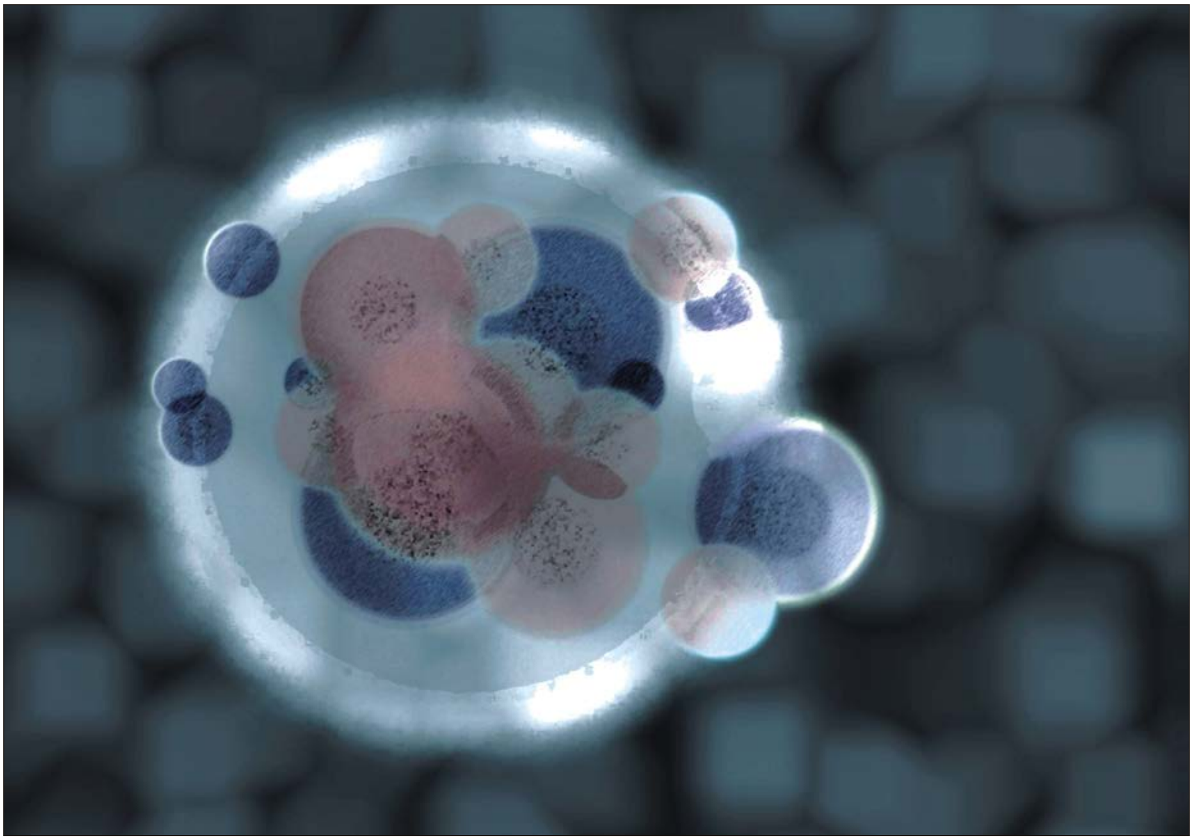

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Analysis of Heavy Metal Concentrations in Tilapia Fish (*Oreochromis niloticus*) From Four Selected Markets in Selangor, Peninsular Malaysia

Abdulali K.A. Taweel, M. Shuhaimi-Othman and A.K. Ahmad
School of Environmental and Natural Resource Sciences, Faculty of Science and Technology, University Kebangsaan Malaysia, Bangi, 43600, Selangor

Abstract: Concentrations of cadmium (Cd), zinc (Zn), lead (Pb), nickel (Ni) and cobalt (Co) were measured in various organs (such as in liver, gill and muscle) of Tilapia fish (*Oreochromis niloticus*) which were collected from Serdang night market, Bangi night and wet markets and Kajang wet market, in Selangor, Peninsular Malaysia. The concentration of metals was measured by inductively-coupled plasma mass spectrometry (ICP-MS). The levels of metal varied as it depended on various tissues. Zinc was the dominant metal measured during this study. In general, results indicated that all studied heavy metals concentrations were significantly higher ($p<0.05$, two way ANOVA) in liver samples collected from all sites than gill and muscle, except for the zinc in fish liver from Serdang night market (41.87 $\mu g\ g^{-1}$ dry weight (DW)) less than zinc in fish gill in the same market (55.72 $\mu g\ g^{-1}$ DW). The highest Cd, Pb and Co concentration in fish liver were observed in Kajang wet market which is 0.44, 0.72 and 02.86 $\mu g\ g^{-1}$ DW, respectively. Meanwhile, in fish gill, levels of Pb and Co in Serdang night market were the lowest among all sites which is 0.34 and 0.31 $\mu g\ g^{-1}$ DW, while the highest in gill from Bangi wet market which is 0.63 and 0.41 $\mu g\ g^{-1}$ DW, respectively. Cd, Zn, Pb and Co levels in fish muscle from Serdang night market were the lowest (0.02, 11.36, 0.10 and 0.25 $\mu g\ g^{-1}$ DW, respectively), whereas the lowest Ni level was noted in fish muscle from Kajang wet market (03.84 $\mu g\ g^{-1}$ DW). On the other hand, the highest Cd and Zn level were in Bangi wet market (0.03 and 16.72 $\mu g\ g^{-1}$ dw, respectively). The results obtained in this study were compared with those reported in all other areas of earlier studies. All studied heavy metals levels are below the limits for fish proposed by World Health Organization and safe within the limits for human consumption in the edible part of studied fish.

Key words: Contamination, four markets, heavy metals, ICP-MS, Tilapia fish

INTRODUCTION

Fish are one of the most widely distributed organisms in the aquatic environment and considered as one of the main protein sources of food for human (Rashed, 2001). Freshwater fishes are similar to sea water fishes which play an important role in determining resident's diet (Ahmad *et al*., 2009). Heavy metals are an important group of chemical pollutants, whereby food is the main route for entry into our body; some heavy metals irreversibly are bound to human body tissues, e.g., cadmium to kidneys and lead to bones (Kaplan *et al*., 2011). Fish constitute a major source of heavy metals in food (Sivaperumal *et al*., 2007). High level of metals in environment may lead to an excessive accumulation which cause problem to human, animal and plants (Al-Khateeb and Leilah, 2005). Fish has been found to be excellent indicator for the heavy metal contamination level in aquatic system because it occupies different food chain levels (Karadede-Akin and Unlu, 2007). A major concern and attention about the health effects people due to consumption of foodstuff contaminated with metals were paid (Iwegbue *et al*., 2008). Heavy metals occur in the environment both as a result of natural processes as well as contaminants from human beings activities (Franc *et al*., 2005). Some heavy metals are known as potentially toxic include arsenic, lead, aluminium and cadmium, others are essential such as nickel, zinc and chromium (Abduljaleel and Shuhaimi-Othman, 2011). Heavy Metal has positive and negative effect on human health and surroundings (Abduljaleel *et al*., 2011). The accumulation of heavy metals in fish has been extensively studied and well documented. However, the research has been generally focused on the muscle tissue, while the distribution pattern among other tissues, such as liver and gills have been mainly neglected (Jaric *et al*., 2011). Furthermore, the contaminants also concentrate in some of the organs of fish and can cause lethal and a range of sub lethal effects (Ozmen *et al*., 2008). It was known that active metabolite body parts such as liver, gill and kidney concentrate more on an amount of heavy metal than other parts like muscle (Dural *et al*., 2007). Fishes are often seen

Corresponding Author: Abdulali K.A. Taweel, School of Environmental and Natural Resource Sciences, Faculty of Science and Technology, University Kebangsaan Malaysia, Bangi, 43600, Selangor

at the top of the aquatic food chain and may accumulate large amount of heavy metals from its environment (Mansour and Sidky, 2002). In addition, fishes are one of the most indicative aspects in freshwater ambience, for the evaluation of heavy metals pollution and health risk potential of human consumption (Papagiannis *et al*., 2004). Tilapia *Oreochromis niloticus* as commercially important fish species (Christopher *et al*., 2009) can survive at adverse environmental conditions because their resistance to disease is strong, their respiratory demands are slight so that they can tolerate low oxygen, high ammonia levels and wide range of salinities (Zhou *et al*., 1998). Tilapia fish have a greater capacity for metal bioaccumulation than tiger prawn (Mokhtar *et al*., 2009). Heavy metals are accumulated through different organs of the fish because of the affinity between them. In this process, all heavy metals are concentrating at a different levels in different organs of the fish body parts (Rao and Padmaja, 2000). Most studies have been concerted only on the accumulation of heavy metals in the edible part (muscle), in view of the fact that it is the main fish part that is consumed by human beings (Keskin *et al*., 2007). Muscle is not always a good indicator of the entire body fish contaminations and therefore, it is vital to analyze other body parts as well, such as the gills and liver (Has-Schon *et al*., 2006). Due to the existence of metal-binding proteins in some tissues, for an example metallothioneins in the liver can accumulate significantly higher heavy metal concentration than the muscles (Ploetz *et al*., 2007; Uysal *et al*., 2008a). The metal content in the fish dorsal muscle was estimated because of its significance for human consumption and the fish liver was also measured since this organ tends to concentrate metals, it is also a good indicator of chronic exposure to heavy metals because it is the site of metal metabolism (Usero *et al*., 2003). Whereas, the concentration of metals in gills represents the concentration of metals in water (Uysal *et al*., 2008b). Malaysia as a developing country is facing various forms of contaminations risk (Ahmad and Shuhaimi-Othman, 2010). Hence, the main aim of this study was to evaluate heavy metal contamination and to compare metal levels within and between liver, gill and muscle tissues of tilapia fish (*Oreochromis niloticus*) in four local markets in Malaysia in February 2010.

MATERIALS AND METHODS

Area of study: Four local markets have been selected to study in Selangor (Malaysia) as shown in Fig. 1. The

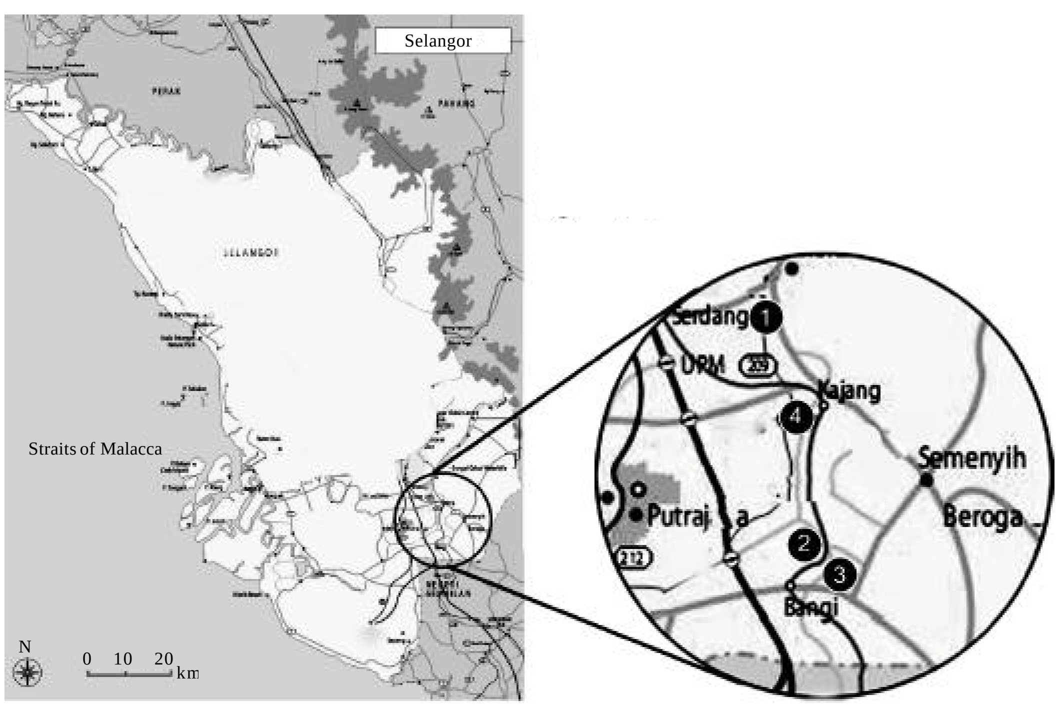

Fig. 1: The locations of sampling sites; 1: Night market at Serdang town, 2: Night market at Bangi town, 3: Wet market at Bangi town, 4: Wet market at Kajang town

markets included Night market-Serdang (station 1) which is situated at 03°00` 59.30``N and 101°42` 46.07``E in Serdang town, Night market Bangi (station 2) which is situated at 02°56`32.60``N and 101°45` 51.83``E in Bangi town, Wet market Bangi (station 3) which is situated at 02°56` 40.25``N and 101°46` 09.13``E in Bangi town and Wet market-Kajang (station 4) which is situated at 02°59`29.18``N and 101°47` 32.85``E in Kajang town.

Reagents: All reagents were of analytical reagent grade. Double deionised water was used for all dilutions. Nitric acid, HNO_3 (65%) and hydrogen peroxide, H_2O_2 (30%) were of ultrapure quality (Merck, Darmstadt, Germany). The element standard solutions from Merck company to make the calibration were prepared by diluting the stock solutions of 1000 mg L^{-1} of each element (Kasmari, 2007). The certified standard reference materials of marine biota sample (SRM2976, freeze-dried mussel tissue, National Institute of Standards and Technology, USA) were used.

Collection of fish samples: Fresh fish samples were obtained directly from each market/station. The fish are commonly consumed by the local population in Malaysia. Total size and weight of the samples were measured; fish length ranged between 20-22 cm and the wet weight ranged between 220-240 g. Fish were carefully conserved with ice in clean polyethylene bags. Ice was used to minimize the tissue decay and to maintain moist conditions during transportation (Eastwood, 2000). Fish were placed in an isolated container during transportation and immediately taken to the toxicity laboratory at Faculty of Sciences and Technology-University Kebangsaan Malaysia, Bangi, Malaysia, where the samples were washed by using deionized water, identified and then deep frozen at -20°C while awaiting dissection on the following day until it is prepared for analysis. The sampled fish were the mostly consumed sizes.

Sample preparation and analysis: The frozen fresh fish samples were thawed at room temperature, only boneless tissues were taken for the metal analysis, such as liver, gills and muscles samples of the fish. The organs were removed with a stainless steel knife, homogenized and weighed and then individual samples were dried to the constant weight at 80°C for two days in acid-washed petri dishes. When samples reached constant weight, samples were allowed to cool in the desiccators and crushed into a fine powder by using a porcelain mortar and pestle. The samples portions were 0.5 g DW powdered form of muscle and gill and 0.1 g DW powdered form of liver (because the liver is tiny compared to the gills and muscles in the fish body) in duplicate then it was digested by using closed vessel microwave digestion in a microwave oven (Milestone model Start D, Italy), by using ultra pure nitric acid (65%) hydrogen peroxide (35%) mixture 3:1 in ratio at a temperature (150°C) for 20 min, following a cooling to room temperature for 35 min in the microwave (Taghipour and Aziz, 2010; Taweel *et al*., 2011). Study reported that the microwave digestion method was an accurate method for samples digestion compared with others methods such as dry ashing and wet digestion (Mendil *et al*., 2010). Hydrogen peroxide was added to the samples with nitric acid as it decreases nitrous vapours and speeds up the digestion of organic substances by elevating the reaction temperature (Dig-Acids, 2001). Potential presence of heavy metals in chemicals which used in digestion was determined. Blanks were used simultaneously in each batch of analysis to authenticate the analytical quality. The digested samples were diluted with deionized water to a total volume of 25 mL for liver and 50 mL for gills and muscles, then filtering the samples through 0.45 μm Whatman filter paper. The analysis was performed by inductively-coupled plasma mass spectrometry (ICP-MS) (model ELAN 9000 Perkin Elmer ICP-MS, USA). It included the assessment of concentrations of the following five heavy metals cadmium (Cd), zinc (Zn), lead (Pb), nickel (Ni) and cobalt (Co). The quality of the analytical process was confirmed by the analysis of standard reference materials of mussel. The concentrations were found to be within 90-110% of the certified values for all measured heavy metals. The concentrations of heavy metal were expressed as μg g^{-1} DW of fish. All the glassware and plastics were soaked overnight in 10% (v/v) nitric acid and rinsed with distilled and deionised water and dried before being used (Khansari *et al*., 2005). The operational parameter settings were used in ELAN 9000 Perkin Elmer as in Table 1.

Statistical analyses: Statistical analyses were conducted to determine the differences in the heavy metal contamination of the fish of the four studied sites and in the different three organs were evaluated by two-way Analysis of Variance ANOVA. Turkey's test for multiple

Table 1: Operational parameter settings were used in ELAN 9000 Perkin Elmer

Characteristics	Instruments condition
RF generator	40 MHz
RF power	1000 W
Spray chamber	Ryton Scott
Nebulizer	Cross-flow
Plasma gas flow	15.0 L min^{-1}
Auxiliary gas flow	1.0 L min^{-1}
Nebulizer gas flow	0.60 L min^{-1}
Sampler and skimmer cone	Nickel

comparisons was used to evaluate heavy metals mean differences between sites and between fish organs. The statistical significance level was set at $p<0.05$. All test procedures were performed in a computer program SPSS version 18.

RESULTS AND DISCUSSION

Figure 2 shows heavy metals (Pb, Cd, Zn, Co and Ni) concentration in liver, gills and muscles of Tilapia fish from four studied markets. Heavy metals concentration

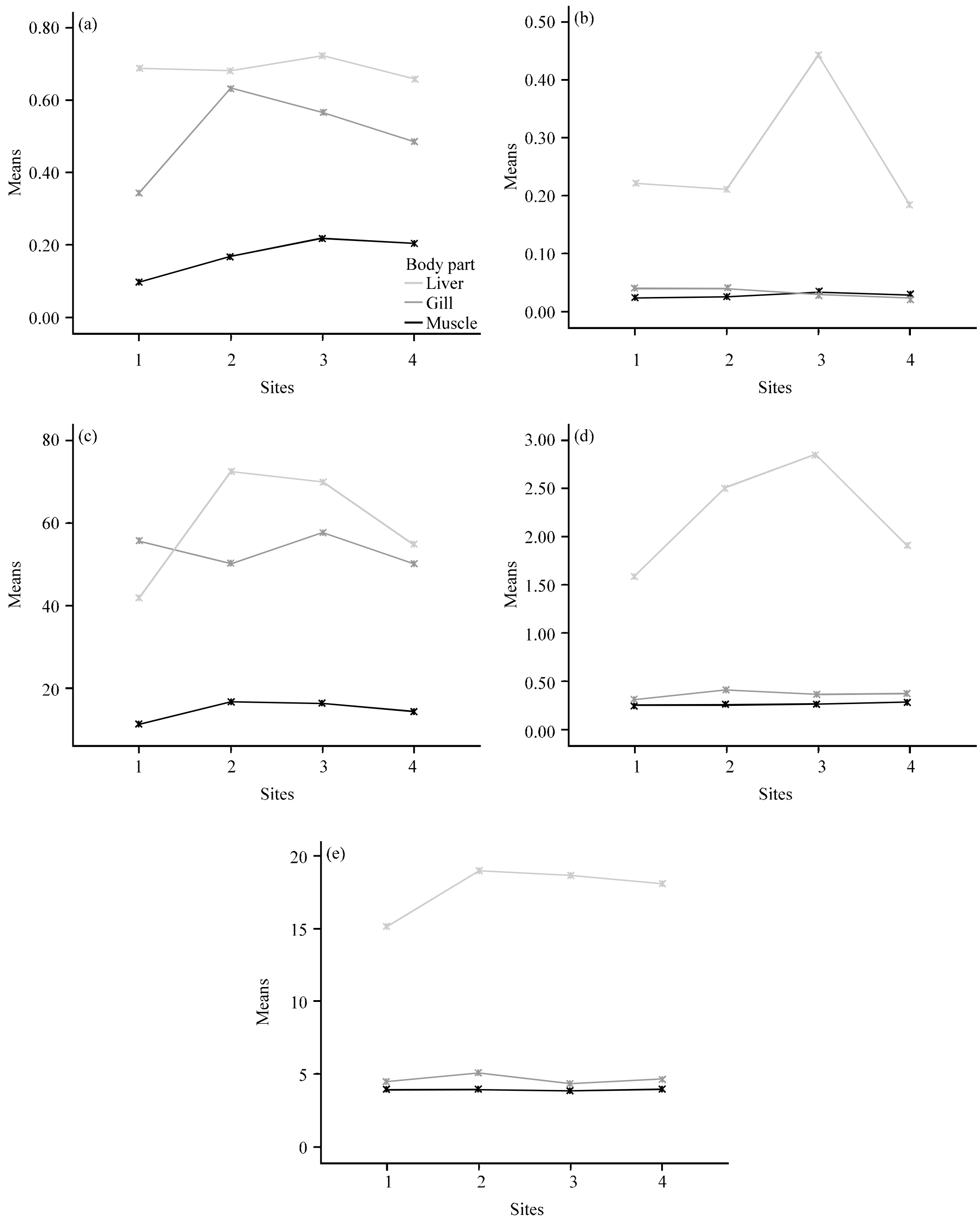

Fig. 2(a-e): Heavy metals (a) Pb, (b) Cd, (c) Zn, (d) Co and (e) Ni concentration in liver, gills and muscles of tilapia fish from four studied markets, 1: Serdang night market, 2: Bangi night market, 3: Kajang wet market and 4: Bangi wet market expressed as μg g^{-1} DW in tilapia organs; liver, gill and muscle, the results of heavy metals from four representative sampling sites were presented in Table 2-4, respectively. Concentrations of heavy metals detected in the liver, gill and muscle samples showed different capabilities for accumulating. On the other hand, there were great variations among heavy metal accumulation amount of investigated tissues. In general, among all studied parts of tilapia fish, liver accumulated and concentrated the highest concentration of all studied heavy metals which were Cd, Zn, Pb, Ni and Co. However, Zn levels in gills from Serdang night market were higher than Zn in liver and muscle in the same market. Meanwhile, fish muscles exhibited the lowest concentrations of all studied heavy metals. Similar situations were reported by some researchers (Dural *et al*., 2007; Taweel *et al*., 2011), except Cd levels in fish gill from Kajang wet market were equal with muscle level in the same market, while Cd levels in fish gill from Bangi wet market were lower than Cd in muscle from the same market. The concentrations of heavy metals which were analyzed in the muscle of tilapia fish (*Oreochromis niloticus*) were lower than the maximum permitted concentrations proposed by FAO and WHO (1984) and MFR (1985). For all the metals, significant differences at $p<0.05$ were obtained between fish liver and other studied fish body organs. In addition, the mean concentration of metals in the gills was arranged in increasing order of Zn>Ni>Pb>Co>Cd. Meanwhile, mean concentrations of heavy metals in the fish liver and muscle obtained from all markets was in the increasing order of scales ranked as follows: Zn>Ni>Co>Pb>Cd. Such ranking of metal concentrations were similar to results were obtained in fish from the Saricay, South-West Anatolia (Yilmaz *et al*., 2007).

Cadmium: The highest cadmium concentration in liver was found in fish collected from Kajang wet market (0.44 μg g^{-1} DW), while the lowest was found in Bangi wet market (0.18±0.01 μg g^{-1}). Cadmium concentrations in fish liver from this study were similar to other reported fish such as from southern Atlantic coast of Spain (Usero *et al*., 2003). While, Azmat *et al*. (2006) found Cd levels in fish were between 0.16 to 0.62 μg g^{-1}. Cd in fish muscle was not significantly different ($p<0.05$) among all sites (0.02, 0.03, 0.03, 0.03 μg g^{-1} DW), respectively, these results were similar to results (0.02 and 0.03 μg g^{-1} DW) were obtained by Usero *et al*. (2003). Cd levels in fish gill were between 0.02-0.04 μg g^{-1} DW, whereas (Bhattacharya *et al*., 2006) found Cd concentration in fish gill between 0.41-1.01 μg g^{-1} DW.

Table 2: Heavy metals concentration (μg g^{-1} DW) in liver of tilapia fish

Metal	Serdang night market	Bangi night market	Kajang wet market	Bangi wet market	Other study
Cd	0.22±0.050^{a}	0.21±0.020^{a}	0.44±0.090^{b}	0.18±0.010^{a}	0.43, 0.15[1]
Zn	41.87±09.93^{a}	72.60±17.98^{c}	70.11±11.51^{c}	54.96±07.60^{b}	*42.53[2]
Pb	0.67±0.230^{a}	0.68±0.110^{a}	0.72±0.060^{a}	0.66±0.140^{a}	01.90[3]
Ni	15.16±0.960^{a}	18.99±01.12^{b}	18.66±01.06^{b}	18.09±01.90^{b}	11.40[3]
Co	01.58±0.130^{a}	02.51±0.290^{b}	02.86±0.860^{b}	01.91±0.310^{a}	02.50[3]

Values are Mean±SD, Values in each row with the same superscript are not significantly different at $p<0.05$, 1: Usero *et al*. (2003), 2: Yilmaz *et al*. (2007), 3: Abu Hilal and Ismail (2008). *Original value that is measured on the basis of wet weight has been converted to corresponding (*) value on the basis of dry weight

Table 3: Heavy metal concentration (μg g^{-1} DW) in the gill of tilapia fish

Metal	Serdang night market	Bangi night market	Kajang wet market	Bangi wet market	Other study
Cd	0.04±0.01^{a}	0.04±0.01^{a}	0.03±0.00^{a}	0.02±0.00^{a}	0.14[1]
Zn	55.72±7.56^{a}	50.21±3.86^{a}	57.74±6.85^{a}	50.27±3.49^{a}	62.39[1]
Pb	0.34±0.01^{a}	0.63±0.15^{bb}	0.57±0.08^{cb}	0.49±0.11^{ab}	0.51[2]
Ni	04.47±0.20^{a}	05.07±0.79^{a}	04.31±0.44^{a}	04.68±0.54^{a}	*02.30[1]
Co	0.31±0.02^{a}	0.41±0.04^{a}	0.36±0.04^{a}	0.38±0.02^{a}	*0.54-108[3]

Values are Mean±SD, Values in each row with the same superscript are not significantly different at $p<0.05$. *Original value that is measured on the basis of wet weight has been converted to corresponding (*) value on the basis of dry weight, 1: Jaric *et al*. (2011), 2: Zyadah (1999), 3: Uysal *et al*. (2008a,b)

Table 4: Heavy metals concentration (μg g^{-1} DW) in muscle of tilapia fish

Metal	Serdang night market	Bangi night market	Kajang wet market	Bangi wet market	FAO and WHO (1984)*	MFR (1985)**	Other study
Cd	0.02±0.000^{a}	0.03±0.010^{a}	0.03±0.010^{a}	0.03±0.00^{a}	1 μg g^{-1}	1 μg g^{-1}	0.021
Zn	11.36±01.28^{a}	16.72±03.39^{a}	16.42±04.11^{a}	14.35±0.72^{a}	100 μg g^{-1}	100 μg g^{-1}	16[1]
Pb	0.10±0.020^{a}	0.17±0.040^{b}	0.22±0.060^{b}	0.21±0.03^{b}	2 μg g^{-1}	2 μg g^{-1}	0.17[1]
Ni	3.95±0.290^{a}	03.97±0.170^{a}	03.84±0.410^{a}	03.97±0.25^{a}	-------	------	03.60[2]
Co	0.25±0.220^{a}	0.26±0.020^{a}	0.26±0.020^{a}	0.29±0.02^{a}	-------	-----	0.27[3]

Values are Mean±SD, Values in each row with the same superscript letter are not significantly different at $p<0.05$. *World Health Organization, **Malaysian Food Regulations (values are in μg g^{-1}), 1: Chi *et al*. (2007), 2: Tuzen (2009), 3: Sivaperumal *et al*. (2007)

Zinc: Zinc concentration in fish liver varied significantly among almost all markets, the findings were 41.87, 72.60, 54.96 and 70.11, respectively as the same markets order mentioned previously whereas, Yilmaz *et al.* (2007) recorded 9.450 $\mu g\ g^{-1}$ wet wt which corresponding 42.53 $\mu g\ g^{-1}$ DW Zn level in gill and muscle from all markets not significantly varied, Zn values in muscle between 11.36 to 16.72 $\mu g\ g^{-1}$ DW whereas, Dural *et al.* (2007) reported 8.82 and 25.21 $\mu g\ g^{-1}$ DW in autumn and winter season, respectively, while (Etesin and Benson, 2007), reported that Zn levels in muscle tissues were 7.78±5.40, 11.48±0.20 and 14.00±3.12 mg kg^{-1} DW at IAS, DRN and OBG stations, respectively. Zn values in gill ranged between 50.21 and 57.74 $\mu g\ g^{-1}$ DW whereas, Dural *et al.* (2007) reported 31.01 and 51.01 $\mu g\ g^{-1}$ DW in autumn and winter seasons, respectively.

Lead: Lead values in fish liver and muscle from all studied sites were not completely statistically significant ($p<0.05$), whereas, the values in gill varied significantly between all markets, these values variations of heavy metals in gills reflect the pollution variety of water from where fish were caught which depended on environmental medias of the fish that sell in the different markets. The Pb levels in fish muscle from all sites were between 0.10-0.22 $\mu g\ g^{-1}$ DW while (Azmat *et al.*, 2006) recorded Pb in fish were 0.19-0.79 $\mu g\ g^{-1}$.

Nickel: Nickel concentration in fish liver collected from Serdang night market was the lowest 15.16 $\mu g\ g^{-1}$ DW, while (Eastwood and Couture, 2002) got this result 14.31 $\mu g\ g^{-1}$ DW, meanwhile Bangi night market, Kajang wet markets and Bangi wet market had approximately the same value of fish liver content of Ni (18.99, 18.66 and 18.09 $\mu g\ g^{-1}$ DW), respectively, while Abu Hilal and Ismail (2008) were reported this result 11.40 $\mu g\ g^{-1}$ DW in Fish from the Gulf of Aqaba red sea. Ni level in fish gill and muscle did not vary significantly among all markets.

Cobalt: Cobalt level in fish liver from Serdang Night market and Bangi Night market was significantly different ($p<0.05$) 01.58 and 02.51 $\mu g\ g^{-1}$ DW, respectively, on the other hand Co concentration in fish liver from Kajang Wet market and Bangi Wet market varied significantly 02.86 and 01.91 $\mu g\ g^{-1}$ DW, respectively. In contrary the Co levels in fish muscle were not significantly different among Serdang market, night market Bangi, Kajang market and wet market Bangi (0.25, 0.26, 0.26 and 0.29 $\mu g\ g^{-1}$ DW, respectively while (Sivaperumal *et al.*, 2007) reported 0.27 $\mu g\ g^{-1}$ DW.

CONCLUSION

This study indicated the differences in heavy metals found in liver, gill and muscle of Tilapia fish (*Oreochromis niloticus*) in four markets in Selangor Malaysia. It can be overall concluded that Cd, Pb, Zn, Ni and Co contents in muscle tissue (edible part) analyzed in this study were well below the limits set by either the FAO and WHO (1984) and/or MFR (1985) for human consumption. Zinc was the highest in liver, gills and muscle of analysed fish in all studied sites. On the other hand, cadmium was generally the lowest and the values were similar to results reported in all other previous studies. In contrast, Cd, Pb, Zn, Ni and Co in liver levels of Tilapia fish (*Oreochromis niloticus*) exceeded regulatory limits. Consumption of muscle tissue may not pose as a threat to human health and are completely known to be safe. Furthermore, consumption of Tilapia fish (*Oreochromis niloticus*) livers may pose as a human health risk. Hence, further studies on bioaccumulation of other chemicals may help elucidate the impact of contaminants on the human health.

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