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## Detection of Heavy Metal Residues in the Muscle and Skin of Tilapia

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### Abstract

A study to detect the presence of heavy metals, which are Lead (Pb), Copper (Cu) and Zinc (Zn) in Tilapia caught from 3 waterways in Universiti Putra Malaysia, Serdang, Selangor was conducted. Water samples from the study sites were also sampled and analysed. These locations were in the academic areas of Universiti Putra Malaysia, Serdang campus near the agricultural and housing areas. Results showed that the mean concentration of Cu, in the water was 0.04 µg/mL and Pb and Zn were 0.03 µg/mL. The concentration of the elements tested was found to be lower than the recommended limits set by FAO/WHO but the concentration of Pb almost breached the limit of 0.05 µg/mL. The mean concentration of Pb in the fish muscles (0.64 µg/g) did not exceed the permissible limits set by FAO/WHO (1.5 µg/g) and the Malaysian Food Regulation (2.0 µg/g). The concentration of Cu in the fish muscle was much lower (2.13 µg/g) than the permissible limits set by the WHO (10.0 µg/g) and the Malaysian Food Regulation (30.0 µg/g). The highest element that had accumulated in the fish muscle was Zn (8.28 µg/g). However, the concentration of Zn did not exceed the permissible limits set by FAO/WHO (150.0 µg/g) and the Malaysian Food Regulation (100.0 µg/g). In the fish skin, the concentration of Cu and Pb were quite high. The concentration for Pb in the skin (6.77 µg/g) exceeded both the permissible limits set by FAO/WHO (1.5 µg/g) and the Malaysian Food Regulation (2.0 µg/g) while the concentration of Cu in the skin only exceeded the permissible limits set by FAO/WHO (10.0 µg/g). Among the three elements studied, Zn concentration was highest in the fish skin (45.8 µg/g). However, the Zn levels did not exceed the permissible limits of FAO/WHO and the Malaysian Food Regulation. Therefore, it can be concluded that the Tilapia caught from the waterways were not suitable for animal and human consumption because the concentration Pb in the fish skin was too high. Copper was also found to be present in the skin at concentration that can pose health hazard. However, these fishes can be said to be safe to be consumed if the skin were to be removed.

**Key words:** Heavy metal, residue, skin, muscle, Tilapia, waterways, permissible limits

## Introduction

Pesticides and herbicides derived from agricultural operations and industrial effluents such as metals can ultimately find their way into a variety of different water bodies and produce toxic effects in aquatic organisms. Toxic metals that accumulated to hazardous levels in aquatic biota can pose health problem of public concern. Drinking of water and/or consumption of fish from excessively polluted water could lead to health hazards to man. Pollutants can enter fish through five routes via gills, oral consumption of food and water, skin, and food or non-food particles.

Following absorption, the pollutant is carried in the blood stream to the liver for transformation and/or storage, or either stored in a storage point (tissues) inside the fish's body. The rate of absorption and the dynamic process associated with the pollutant's elimination by the fish determines the concentration of the pollutant at any given tissue.

Heavy metals in the aquatic environment are ranked as major polluting chemicals in both developed and developing countries. It is also a major concern worldwide due to their threat to plant and animal life, thus disturbing the ecological balance of nature.

Tilapia was chosen for this study because of its abundance and ubiquitous distribution. Other than that, the public is seen catching these fishes at the study areas for consumption using cast nets and/or rod and line where this species is abundant together with other fishes such as Suckermouth Catfish (*Hypostomus plecostomus*). Furthermore, Tilapia can successfully spawn, is fast growing and highly resistant to diseases. These superior characteristics lead to its wide distribution in rivers, reservoirs and fishponds which attract fishing enthusiasts to fish this fish.

This study highlights the public health significance upon consumption of fish with heavy metal residue from polluted waterways. Previous studies on heavy metal residues in fish were on the residues of metals in tissues that were inedible such as gills, intestines, liver, and tail of fish (Zheng Zhang *et al.*, 2007, Olowu *et al.*, 2010). Studies on heavy metal residues in the muscle and skin of fish are very limited.

The specific objective of this study was to detect the presence of heavy metals (Cu, Pb, Zn) in the muscle and skin of Tilapia and to compare the concentration of these heavy metals in the fish muscle and skin with available standards.

## Materials and Methods

### *Experimental Animals*

Ten juvenile Tilapias were caught with a rod and line from three waterways, two at Jalan Sapucaya and one at Ladang 2 in the Serdang campus of Universiti Putra Malaysia. These waterways drain water from the academic areas which include Faculty of Veterinary Medicine, Faculty of Biotechnology, Faculty of Environmental Studies, Faculty of Agriculture, and Faculty of Forestry and nearby housing areas including farms and orchards. It was a rainy season at the time of

sampling. The water pH and temperature were 6.62 and 29.8°C, respectively, in the three waterways. The fishes were later transported to the Analytical Laboratory of Department of Soil Science, Faculty of Agriculture, Universiti Putra Malaysia.

### ***Sampling***

In the laboratory, the fish were weighed using an electronic balance and the values were recorded to the nearest gram before 10 muscle samples and 10 skin samples were dissected from the fish. Water samples were also collected from each waterway using plastic containers. Locations of water samplings were based on evidence of local public seen fishing there. The temperature and pH of the water in the waterways were also taken on the same day. All samples were then analysed for heavy metal concentration using a Perkin Elmer 5100 PC Atomic Absorption Spectrophotometer.

### ***Sample Storage***

The samples were thoroughly cleaned with distilled water to remove debris that adhered on the surface of the fish body and gills. Each sample was then wrapped in aluminium foil and kept overnight in a freezer at -10°C (Olowu *et al.*, 2010).

### ***Sample Preparation***

The samples were then thawed at room temperature after been frozen for 24 h and defrosted before the samples were unwrapped. The scales of the fish were removed using scale remover before the fish were dissected using stainless steel scalpel blades attached to a scalpel holder (Zheng Zhang *et al.*, 2007). Muscle tissues at the trunk, caudal peduncle and also the belly were dissected and removed from the bones. After removing the whole chunk of muscle tissue from the bones, the skin was separated from the muscle using scalpel blades and forceps. Dissected samples were placed in small air tight plastic containers (5 × 3cm) and labeled accordingly. Later, all the samples were chilled overnight at 4°C.

### ***Sample Digestion***

Three grams of fish muscle tissue and 0.4g of fish skin were weighed using an electronic balance. The samples were oven dried for 1 h at 150°C to obtain the dry weight. Later, the samples were placed in 200-mL beakers containing 100 mL HNO<sub>3</sub> and 300 mL HCl acids. The samples were then left overnight. All the procedures were conducted in a fume cupboard.

After 12 h, the samples were placed on a stirring hot plate and heated at 150°C for 1 h. After 1 h, the samples were left to cool for half an h at room temperature and later poured into 100 mL volumetric flask. Distilled water was added until the final volume of 100 mL and the volumetric flask was covered with "Parafilm". The flask was shaken gently and labelled.

The sample digests were later transferred into 100-mL plastic bottles prior to analysis with a Perkin Elmer 5100 PC Atomic Absorption Spectrophotometer to detect the presence of heavy metals in the tissues.

### **Detection of Heavy Metals**

The Atomic Absorption Spectrophotometer (AAS) used for this study is a Perkin Elmer 5100 PC, connected to a computer equipped with software (AA WinLab Analyst) for heavy metal detection. Before operating, the AAS was calibrated with three standard solutions and a blank solution which was distilled water. The three standard solutions were T1, T2 and T3, standards for Cu, Zn and Pb respectively, in 100-mL volumetric flasks. After calibrating with the standard solutions, the machine was calibrated with the blank solution before analyzing the prepared samples. A cathode lamp, depending on the element to be tested was fixed on the machine.

A capillary aspiration tube connected to the machine was dipped into a sample to be analyzed and the sample was aspirated into a flame, atomized and detected by the machine.

### **Results**

The present study showed that there were heavy metal residues in Tilapia in the waterways in Universiti Putra Malaysia, Serdang, Selangor. All the elements studied (Cu, Pb and Zn) had been successfully detected in the fish muscles and skin. Copper and Pb were observed to be present at high concentrations in the fish skin of the Tilapia. However, the results revealed that the Tilapia studied can be considered safe for consumption if the fish skin is removed.

### **Discussion**

Tilapias is a type of fish that eats almost everything and most of the time scavenge food from the river beds and the water column. Aquatic microflora and fauna which are constituted as fish food have capabilities of accumulating or incorporating heavy metals into their living cells from the environment (Fostner and Wittman, 1981, Ibrahim and Sa'id, 2010). The mean values of Cu, Zn and Pb in the water samples were 0.04 µg/mL, 0.03 µg/mL and 0.03 µg/mL, respectively. None of these values exceeded the permissible limits for human consumption by the World Health Organization. For this study, only surface water samples were collected. The results could have differed if water samples were collected from the bottom of the waterways and analyzed.

Food for the fishes is mainly found at the bottom and there are possibilities that the heavy metals can accumulate at this site of waterways due to their large atomic numbers. Heavy metal concentration is usually high in sediments or riverbeds and this may also be attributed to human activities such as discharge of untreated sewage and uses of metals and industrial materials that contain metals as well as the ability of the sediment to act as sink (Okeye *et al.*, 1991; Olowu *et al.* 2010).

Bioaccumulation is a process where an organism concentrates metals in its body from surrounding food or medium, either by absorption or ingestion (Fostner and Wittman, 1981). When bioaccumulation occurs, fish can regulate metal concentration to a certain limit (Health, 1991). Results from this study showed that the heavy metals tended to be bioaccumulated in the skin rather than the muscle.

Lead had the least bioaccumulation in the fish muscle studied. The content of Pb in the fish muscle was lower than the permissible limits set by FAO/WHO and the Malaysian Food Regulation although the level of Pb in the fish tissue was the lowest; it was close to the permissible limits set. The second highest metal bioaccumulated in the fish muscle was Cu. The content of Cu in the fish muscle was much lower than the permissible limits set by FAO/WHO and the Malaysian Food Regulation. Zinc was the element highly bioaccumulated in the fish muscle. Even though the content of Zn in the fish muscle was the highest, its level of bioaccumulation was very much lower than the permissible limits set by FAO/WHO and the Malaysian Food Regulation.

Lead was the least bioaccumulated element found in the skin when compared to other elements tested. Although the content of Pb was lowest in the fish skin, it exceeded the permissible levels set by the FAO/WHO and the Malaysian Food Regulation. The second highest metal bioaccumulated in the fish skin was Cu and it also exceeded the permissible levels set by FAO/WHO and the Malaysian Food Regulation. Zinc was also the highest bioaccumulated element in the fish skin studied. Even though Zn had the highest level of bioaccumulation in the fish skin, it still did not exceed the permissible limits set by FAO/WHO and the Malaysian Food Regulation.

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