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Accumulation of Metals in the Gills of Tilapia Fingerlings (*Oreochromis niloticus*) from *in vitro* Toxicology Study

B.Y. Kamaruzzaman, B. Akbar, K.C.A. Jalal and S. Shahbudin
International Islamic University Malaysia, Jalan Istana, Bandar Indera Mahkota, 25200, Kuantan, Pahang, Malaysia

Abstract: A laboratory experiment was conducted to determine the different level of accumulation of zinc (Zn), copper (Cu) and lead (Pb) in 21 days fingerlings tilapia (*Oreochromis niloticus*). The concentration of these metals in the gills was detected through Inductively-Coupled Plasma Mass Spectrometer (ICP-MS). It was observed that the tilapia fingerlings can accumulate up to 3000 ppb of Zn after 21 days of exposure. The accumulation rate of Cu at 7 to 14 days was between 0.01 to 0.02 μg kg^{-1} while steady increase (0.06 ppb) was detected when exposure period extended to next 7 days. Meanwhile, gradual increase in Zn accumulation was observed at all the time. The fish had accumulated up to 142 μg kg^{-1} of Pb at the highest concentration in 30 days of exposure proved that there is a elevated three fold increase in Pb uptake compared with first ten and 20 days of exposure. It was also proven from this study that fishes exposed to longer period with minimal concentration tend to accumulate less heavy metal in gills since the heavy metals are transported to other parts of the body.

Key words: Bioaccumulation, tilapia fingerlings, gill, heavy metal

INTRODUCTION

Over the last few decades, there has been growing interest in determining heavy metal levels in the marine environment and attention was drawn to the measurement of contamination levels in public food supplies, particularly fish (Kalay *et al*., 1999; Rose *et al*., 1999; Tariq *et al*., 1993). Metals are non-biodegradable and are considered as major environmental pollutants causing cytotoxic, mutagenic and carcinogenic effects in animals (Rauf *et al*., 2009). Heavy metals such as copper (Cu) and zinc (Zn) are essential for fish metabolism while some others such as lead (Pb) and cadmium (Cd) have known role in biological systems (Moraes *et al*., 2003). However, in elevated levels they tend to accumulate in fish body and later it forms threats to human health through food chain. Aquatic organisms have the ability to accumulate heavy metals from various sources including sediments, soil erosion and runoff, air depositions of dust and aerosol and discharges of waste water (Labonne *et al*., 2001; Goodwin *et al*., 2003). Therefore, accumulative capacity of aquatic organisms can pose a long lasting effect on biogeochemical cycling in the ecosphere. Heavy metals can also adversely affect the growth rate in different fishes (Hayat *et al*., 2007). Previous studies showed that accumulation of heavy metals mostly depend on their concentration in the medium and their exposure period (Moraes *et al*., 2003). It is difficult to visualize the pattern of metal accumulation in fish in the environment as there

Corresponding Author: B.Y. Kamaruzzaman, International Islamic University Malaysia, Jalan Istana, Bandar Indera Mahkota, 25200, Kuantan, Pahang, Malaysia

are multiple potential pollutants often encountered as complexes mixtures (Allison *et al.*, 2002). Other factors that are also to be considered are the seasonal variations, environment conditions, fish diet and influences on the enzyme systems that activate and detoxify the pollutants. Furthermore, fish is not sessile, complicating any *in vivo* bio-monitoring. Upon taking these points into considerations, the assessment of heavy metal toxicity in fish should be carried out *in vitro* to find out the more accurate information on the changes in the accumulation pattern of heavy metals in the fish.

It is also important to note that, the growing rate of anthropogenic waste input into the natural environmental system leads to bioaccumulation of heavy metals in biota and their levels in economically important fin fish and shell fishes have become a matter of great concern. Hence, it is important to establish the levels of heavy metal tolerance by these organisms in order to maintain the good quality environment. In this present study, we investigated the concentrations of 3 metals (Cu, Zn and Pb) in gill samples of Tilapia fingerlings. Tilapia fingerlings were chosen based on pervious study (Vijayendran *et al.*, 2006). Fish gills are sensitive respiratory and ion-regulatory membranes and are the first point of contact between waterborne metals and a fish. Furthermore, the gills are continuously exposed to ambient water and in any changes in water quality will rapidly reflect in the fish gill structure (Playle, 1998). Thus, the aim of this study was to determine the selected heavy metal (Cu, Zn and Pb) levels in gills and the pattern of accumulation at 7, 14 and 21 days exposure, respectively.

MATERIALS AND METHODS

Analytical Methods

The 96 h median lethal concentration (96 h LC_{50}) of Cu, Zn and Pb were prepared prior to laboratory experiment. The 96h LC_{50} values obtained (1.819 mg L^{-1} for Cu, 5.906 mg L^{-1} for Zn and 2.731 mg L^{-1} for Pb) were then used to design a sub-lethal concentration whereby the 96 h LC_{50} value was reduced to 25% from highest concentration towards the lowest concentration. A flow through system was applied with 15 fingerlings of tilapia species have been exposed in each tank with tri-replicate for 21 days. At interval 7, 14 and 21 days, fishes were harvested for heavy metal analysis.

Open Acid Digestion

An Inductively-Coupled Plasma Mass Spectrometer (ICP-MS) was used for the quick and precise determinations of Cu, Zn and Pb in the digested fish gills (Vijayendran *et al.*, 2006). Briefly, the killed fishes were first cleaned with de-ionized distilled water and the total length and body weight were then measured. The gills obtained from the fish were vigorously rinsed in deionised water, bottled and immediately stored at −70°C to avoid any contamination. The crushed dry tissue were placed in a mixture of 1 mL concentrated HCI and 1 mL 65% HNO_3 and left to preliminary digestion for 3 h in the teflon beaker. The samples mixture were then heated on the hot plate until completely digested. One milliliter of H_2O_2 was added to the sample in order to break down any recalcitrant lipid material. After cooling to the room temperature, the content of the vessel was thoroughly transferred into a 50 mL polypropylene test tube with deionized double distilled water. A clear solution with no residue was obtained at this stage. The precision assessed by replicate analyses was within 3%. The accuracy was also examined by analyzing, in duplicate a dogfish muscle tissue (DOLT-3) and the results coincided with the certified values within a difference of ±3%.

Statistical Analysis

Statistical analysis of data was carried out using Microsoft Excel programme. Data obtained from the ICPMS reading were plotted on graphs to check the patterns of different heavy metal accumulation in fingerlings. Normal distribution or close to normal distribution was observed and hence, no transformation were done for statistical analysis. The obtained data were also compared by two ways Analysis of Variance (ANOVA) with blocking.

RESULTS

This experiment was designed to measure the accumulation of lead, copper and zinc in the tilapia fish gills after exposure for 21 days. Analysis of the certified materials (National Research Council Canada) of all 3 heavy metals was found to be within 10-25% of expected values (75-90% recovery). For the Cu, there was no much differences between mean metal accumulation at 7 and 14 days. The accumulation rate was between 0.01 to 0.02 $\mu g\ kg^{-1}$ at the highest concentration. However, there was a steady increase occurred when exposure period was extended for another 7 days, whereby at 21 days, 0.06 $\mu g\ kg^{-1}$ of Cu was detected in the gills of the fish (Fig. 1).

Although, there was a steady increase in the accumulation level of Zn noted at all the time between 7 and 14 days (the accumulation rate was 500 $\mu g\ kg^{-1}$), dramatic increase of Zn was noted at 21 days of exposure (3000 $\mu g\ kg^{-1}$) (Fig. 2).

The fish had accumulated up to 142 $\mu g\ kg^{-1}$ of Pb at the highest concentration in 30 days of exposure. There is no much differences between the metal uptake of tilapia fish at the highest concentration in 10 days and 20 days whereby 50 $\mu g\ kg^{-1}$ of lead was reported at day 10 and it increased only 2 to 52 $\mu g\ kg^{-1}$ at 20 days of exposure. However, there was an elevated increase in metal uptake reported at the days of 30 where the concentration has increased nearly 3 folds upto 142 $\mu g\ kg^{-1}$ (Fig. 3).

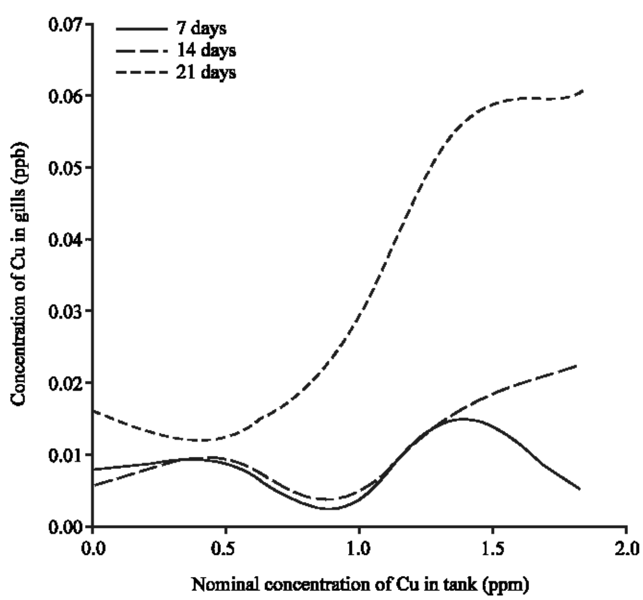

Fig. 1: Mean metal concentration of Cu in gills of Tilapia fingerlings during different days of exposure

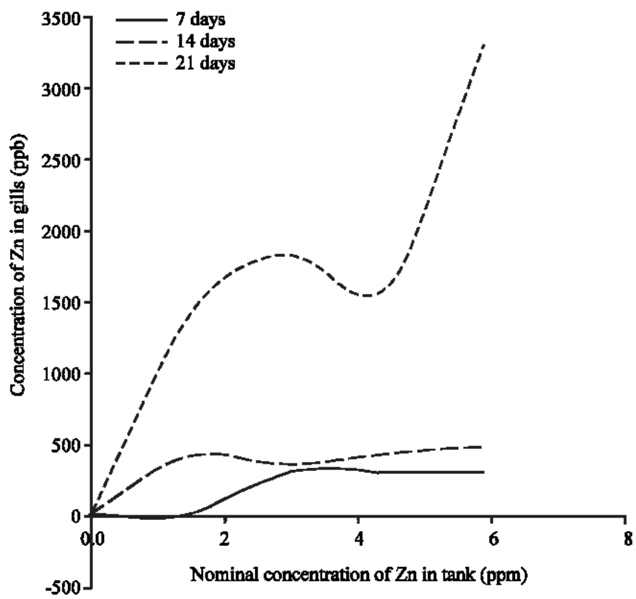

Fig. 2: Mean metal concentration of Zn in gills of Tilapia fingerlings during different days of exposure

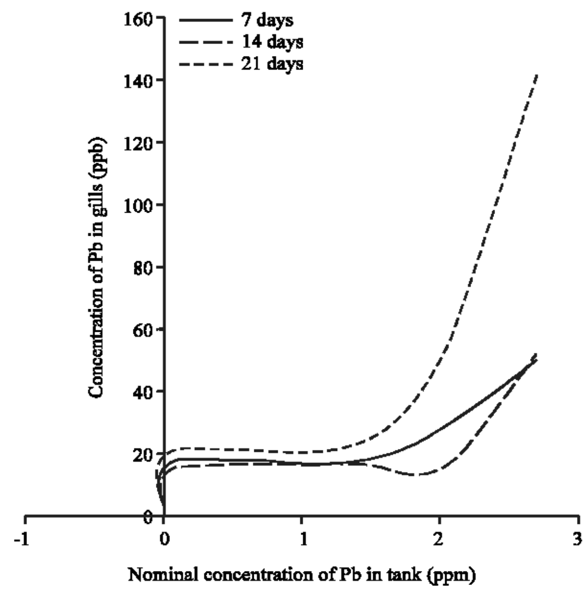

Fig. 3: Mean metal concentration of Pb in gills of Tilapia fingerlings during different days of exposure

Overall, there was no statistically significant correlations ($p>0.05$) between days of exposure and the bioaccumulation of metal in the body of the fish but there were statically significant statistical differences was observed when different concentration of toxicant was exposed to the fish.

DISCUSSION

It was observed that fish responded more towards the increased concentration of selected heavy metals than the time frame of exposure. It might be due to, when a toxic substance is introduced in to the environment, 3 major steps can be identified before the fish start to show response to the pollutant. They were:

- Chemical and physico-chemical process in which the pollutants react with other component in the water and become available to the organism
- Physiological process, including absorption, transport, distribution, metabolic transformation, accumulation and excretion
- In-toxication process, including combination with the receptors (Tao *et al*., 1999)

So, based on these facts, it was found that, the metal might be transported to other parts of the organ in its body and deposited there. There is in-toxic mechanism practiced by the fish to defend itself from the toxicity of the pollutants. Bervoets *et al.* (2001) reported that gill accumulate more concentration of heavymetal than liver followed by muscle and gut region for short time exposure. However, for long term exposure, the heavy metal level was in this decreasing order gut>kidney>liver> gills.

Knowledge of heavy metal concentrations in fish is important with respect to nature of management and human consumption of fish. In the study, heavy metal concentrations in the tissue of freshwater fish vary considerably among different studies (Hayat *et al*., 2007; Chattopadhyay *et al*., 2002; Papagiannis *et al*., 2004), probably due to differences in metal concentrations and chemical characteristics of water from which fishes were sampled. In river, fishes are often at the top of the food chain and have the tendency to concentrate heavy metals from water at higher rate (Mansour and Sidky, 2002). Therefore, bioaccumulation of metals in fish can be considered as an index of metal pollution in the aquatic bodies (Javed, 2005; Tawari-Fufeyin and Ekaye, 2007; Karadede-Akin and Unlu, 2007) that could be a useful tool to study the biological role of metals present at higher concentrations in fish (Dural *et al*., 2007). Therefore, the toxicity tests (Bioassay) are necessary in water pollution evaluation because chemical and physical measurements alone are not sufficient to assess potential effects on aquatic biota (Tarzwell, 1971).

CONCLUSION

Prediction of accumulated levels of any metal in tissues is possible only when the relative importance of each exposure route is known from the environment. In this study, it was clearly shown that the heavy metal exposure via water borne has a significant impact on the gills of the fish. It was also apparent that fishes exposed to longer period with minimal concentration tend to accumulate less heavy metals in gills as they are transported to other parts of the body. Knowledge of these outputs made a possible to give a better description of the variation and the pattern of metal accumulation in the tissues. Detailed study need to be carried out to know the various physiological changes undergone by the fish during different concentration of heavy metal exposure.

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