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PRELIMINARY STUDY ON ACCUMULATION AND DEPURATION OF COPPER, ZINC, AND LEAD IN TILAPIA (*Oreochromis mossambicus*) UNDER LABORATORY CONDITIONS

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Abstract: Tilapia (*Oreochromis mossambicus*) was studied for the uptake and elimination of single metals namely Copper (Cu), Zinc (Zn) and Lead (Pb) under laboratory conditions. Tilapia was acclimatised at 25 ± 2°C for 4 days. The dissolved oxygen and pH of water samples were maintained at 6 - 7 mg/L and 7.6 ± 0.1 respectively. The exposures to Cu (0, 5, 10, 15 and 20 mg/l), Zn (0, 10, 15, 20 and 25 mg/L) and Pb (0, 1, 2, 3 and 4 mg/L) over a period of 4 days were followed by 4 days of depuration. The accumulation and depuration patterns of Cu and Zn were not apparent and statistical analysis (*t*-test) did not show any significant difference ($p>0.05$) for both phases. The results indicate that tilapia can be used as a biomonitor for Pb as there was significant difference ($p<0.05$) between accumulation and depuration of Pb, particularly for higher exposures (3 mg/L and 4 mg/L Pb).

KEYWORDS: Tilapia, metals, accumulation, depuration, biomonitor.

Introduction

Among many aquatic organisms, fish have been appreciated as valuable biomonitors of bioaccumulation of toxic compounds. An ideal bioindicator should be sessile or sedentary, thus being representative of the study area, hardy, can tolerate high concentrations of pollutants and large ranges of salinity, permitting laboratory studies and profuse in-study areas, can be identified easily and sampled and should provide adequate tissue for analysis (Gay and Maher, 2003).

Tilapia (*Oreochromis* spp.), a native freshwater fish of Africa, can survive at adverse environmental conditions. Their respiratory demands are slight so they can tolerate low oxygen and high ammonia levels (Trewavas, 1983). Some freshwater tilapias are able to survive and grow over a wide range of salinities (Watanabe et al., 1987). Tilapia are considered as an easily grown fish species since they eat a variety of foods and grow well in poor-quality water with low dissolved-oxygen level (Mair and Roberts, 1988).

Metals, one of the commonly-studied contaminants, can be taken up by fish from water, food, sediments, and suspended particulate material. Studies have revealed that accumulation of heavy metals in tissue is dependent upon concentrations and exposure period, and other environmental factors such as salinity, pH, hardness and temperature (Canli and Atli, 2003). Investigations of metals accumulation and assessment of fish as potential biomonitors under field and experimental conditions have been done on various species of fish such as Rainbow Trout (*Oncorhynchus mykiss*), Catfish (*Clarias gariepinus*), Grey Mullet (*Mugil cephalus L.*), Sea Bream (*Sparus aurata L.*) and other types of fish (Hansen et al., 2002; Olaifa et al., 2004; Yilmaz, 2005). Researches pertaining to tilapia species, among others, include determination of heavy metals in various tilapia species in order to examine the safety of these fish for consumption (Zhou et al., 1998; Allinson et al., 2002; Begum et al., 2005). Metals were also found to accumulate in different levels in different organs of tilapia.

Toxicity tests were also performed on various species of tilapia, such as Nile tilapia (*Oreochromis niloticus*), *Tilapia mossambica* and *Tilapia zillii.* Pelgrom et al. (1995a) exposed mature female tilapia to single Cu and Cd and studied the interaction between Cu-Cd. The study indicated that distribution of Cu and Cd was metal and organ specific. Toxicity studies on *Tilapia mossambica* and Nile tilapia have been conducted to evaluate the potential of these tilapia species as biomonitors and also to study the impact of the exposed metals (Almeida et al., 2001; Suhendrayatna et al., 2001; Richard and Benjamin, 2005). Accumulation patterns of contaminants in fish depend both on uptake and elimination rates (Hakanson, 1984). Heavy metals were accumulated in tilapia after they were fed with metal-contaminated sludge (Wong and Chiu, 1993).

In Malaysia, tilapia are considered as one of the commercial fish for both the fisheries and the local inhabitants as a potential source of food. Yap et al. (2005) have reported that tilapia *Oreochromis mossambicus*, collected from a pond receiving semi-untreated domestic wastes, did not possess health effects for the consumer. Genetic structure of *Oreochromis* spp. (Tilapia) populations was studied by Bhassu et al. (2004). Reports and scientific data on the capability of tilapia as biomonitors in Malaysia are still limited.

This study aims to investigate the accumulation and depuration patterns of Cu, Zn and Pb in tilapia at different exposed concentrations and to evaluate whether tilapia can be used as a suitable biomonitoring species.

Materials and Methods

Sample Collection

Tilapia were obtained from Agriculture Research Centre, Semenggok, Kuching. Wet weights of the samples were determined. Prior to exposure test, 9 individuals were analysed for background metals.

Experimental Design

In the laboratory, a total of 175 individual specimens were acclimatised in a holding tank for 4 days. All fish were fed with a control diet based on a commercial feed. After acclimatisation, similar size (length: 3-4 cm, weight: 10-15 g) of tilapia was placed in 5 different experimental tanks (n=35 individuals per tank). Test solutions (20 L) were poured into the tanks which were constantly aerated at 25 ± 2°C. Dissolved oxygen and pH were maintained at 6 - 7 mg/L and 7.6 ± 0.1 respectively.

The accumulation study consisted of single exposure with three different single elements, namely Cu, Zn and Pb. These metals were chosen considering the fact that they are commonly found in freshwater systems and widely discharged from various industrial activities. The experiment was designed to allow 4 days of metals uptake and 4 days of elimination period in metal-free water tank with clean water. Metal solutions were prepared using dechlorinated tap water as diluents from stock solutions (1000 mg/L) of copper sulfate ($CuSO_4.5\ H_2O$), Zn sulfate ($ZnSO_4.7\ H_2O$), and Pb (II) nitrate [$Pb(NO_3)_2$]. Experimental tanks with metal solutions were allowed to equilibrate for 48 hours before the experiment started to minimise adsorption and consequent loss of the metals (Suhaimi-Othman and Pascoe, 2007). Exposure treatments consisted of 4 different concentrations of metals and 1 control. Table 1 shows the concentrations of Cu, Zn, and Pb used in the test. Sampling was conducted at 24, 48, 72 and 96 h for metal analysis. None of the fish were fed during the accumulation and depuration period in order to empty the gut. Fish were monitored from the test iniation to the ending of test for mortality.

Table 1: Concentration of metals (mg/L) used in the toxicity test

	Cu	Zn	Pb
Treatment1 (Control)	0.0	0.0	0.0
Treatment 2	5.0	10.0	1.0
Treatment 3	10.0	15.0	2.0
Treatment 4	15.0	20.0	3.0
Treatment 5	20.0	25.0	4.0

Chemical Analysis

Water samples were taken after 96 hours (at the end of the accumulation test) and filtered using 0.45 µm membrane filter. Samples were acidified with concentrated nitric acid until pH<2. After acidification, the water samples were analysed for Cu, Zn and Pb by direct injection with Flame Atomic Absorption Spectrophotometer (FAAS).

Tilapia samples were taken out every 24 h throughout the uptake and depuration phases for metals analysis. Flesh samples were separated from the fish and were kept in freezer. The samples were freeze-dried and ground with pestle and mortar to a fine powder. Powdered samples were digested according to the procedures proposed by Dalziel and Baker (1983). Tissue samples of 0.5 g were weighed and placed in beakers and 10 mL of freshly-prepared nitric acid and hydrogen peroxide (1:1) v/v were added and set aside till the initial reactions subsided. A beaker was placed on a hot plate and gently boiled at a temperature not exceeding 120ºC for about 2 hours to reduce the volume to 3-4 mL. Next, it was cooled, filtered and transferred to a 25 mL volumetric flask and made up to volume with deionized water. Then, samples were tested for Cu, Zn, and Pb using FAAS. All metal concentrations are expressed in dry weight (mg/kg).

Statistical Analysis

Two-way ANOVA without replication and paired comparison using Student's *t*-test ($p < 0.05$) were used to test for differences of Cu, Zn and Pb accumulation and depuration between treatment groups.

Results and Discussion

Accumulation and Depuration of Cu, Zn and Pb

Figures 1-3 shows the accumulation and depuration of Cu, Zn and Pb in tilapia respectively. The mean concentrations of Cu, Zn and Pb in tilapia prior to exposure were 3.12 mg/kg, 30.84 m/kg and 1.52 mg/kg respectively. The mean measured copper concentration in the flesh of tilapia (with standard deviation) for 0.0 mg/L, 5.0 mg/L, 10.0 mg/L, 15.0 mg/L and 20.0 mg/L Cu exposures were 5.92 mg/kg (0.68), 63.44 mg/kg (2.99), 67.92 mg/kg (2.38), 81.10 mg/kg (2.40), and 92.73 mg/kg (2.52) respectively. In general, accumulation of Cu in tissues of tilapia increased up to 96 h for 5 mg/L to 20 mg/L (Figure 1). Depuration rate of Cu was slow and did not significantly ($p > 0.05$) decrease for all the exposure treatments. This indicates that Cu was not completely eliminated from tilapia. Furthermore, statistical analysis (*t*-test) did not show any significant difference (p >

0.05) between accumulation and depuration phases of Cu for treatments 2, 3, 4 and 5. This shows that accumulation and depuration of Cu did not occur in the tilapia. Study by Richard and Benjamin (2005) showed that Nile tilapia does not recover quickly from dietary Cu exposure and accumulated excess Cu in the liver and intestine. Cu is known as an essential metal which can be regulated by fish in their bodies. Study by Pelgrom et al. (1995b) demonstrated that tilapia were more Cu-tolerant fish when compared to the Cu-sensitive rainbow-trout. Tilapia collected from ponds in Hong Kong also indicated to be Cu-resistant based on comparison made between 96-h LC_{50} values for tilapia and common carp (Lam et al., 1998). Several studies have been devoted to study the exposure of Cu in order to relate the biological function of Cu in tilapia and other fish species (Hansen et al., 2002; De Boeck et al., 2004). Statistical analysis (ANOVA) showed significant difference ($P<0.05$) in the accumulation and depuration of Cu in tilapia compared to that in control for each treatment.

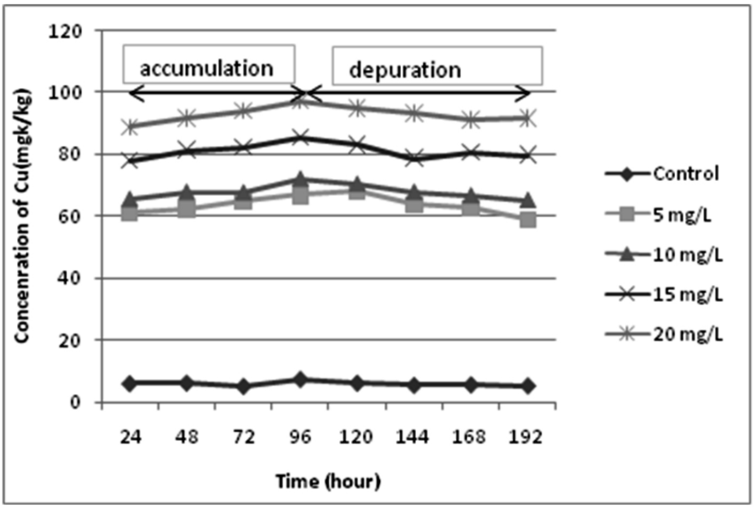

Figure 1. Accumulation and depuration of Cu in tilapia with different exposure

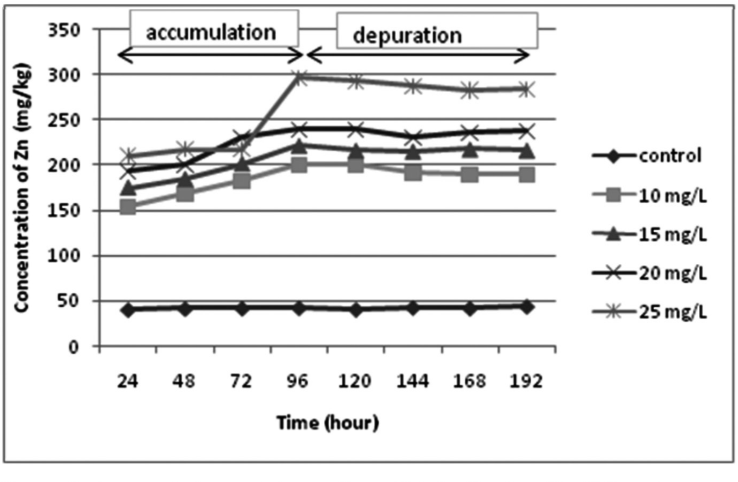

Figure 2. Accumulation and depuration of Zn in tilapia with different exposure

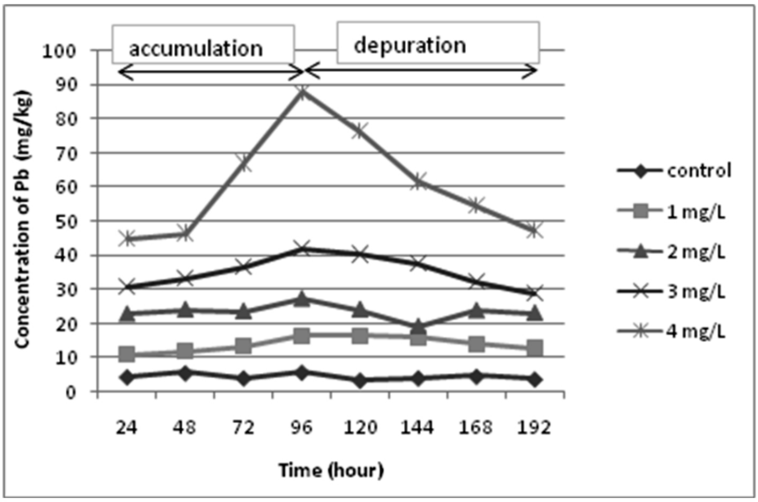

Figure 3. Accumulation and depuration of Pb in tilapia with different exposure

Figure 2 represents the concentrations of Zn in tilapia at different time of exposures. Mean measured concentrations of Zn were 41.85 ± 4.50 mg/kg (for 0.0 mg/L), 185.08 ± 1.70 mg/kg (for 10.0 mg/L), 205.96 ± 0.75 mg/kg (for 15.0 mg/L), 226.38 ± 1.20 mg/kg (for 20.0 mg/L), and 261.35 ± 1.25 mg/kg (for 25.0 mg/L). The accumulation of Zn in tilapia increased with increasing exposure concentrations except for treatment 5 (25.0 mg/L) where the concentration of Zn slightly decreased from 48 h to 72 h exposure time. However, after 72 h the concentration of Zn rose until 96 h.

The concentration of Zn in tilapia at 192 h was found to be higher than the concentrations of Zn in tilapia at 24 h for all the exposure concentrations. There was no significant difference (*t*-test, $p > 0.05$) before and after exposure for Zn in all the treatments. This is an indication to show that there was no accumulation and depuration occurring in the tilapia. Spiked concentrations of Zn were retained and were not cleared from the tissues of tilapia throughout the exposure and elimination duration. The differences in elimination of Zn may be attributed to the differences in regulation of Zn in tilapia. This result showed that tilapia was unable to regulate Zn effectively. Laboratory bioassay conducted on *Tilapia zilii* revealed that tilapia were more susceptible to Zn and resistant to Zn toxicity at lower temperature (9.3 ± 1.5ºC) compared to higher temperature (25 ± 1ºC) studied (Hilmy et al., 1987).

The concentrations of Pb in tilapia for 0.0 mg/L, 1.0 mg/L, 2.0 mg/L, 3.0 mg/L and 4.0 mg/L Pb exposures is depicted in Figure 3. The concentrations of Pb in tilapia for 1.0 mg/L, 2.0 mg/L and 3.0 mg/L increased gradually during accumulation and decreased gradually during depuration period. For 4.0 mg/L Pb exposure, the concentration of Pb in tilapia have drastically increased during accumulation period and rapidly decreased during depuration period. The mean measured concentrations of Pb were 4.38 ± 0.83 mg/kg for 0.0 mg/L, 13.88 ± 2.12 mg/kg for 1.0 mg/L, 23.54 ± 2.24 mg/kg for 2.0 mg/L, 35.19 ± 4.65 mg/kg for 3.0 mg/L, and 60.73 ± 15.50 mg/kg for 4.0 mg/L.

The concentration of Pb in tissues of tilapia increased with increasing exposure time, except for 2.0 mg/L Pb exposure. Elimination of Pb was slow for all the exposed concentrations with increasing elimination time. The accumulation and depuration were significant ($p<0.05$) for treatments 4 ($p=0.075$) and 5 ($p=0.018$). But, treatments 2 and 3 did not show any significant differences (*t*-test, $p > 0.05$). This shows that tilapia can accumulate high concentrations of Pb and tilapia can be highly resistant to Pb. Even though previous authors have carried out toxicity studies of Cu and Zn on tilapia, there is lack of knowledge about the effects of Pb. Patel et al. (2006) reported that kidney of rainbow trout acts as a high-capacity sink for Pb under short-term acute exposures. Statistical analyses (ANOVA) showed significant difference ($P<0.05$) in Pb accumulation and depuration of tilapia compared to that of tilapia in control water for all the exposures.

Uptake of metals at 96 hour

Figures 4 - 6 shows the accumulation of metals in tilapia versus mean concentrations of metals in water at 96 h. The ranges of Cu, Zn and Pb uptake at 96 h were 67 - 97 mg/kg, 201 - 297 mg/kg and 16 – 88 mg/kg respectively. There was direct accumulation of Pb by tilapia as shown in Figure 6. Accumulation of Pb in tilapia was proportional to the concentration of Pb in water ($r=0.94$). There was no direct accumulation of Cu and Zn in water compared with that of the metals in tilapia at 96 h (Figures 4 and 5).

Metal uptake by aquatic organisms is a two-phased process, which involves initial rapid adsorption or binding to the surface, followed by a slower transport into the cell interior (Foulkes, 1988). Mechanisms of uptake, bioconcentration and relationship between body concentration and observed toxicity are vital when addressing the effects of pollutants (Suhaimi-Othman and Pascoe, 2007). During the accumulation test, concentrations of copper, Zn and Pb in tissues increased with exposure to levels greater than those recorded in fish collected from the control tanks. However, for depuration phase in clean water, concentrations of copper, Zn and Pb in tissues showed discrepancies in their elimination pattern.

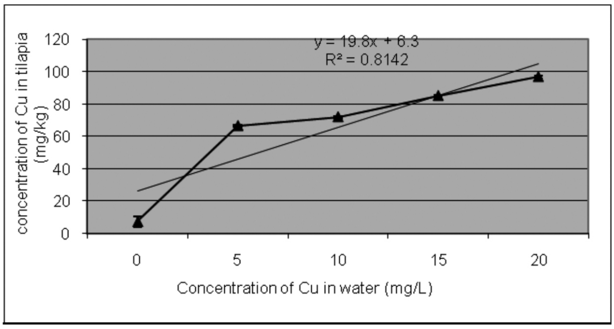

Figure 4: Mean concentration of Cu versus mean concentration of Cu in tilapia at 96 h

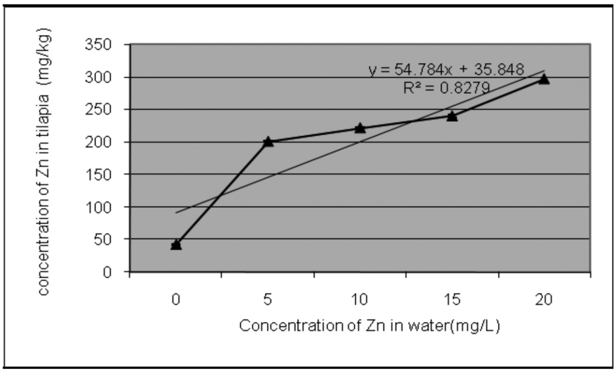

Figure 5: Mean concentration of Zn versus mean concentration of Zn in tilapia at 96 h

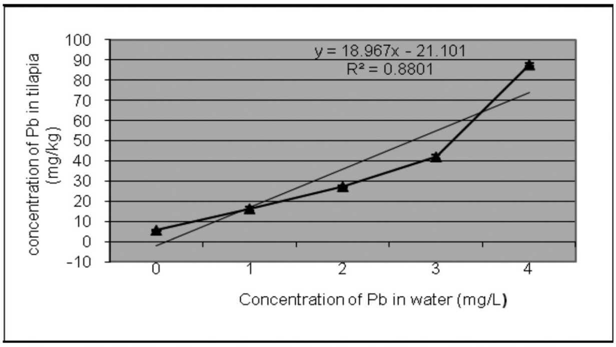

Figure 6: Mean concentration of Pb versus mean concentration of Pb in tilapia at 96 h

This study demonstrated that the short-term exposure (maximum up to 96 hours) of fish to the metal resulted in significant bioaccumulation, with significantly higher Cu, Zn and Pb concentrations in tilapia tissues when compared to the control levels. The differences in accumulation and depuration of metals probably related to the differences in regulation of these elements in tilapia and also their highly resistant nature against metal toxicity. Whether the metal levels exposed caused toxic effect on tilapia, can be concluded using hematological values. Ishikawa et al. (2007) revealed that mercury concentrations exposed was not toxic to tilapia based on their hematological values.

Conclusion

Based on the outcomes of this study, it can be concluded that tilapia could be a useful biomonitor for Pb at high levels. Although tilapia showed ability to accumulate Cu and Zn, the accumulation and depuration patterns were fluctuating and not significant. Continuous study should be undertaken to establish tilapia as indicator in the local environment as tilapia fulfilled the criteria as a biomonitor. Hematological parameters should be taken into account for future studies in order to further elucidate the effects and changes resulting from the metal exposure.

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