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ACCUMULATION OF COPPER, LEAD AND CADMIUM IN FLESH TISSUE OF TILAPIA (*OREOCHROMIS NILOTICUS*)

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Abstract

A study was conducted to evaluate the uptake and accumulation of waterborne copper (Cu), lead (Pb) and cadmium (Cd) in flesh tissue of Tilapia (Oreochromis niloticus). Single breed fingerlings (7.8 ± 1.3 g) of Tilapia were obtained from Aquaculture No.1 (Bac Ninh, Viet Nam). Four groups of fish (40 to 45 each) were maintained in 100 liters of water in glass tanks. Tilapia were treated with the different concentrations of Cu, Pb (0, 0.02, 0.05, 0.2 mg/l) and Cd (0, 0.005, 0.01, 0.05 mg/l) for 60 days. Fish sampling was done on day zero and every 15 days. The copper, lead and cadmium concentration in flesh tissue in the experimental tanks increased with increasing exposure time. Statistical analyses indicated Cu, Pb and Cd levels in all treatment samples were significantly different from control (p<0,01), but they did not differ between samples (p > 0.05). When compared to the day zero values, the levels of accumulation of cadmium at 60 days was highest.

Keywords: Copper; Lead; Cadmium; Flesh; Tilapia.

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1. Introdution

According to the survey results from previous researches, the concentrations of some heavy metals in water and sediment in several fish farming areas in Hanoi exceeded the National technical regulation of surface water quality (QCVN 08:2015/ BTNMT) [1,2]. This would be the main reasons causing the accumulation of heavy metals in fish raised in these water bodies.

Cadmium and lead have no known role in biological systems, whereas copper is essential components of enzymes or metalloproteins in fish metabolism. Heavy metals accumulate in the tissues of aquatic animals and may become toxic when accumulation reaches a substantially high level. Uptake of heavy metals through food chain in aquatic organisms may cause various pathological disorders like hypertension, sporadic fever, renal damage or cramps in human. Biomagnifications, contaminated water and contamination in food web are also cause of deposition of heavy metals in fishes [1, 2, 3].

During the last few decades, great attention has been paid to the possible dangers of many environmental pollutants due to the consumption of contaminated

fish. Tilapia is the most popular and highly economic fish. In the present research, *Oreochromis niloticus* (Tilapia) was selected due to its adoption in polluted aquatic environment. The purpose of this research is to quantify the accumulation of copper, lead and cadmium in flesh tissue with various heavy metals concentrations.

2. Methodology

2.1. Experimental design

The Tilapia (average weight of 7.8 ± 1.3 g) were collected from Research Institute for Aquaculture No.1 (Bac Ninh) and acclimated to laboratory conditions for a week. Forty individuals per glass tank (100 litters) were used for the experiments. The fish were fed with standard powdered feed for whole experimental period, twice per day and the amount of feed accounts for 5% of the weight of the fish cultured. Water in tanks was replaced every two days. pH of cultured water from 6.5 to 7.5 and aerated. Fish were divided into four groups with the first group serving as control and other groups as experimental groups. The heavy metals concentration of experimental groups as follow [4]:

Tanks	Cu (mg/l)	Pb (mg/l)	Cd (mg/l)
Control	0 ≤ 0.001	0 ≤ 0.001	0 ≤ 0.001
Tank 1	0.02	0.02	0.005
Tank 2	0.05	0.05	0.01
Tank 3	0.20	0.20	0.05

2.2. Sample preparation and metal analysis

Flesh tissue of five fish from each aquarium was done on day zero and every 15 days thereafter from all tanks for 60 days.

An aliquot of 50 ml of each water sample was taken into a 100 ml - Erlenmeyer flask, 5 ml suprapur HNO_3 (60 %) were added and the mixture was heated on a hot plate to 95 °C for 4 h. Upon cooling to room temperature, the sample was adjusted to 50 ml with bidistilled water in a volumetric flask; finally, the sample was filtered through a syringe filter of 0.45 - µm pore size (Whatman, Singapore).

Upon arrival at the laboratory, the fish were immediately excised and muscle tissues were prepared for metal analysis by wet digestion [5]. Briefly, the samples, 0.1 - 0.2 g wet weight each, were cut into small pieces and digested in 5 mL aqua regia in 20 mL borosilicate glass tubes for 1 h at 60 °C, followed by 3 h at 120 °C. The samples were cooled to room temperature and 500 µl each of concentration H_2O_2 were added, followed by heating to 120 °C in a hot block until clear solutions were obtained, taking about 1 h. The digestates were diluted to 20 mL each with bidistilled water and filtered through 0.45 µm cellulose syringe filters. Samples were then ready for measuring heavy metals by inductively coupled plasma mass spectrometry (ICP - MS, ELAN 9000; Perkin - Elmer SCIEX, Waltham, MA, USA); detection limits for Cu was 1 µg/L, for Pb, Cd was 0,001 µg/L, respectively. The analytical method was validated with certified standard reference materials from oyster and fish liver (Graham B. Jackson Pty., Ltd, Dandenong, Victoria, Australia). Recoveries were within the certification range, i.e., 93 % for Cd, 90 % for Pb and 92 % for Cu. Procedural blanks consisting of aqua regia were below detection limits. The results were reported in mg/kg for fish wet weight. All reagents used were of analytical grade (Merck, Darmstadt, Germany). The results were given as µg/g.ww (wet weight).

2.3. Data analysis

The data were presented as means ± SEM. Two way analysis of variance was used to determine whether differences in metal concentrations among tanks and sampling times and among heavy metals and species were significant. When significant differences were found, the Student - Newman - Keuls test was applied (GraphPad Software, San Diego, CA).

3. Results and Discussion

3.1. Exposure to copper

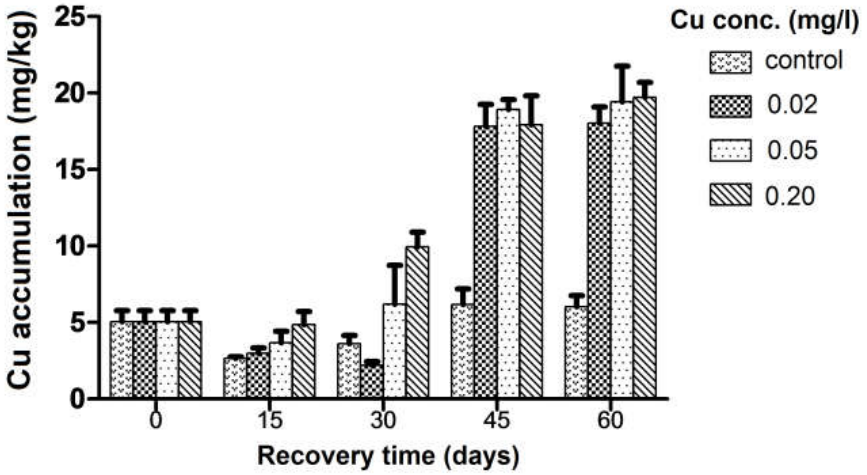

Figure 1: Cu accumulation (mg/kg wet weight) at different time in the flesh

Data is presented as ± SEM (n = 5)

The amount of Cu uptake and absorption in the flesh tissues was presented in Figure 1. Statistical analyses indicated copper level in all treatment samples were significantly different from control ($p < 0.01$). The copper level in all treatment samples was so high, but they did not significantly different ($p > 0.05$).

The mean measured copper (with SEM) of all samples collected at 60 days for 0.02 mg/l, 0.05 mg/l, 0.2 mg/l Cu and control nominal exposures were 18.03 (0.47), 19.43 (1.04), 19.71 (0.44) and 6,03 (0.32) mg/kg. This result showed that high dose exposure resulted into high uptake and accumulation. Our findings were similar to those of Carriquiriborde and Ronco (2008), Wu et al., (2008) and Jeng-Wei Tsai et al. (2012) [6, 7, 8].

3.2. Exposure to lead

Pb uptake and absorption was similar like as Cu in flesh tissue at all treatment (Fig. 2). At treatments 0.02 mg/l it was significantly different ($p < 0.01$) and at 0.05 mg/l and 0.20 mg/l highly significant different ($p < 0.001$) from control. Lead level in flesh of all treatment samples increased with increasing exposure time. Statistical analyses indicated lead level in all treatment samples were so high, they did not differ between samples collected at 0.02 mg/l treatment and 0.05 mg/l treatment ($p > 0.05$) but between the others ($p < 0.01$).

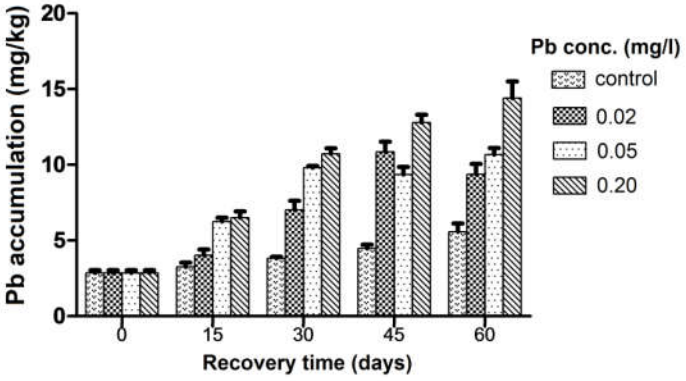

Figure 2: Pb accumulation (mg/kg wet weight) at different time in the flesh

Data is presented as ± SEM (n = 5)

The maximum values of Pb in flesh tissue (Pb mg/kg wet wt.) were calculated for the all treatments at 60 days. The data indicated the following rank order of Pb concentration in flesh tissue at 0.2 mg/l treatment (14.41 ± 0.49 mg/kg wet wt) > 0.05 treatment (10.67 ± 0.19 mg/kg wet wt) > 0.02 treatment (9.37 ± 0.31 mg/kg wet wt). The result was consistent with previous studies (Alves, L. C. 2006; and Ahmed M.S. 2010) that high dose and longer exposure resulted into high uptake and accumulation [9, 10].

3.3. Exposure to cadmium

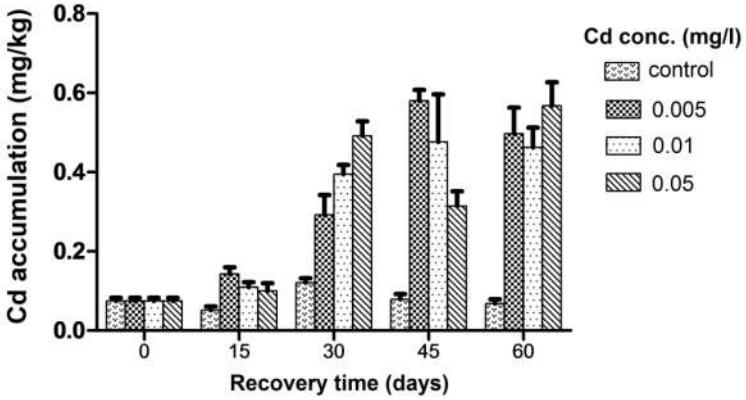

Figure 3: Cd accumulation (mg/kg wet weight) at different time in the flesh

Data is presented as ± SEM (n = 5)

Accumulation Cd after in flesh tissue after 60 days of exposure are shown in Figure 3. Statistical analyses indicated Cd level in all treatment samples were significantly different from control ($p < 0.01$), but they did not differ between samples ($p > 0.05$).

After 60 days exposure, at 0.005, 0.01 and 0.05 mg/l, Cd accumulation values were approximate higher 6.6, 6.2 and 7.5 times than those at day zero, respectively. In case of Cu and Pb, the accumulation levels were lower than those of Cd which were approximately values 3.2 and 3.6; 3.7 and 3.8; 3.9 and

5.0 times, respectively. Similar results previously mentioned by Mustafa (2000) and Al - Nagaawy (2008) who mentioned that the accumulation of essential metals is normally smaller than the accumulation of non - essential metals [11, 12].

4. Conclusion

The results of this study showed that copper, lead and cadmium had different accumulation. The accumulation of all copper, lead and cadmium in flesh tissue increased with increasing the concentration and exposure time. When compared to the day zero values, the levels of accumulation of cadmium at 60 days was highest.

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