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Bioaccumulation test of cadmium and magnesium in the liver of *Oreochromis niloticus* via Scanning electron microscope

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Abstract - In this present study, the bioaccumulation of heavy metal, cadmium (Cd) and major element magnesium (Mg), in the liver tissues of *Oreochromis niloticus*, was investigated after exposing the fish over 96 h period to Cd LC_{50} (0.8 mg/l) and Mg LC_{50} (3.03 mg/l). These tissues were investigated by light and scanning electron microscopy. Scanning electron microscopic X-ray microanalysis of fish liver exposed to Cd or Mg confirmed that these metals were accumulated in liver tissues.

Key words: *Oreochromis niloticus*, Liver, Cadmium, Magnesium and SEM-EDX.

Introduction

The aquatic environment has been continually subjected to numerous chemical contaminants such as heavy metals which have significantly polluted the water sources. Moreover, toxic water pollutants are not easily biodegradable and are a serious health problem of highly industrialized countries. Like illnesses, exposures may also be sub-divided into acute and chronic types (Rand *et al*., 2003).

Biochemical and physiological changes were induced by acute exposure to cadmium or other metals have asserted that fish are highly susceptible to high concentration; the toxic effects of this metal on fish were found to be constant and can be revealed within a few hours of exposure (De La Torre *et al*., 2000). Furthermore, it had been demonstrated that Cd contamination results in the metal entering the body through the blood circulation, then consequently access to other organs including kidney and liver (Pretto *et al*., 2011 and Mc Geer *et al*., 2012).

Increasing the contamination of aquatic ecosystems by metals has caused various morphological, physiological and biochemical changes in aquatic organisms. Generally, heavy metals are potent toxins and their bioaccumulation in tissues leads to several damages including cellular and tissue damage, cell death and dysfunction of a variety of organs.

Therefore, the cellular and tissue damages as well as those related to the histopathology of the considered organs play a significant role in evaluating the toxic potential of contaminants regarding to the living bodies. These damages depend on various factor including the environmental conditions, levels of contaminants, exposure time and type of subjected organism (Oliveira Ribeiro *et al*., 2005; dos Santos *et al*., 2012).

Impact of the bioaccumulation of heavy metals in different organs of fish with ultrastructural studies which can play an important role in the diagnosis of fish diseases caused by the heavy metals (Zeitoun and Mehana, 2014; Aldoghachi, 2016). Previous literature indicated that fish exposed to contaminants (industrial, agricultural, and sewage) suffer from many of the pathological changes in the liver, as the most important organ in metabolism (Triebskorn *et al.*, 2008; Syasina *et al.*, 2012). Exposure to heavy metals may cause histological changes in liver which plays an important role in vital functions of the metabolism. Furthermore, it is the major organ of accumulation, biotransformation and excretion of xenobiotic compounds with morphological alterations occurring in some toxic conditions (Figueiredo-Fernandes *et al.*, 2007; Younis *et al.*, 2013).

Ebrahimi and Taherianfard (2011) declared that exposure to heavy metals can result pathological alterations in liver of two species *Capoeta* sp. and *Cyprinus carpio* in highly polluted areas. Greenfield *et al.* (2008) emphasized that histopathology of fish liver is a monitoring tool which can give an assessment of the impacts induced by environmental stressors on fish populations.

Magnesium as an important constituent of many co-enzymes, is vital to many basic metabolic functions, and also aids in bone growth and the function of nerves, bones, and muscles, including heart rhythm regulation. It is non-toxic for humans, except in large doses. Magnesium does not constitute a public health hazard; before toxic levels occur in drinking water, the taste cannot be tolerated (Raskin, 2007).

The objectives of the present work were: To determine the acute toxicity of cadmium and magnesium in Nile tilapia *Oreochromis niloticus* to obtain information regarding the liver of Nile tilapia in acute cadmium and magnesium exposure; and using electron microscopy in conjunction with X-ray Microanalysis to determine the metal content of the liver tissues.

Materials and Methods

The present study was carried out with *Oreochromis niloticus* which have purchased about 100 fingerling tilapias 8 ± 1 g in mean weight and 8.5 ± 2 cm in length from a commercial aquaculture facility in Serendah, Selangor 48200 Kuala Lumpur, Malaysia. The commencement and termination date of experiment was Nov. 2014-Feb. 2015.

The fishes were acclimated in group of 25 in 50 L glass aquarium filled with tap water for one week, the fishes were fed a dry commercial food (pellets with 25 % of crude protein). Air pump with aquarium was used for aeration system. Temperature, pH, salinity and dissolved oxygen (DO) were recorded daily during the experiment, and the average was 25.5 ± 2 °C, 7.65 ± 0.5, 0.085 ± 0.022 g/l, and 7.2 mg/l respectively.

Tap water in aquaria was replaced every 24 hrs. Afterwards, fingerlings were transferred to assay aquaria (20 x 20 x 40 cm) 5 L glass aquaria, which provided aeration via air pumps and air stone diffusers. Each group was at a stocking density of 10 fish /aquarium.

The chemical materials used in this study was $CdSO_4$ and $MgSO_4$ Analargrade BDH chemicals with 99.5 % purity dissolved in double deionized water, to prepare the stock solution (1000 $mg.l^{-1}$) of metal. A series of five concentrations of each one were prepared by adding a calculated volume from the stock solution with local tap water into test containers. The Tilapias were semi statically exposed to different concentrations (control (0), 0.5, 1, 3 and 5 ppm) of cadmium metal while magnesium was used as the rate of (control (0), 1, 3, 5 and 10 ppm) during 96 hrs. (range determined by preliminary tests) with three simultaneous replicates. No food was supplied during the experiment. Test solutions were replaced by fresh ones of the same respective concentrations every 24 hrs., according to the renewal method recommended in APHA *et al.* (1999). Mortality were recorded at 6, 12, 24, 48, 72 and 96 hrs. of exposure, and dead organisms were removed regularly from the test solutions, for estimating the median lethal concentration (LC_{50}) from the probit transformed concentration-response curves (U.S. EPA, 2002). The 96 hrs. LC_{50} was (0.8 and 3.03 mg/l) for cadmium and magnesium respectively.

Bioaccumulation test *via* Scanning electron microscope with EDX:

Scanning electron microscopy examination for SEM, Fish from the experimental and control groups (n = 3) were anesthetized in ice cold water and sacrificed by cervical decapitation. The liver tissues treatments were fixed in phosphate-buffered 8 % gluteraldehyde (at pH 7.2) for 1 h. and then post-fixed in 4 % osmium tetroxide OsO_4 in the same buffer overnight to increase electron density. Tissues were dehydrated in ascending series of ethanol concentrations and then dehydrated in a grade series of ethanol acetone mixture solutions (Pandey *et al.*, 2008). The percentage of the weight of the mineral contents through the cross-section of liver was quantified by energy dispersive X-ray (EDX) spectroscopy analysis using a scanning electron microscope (JEOL JSM-7001F, Japan) equipped with EDX (OXFORD Instrument X-Max).

Results and Discussion

The results of element composition of liver obtained from dispersive X ray microanalysis (EDX) recorded a slight increase of the weight percentage of metal in treated samples than control samples. This is indicated that metal able to accumulate in liver tissue surfaces. An examination of control sample by EDX in scanning electron microscope showed not detected to the Cd metal (Fig. 1) in compare with exposed samples to Cd ions showed in the spectrum of EDX increasing in the cadmium weight percentage 16.38 % (Fig. 2) and this was an evidence of a cumulative susceptibility to the Cd metal in the fish liver of *O. niloticus* and at the same time this metal has the effect on the reduction of Ca and P as in (Fig. 2).

Anyway, concentrations of metals in the tissues of fish liver reflect the presence of these concentrations indicate the metal storage in longer period (Rao and Padmaja, 2000). Kaoud *et al.* (2011) reported that metal accumulation in the liver of *O. niloticus* treated with cadmium, this bioaccumulation may be attributed to the direct toxic effects of pollutants on hepatocytes.

The present study can be supported by the fact that the differences in chemical composition of the liver depends upon the ambient element exposure. Hence, the stress conditions caused by heavy metal pollutants disturb the elemental composition of the liver and therefore the percentage composition in the liver of Nile tilapia fish can be considered as a reliable pollution indicator with authenticity.

Our results reported increased amount of magnesium weight percentage 9.32 % in microanalysis of EDX (Fig. 3) in compare with control which was 2.2 % (Fig. 1). Study of Begum *et al.* (2005) in three species of fish (*Tilapiani lotica*, *Cirrhina mrigala* and *Clarius batrachus*). They have reported that Mg was accumulated in higher levels and the average concentrations of magnesium component were (2090-2560 mg/kg) in muscle tissues due to the fact that magnesium is one of the major elements. This is similar to study by Oliveira Ribeiro *et al.* (2005) who described that the liver was the most important target organ for heavy metals including magnesium which has been accumulated in range of (646 mg/kg) in *Anguilla anguilla*.

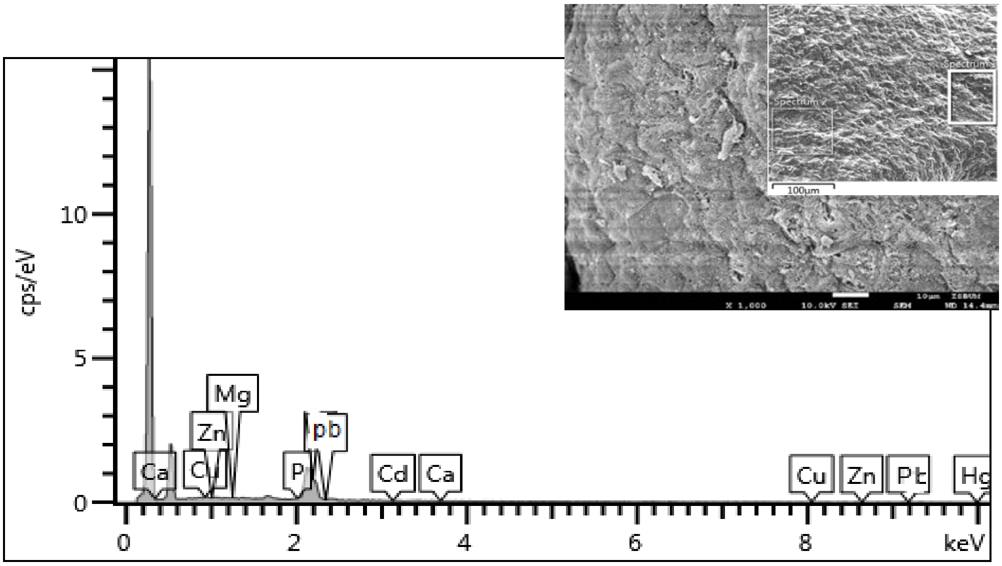

Figure 1. Scanning electronic micrograph and energy dispersive X-ray spectroscopy microanalysis of the control liver tissue. X-ray spectrum shows only essential elements usually present in biological specimens Ca, P, Cu, Zn, and Mg and not detecting of Cd, Pb and Hg in liver tissues.

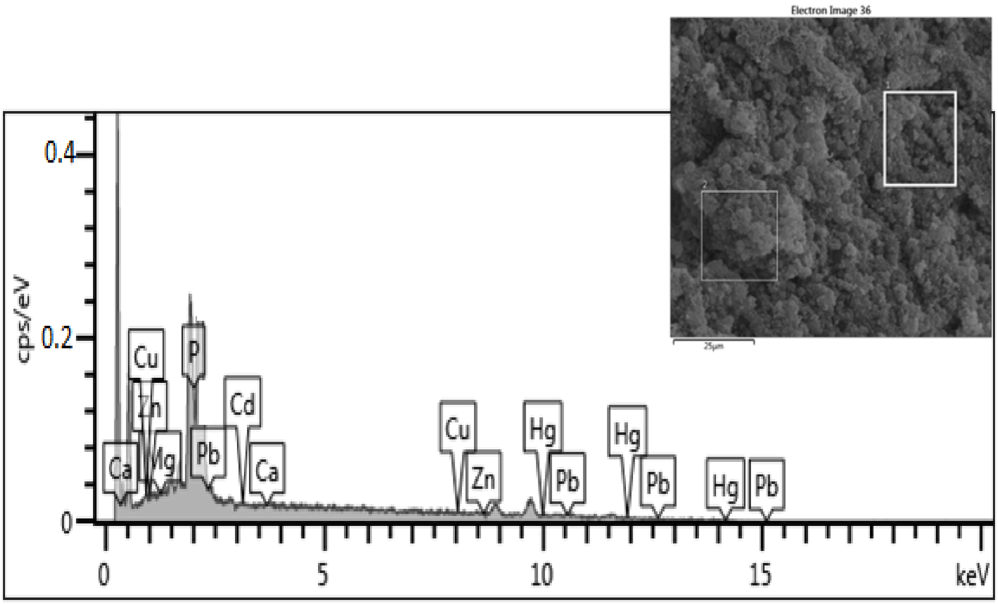

Figure 2. SEM and EDX microanalysis of the liver tissues from *Oreochromis niloticus* after 96 hrs. exposure to Cd ions. Elemental analysis spectrum shows appearance of Cd in weight percentage (16.38 %) in liver tissues with low peaks of essential elements (Ca, P, Zn, Cu and Mg) and no detection of Pb and Hg.

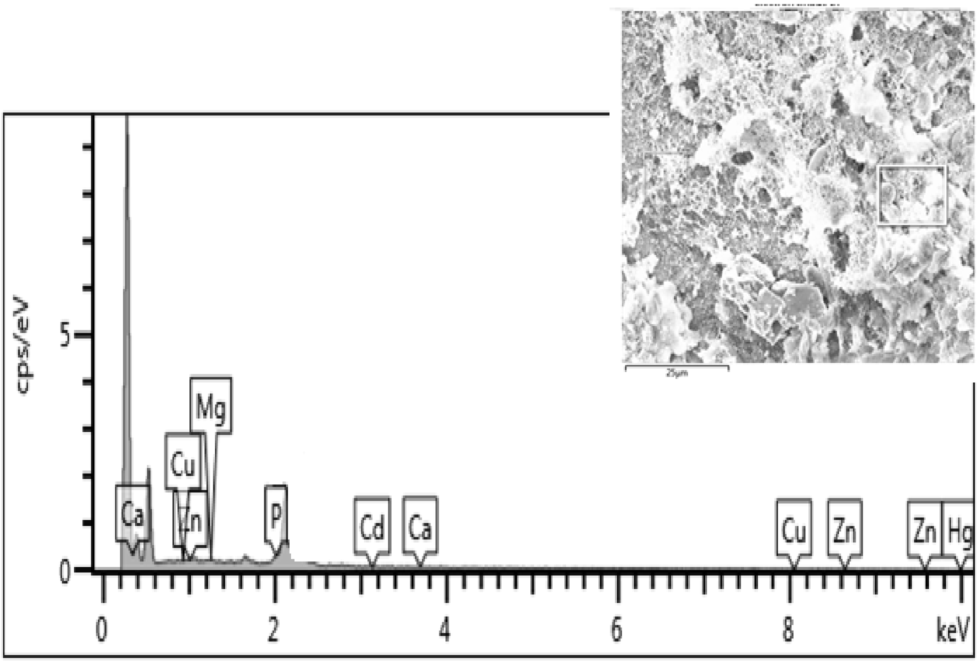

Figure 3. SEM and EDX microanalysis of the liver tissue from *Oreochromis niloticus* after 96 hrs exposure to Mg ions. Elemental analysis spectrum shows appearance of Mg in weight percentage (9.32 %) in liver tissues with low peaks of essential elements (Ca, P, Zn, and Cu) and not detecting of Cd, Pb and Hg.

Magnesium is an important constituent of many enzymes and also supports the bone growth, nerves function, the high concentration of salts especially magnesium sulphate and magnesium citrate is not completely absorbed in the intestine which creates a hypertonic condition that causes diarrhoea and dehydration (Csuros and Csuros, 2002).

The high levels of accumulated heavy metals in liver may be attributed to the sequestering and binding of this metal by metallothionein (MT) (Yacoup, 2007). Special attention is given to heavy metals existing in the aquatic environment because of their toxicity, long persistence and bioaccumulation and non-biodegradable properties in the food chain (Jaric *et al.*, 2011).

Conclusion

The results of the present research revealed that tilapia fish had a higher sensitivity to Cd. Energy dispersive X ray proved the existence of the accumulation of heavy metals in the surface of the exposed fish liver. This work advances a new knowledge as influence of heavy metals in the liver histology of Nile tilapia fish and confirmed that their effects could be observed at different exposure periods; in addition, supporting environmental watch over aquatic systems when polluted by heavy metals. However, there is a need for more additional experiments to enhance understanding of toxicity mechanisms for this element.

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اختبار التراكم الحيوي لعنصر الكادميوم والمغنسيوم في كبد أسماك *Oreochromis niloticus* بواسطة المجهر الألكتروني الماسح

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المستخلص - في هذه الدراسة، تم التحقيق في تراكم العنصر الثقيل الكادميوم والعنصر الرئيسي المغنيسيوم في نسيج الكبد لأسماك *Oreochromis niloticus* بعد تعريض الأسماك لفترة 96 ساعة إلى التركيز نصف المميت للكادميوم (0.8 ملغم/لتر) والتركيز

نصف المميت للمغنسيوم (3.03 ملغم/لتر). تم فحص أنسجة الكبد بواسطة المجهر الألكتروني الماسح. وفحص العناصر بواسطة أشعة أكس للمجهر الألكتروني أكد تراكم عنصر الكادميوم أو المغنسيوم في أنسجة الكبد المعرضة لهذه العناصر.