chloride inyellowfin sea bream (*Acanthopagrus latus*). Iranica J. Energy Envir., 1: 228-233.

Kamaruzzaman, B.Y., B. Akbar, K.C.A. Jalal and S. Shahbudin, 2010. Accumulation of metals in the gills of Tilapia fingerlings (*Oreochromis niloticus*) from *in vitro* toxicology study. J. Fish. Aquat. Sci., 5: 503-509.

Kamaruzzaman, B.Y., M.C. Ong, K. Zaleha and S. Shahbudin, 2008. Levels of heavy metals in green-lipped mussel *Perna veridis* (Linnaeus) from Muar Estuary, Johore, Malaysia. Pak. J. Biol. Sci., 11: 2249-2253.

Khansari, F.E., M. Ghazi-Khansari and M. Abdollahi, 2005. Heavy metals content of canned tuna fish Food Chem., 93: 293-296.

Mebane, C.A., F.S. Dillon and D.P. Hennessy, 2012. Acute toxicity of cadmium, lead, zinc and their mixtures to stream-resident fish and invertebrates. Environ. Toxicol. Chem., 31: 1334-1348.

Nguyen, T.N., 2008. The utilization of soybean products in tilapia feed-A review. Proceedings of the 8th International Symposium on Tilapia in Aquaculture, October 12-14, 2008, Nong Lam University, pp: 53-65.

OECD, 2006. OECD Guideline for the testing of chemicals: Draft proposal for a new guideline; Fish Embryo Toxicity (FET) test. Draft Guideline, May 30, 2006 (1st Version). http://www.oecd.org/env/ehs/testing/36817070.pdf

Omer, S.A., M.A. Elobeid, D. Fouad, M.H. Daghestani and E.M. Al-Olayan *et al*., 2012. Cadmium bioaccumulation and toxicity in tilapia fish (*Oreochromis niloticus*). J. Anim. Vet. Adv., 11: 1601-1606.

Rao, L.M. and R.M.S. Patnaik, 1997. Acute toxicity of Zn, Pb and Cd in the freshwater catfish *Mystus vittatus* (Bloch). Indian J. Fish., 44: 405-408.

Shuhaimi-Othman, M., N. Yakub, N.A. Ramle and A. Abas, 2011. Toxicity of metals to a freshwater ostracod: *Stenocypris major*. J. Toxicol., 10.1155/2011/136104

Shuhaimi-Othman, M., Y. Nadzifah and A.K. Ahmad, 2010. Toxicity of copper and cadmium to freshwater fishes. World Acad. Sci. Eng. Tech., 65: 869-871.

Shuhaimi-Othman, M., Y. Nadzifah, R. Nur-Amalina and A. Ahmad, 2009. Toxicity of copper and nickel to freshwater prawn macrobarchium lanchesteri. Sains Malaysiana, 38: 353-358.

Stephenson, M. and G.L. Mackie, 1988. Total cadmium concentrations in the water and littoral sediments of central Ontario lakes. Water Air Soil Pollut., 38: 121-136.

Straus, D.L., 2003. The acute toxicity of copper to blue tilapia in dilutions of settled pond water. Aquaculture, 219: 233-240.

Taweel, A.K.A., M. Shuhaimi-Othman and A.K. Ahmad, 2012. Analysis of heavy metal concentrations in Tilapia fish (*Oreochromis niloticus*) from four selected markets in Selangor, Peninsular Malaysia. J. Biol. Sci., 12: 138-145.

US EPA, 2002. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms Epa-821-r-02-012. 5th Edn., Environmental Protection Agency, Washington DC., USA.

US EPA, 1998. Ecological effects test guidelines fish acute toxicity test: freshwater and marine. http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Drafts/850-1075.pdf

USEPA, 1999. LC50 software program, version 1.50. Ecological Monitoring Research Division, Environmental Monitoring Systems Laboratory, EPA, Cincinnati, OH., USA.

Verma, S.R., P. Chaudhari and S. Satyanaranyan, 2012. Acute toxicity of copper and cadmium, to the freshwater fish Lebistesreticulatus (Peter). J. Ind. Pollut. Control, 28: 119-124.

Zyadah, M.A. and T.E. Abdel-Baky, 2000. Toxicity and bioaccumulation of copper, zinc and cadmium in some aquatic organisms. Bull. Environ. Contamin. Toxicol., 64: 740-747.