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Full Length Research Paper

Heavy metals concentration in different organs of tilapia fish (*Oreochromis niloticus*) from selected areas of Bangi, Selangor, Malaysia

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The present study was aimed at investigating the six heavy metals: Pb, Cd, Cr, Cu, Ni and Zn measured in the liver, gills and muscles of tilapia fish (*Oreochromis niloticus*) which was collected from five locations around Bangi area, Selangor, Malaysia. The sites included Culture Pond A, Culture Pond B, Langat River, Cempaka Lake and Engineering Lake. The results show that in general, the highest heavy metal concentrations were detected in the liver followed by the gill and the muscle. The heavy metal concentration in the tissues varied significantly depending upon the locations from where the fish was collected. In the liver, the highest Pb was detected in the Langat River (4.8 ± 0.84 µg/g dry weight) followed by that of Engineering Lake (3.28 ± 1.15 µg/g). Copper and Ni levels were observed as the highest in the fish collected from Cempaka Lake with value of 449 ± 37.7 and 20.9±5.7 µg/g, respectively. For Cd, the highest level was detected in the fish from Engineering Lake (0.70 ± 0.17 µg/g), while the highest values of Zn was recorded in those from the Langat River (143 ± 9.8 µg/g). The metal accumulation in the liver of fish was found to be quite high in comparison to the gills and muscles (edible part). However, the concentrations of heavy metals in the muscles of fishes collected from all the sites were within the permissible levels and are safe for the human consumption and public health.

Key words: Heavy metals, tilapia fish organs, Bangi area, five sites.

INTRODUCTION

Tilapia is a fish distributed in the tropical zones around the world. It lives in fresh water and is sold in markets. The tropical freshwater fish, tilapia, is an important cultural fish because it reproduces very easily and does not have feeding problems (Kalay and Canli, 2000). The culture of tilapia has exceeded 800,000 metric tons and is ranked under carps as the second most widely farmed fish group in the world (Thomas Popma, 1999). The tilapia fish is an ideal species of organisms for an assessment study on effects of heavy metal contamination in aquaculture ponds (Mokhtar et al., 2009). Tilapia can survive in bad environmental conditions because their resistance to disease is physically powerful, and their respiratory demands are slight so that they can accept low oxygen and high ammonia levels (Zhou et al., 1998). Fish are often at the top of aquatic food chain and may concentrate large amounts of some metals from the water (Mansour and Sidky, 2002). Some metals are essential to human health. Metals are naturally occurring elements that become contaminants when human activities increase their concentrations above normal levels in the environment (Unger, 2002). Heavy metal pollution is a serious and widespread environmental problem due to their toxicity; heavy metals enter the environment through various natural methods and human activities, and can accumulate in fish and other organisms (Kalay and Canli, 2000). Fish are the final organism in the aquatic food chain and a significant food source for man. Consequentially, heavy metals in aquatic environments are transferred throughout the web chain into humans. It is well known that fish muscle is not an active tissue to accumulate heavy metals, but it was discovered that heavy metal levels in the edible portion (muscle) of some fish in contaminated regions exceeded

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permissible levels. Therefore, determination of heavy metal levels of fish is tremendously important for the health of human beings (Uysal et al., 2008). Metals are very toxic because, as ions or compound form, they are soluble in water where the fish live and may be easily absorbed into the fish and bind to structural proteins and enzymes. In humans, some metals can cause severe physiological and health effects (Wayne and Ho, 1999). Humans are exposed to different levels of heavy metals directly from the water, air and food. Fish consumers may be exposed to relatively higher levels of heavy metals by eating heavy metals contained fish from local rivers, ponds lakes and seas. Edible fish are often contaminated with heavy metals as a result of agricultural technology, industrial pollution, sewage drainage and other sources, which could affect human health and cause chronic diseases (Zyadah and Abdel-Baky, 2000).

Heavy metals such as cadmium, zinc, mercury, chromium and copper cause heavy pollution, particularly in the ponds, lakes and river systems in zones affected by effluents released from industries, sewage and agricultural drains. Among the animal species, fish are inhabitants that cannot escape the detrimental effects of these pollutants (Basha and Rani, 2003). The concentration of the essential elements like Zn and Cu were relatively higher in the muscle and liver tissues than the non-essential metals like Pb and Cd (Etesin and Benson, 2007). Different fish tissues can take heavy metals from surrounding environment, making natural lakes and rivers major sources of fish contamination by heavy metals. This allows humans to be exposed to pollution. Heavy metals were studied through three different fish organs because of the affinity between each of them. Rivers, ponds and lakes are major sources of drinking water. However, the pollution of these natural waters is one of the most critical environmental problems in recent years (Almeida et al., 2002). The Department of Fisheries, Malaysia reported that the production of farming aquaculture products in 2009 was about 472,306.74 tonnes (Malaysia, 2009). Aquaculture production (in metric tons): total (including freshwater), in 2000 in Malaysia was 167,898 in Asia (excluding the Middle East) it was 41,305,773 and in the world it was 45,715,559 (EarthTrends, 2003). The aquaculture industry in Malaysia is growing quickly, particularly that of the production of freshwater aquaculture fish (Ibrahim et al., 2010). The purpose of the present study was to determine some heavy metals concentrations in three different organs of tilapia fish in five sites in the Bangi area. The target of the present study was to measure the concentration of six heavy metals: Pb, Cd, Cr, Cu, Ni and Zn in the liver, gills and muscles of the tilapia fish and make a comparison of their concentration between natural and industrial cultured ponds sites.

MATERIALS AND METHODS

Five locations in Bangi area, Selangor (Malaysia) used for this study included: Culture Pond A which is situated at 02° 59' northern latitude and 101° 52' eastern longitude, located in Semenyih town, Bt14; Culture Pond B which is situated at 03° 00' northern latitude and 101° 53' eastern longitude, located in Semenyih town, Bt18; Langat River which is situated at Bangi town; Cempaka Lake which is situated at 02° 57' northern latitude and 101° 46' eastern longitude, located in Bangi town and Engineering Lake which is situated at 02° 55' and 02° 56' northern latitude and 101° 47' eastern longitude, located at University Kebangsaan Malaysia, Bangi Town (Figure 1).

Sample collection

Three tilapia fish samples were collected randomly from each location, using fishing net to catch the fish from natural sites no. 3, 4 and 5, while at site 1 and 2, the fish were bought from workers on site. The fish length ranged between 20 and 22 cm and the weight ranged between 220 and 240 g. The fish were placed in clean polyethylene bags with ice and immediately taken to laboratory where the samples were deep frozen at -20℃ untill prepared for analysis.

Samples analysis

The samples were taken from -20℃ and were thawed at room temperature, then dissected for analysis using stainless steel scalpels. The liver, gills and muscles on the dorsal surface of the fish were dried in an oven at 80℃ for two days until they reached a constant weight. The samples were then removed from the oven and allowed to cool. Each dried sample was ground using a porcelain mortar and pestle. A half gram dry weight of the powdered form of muscle and gill and 0.1 g dry weight of liver (because liver is small as compared to the gills and muscles in fish body) compared in duplicate were digested using closed vessel microwave digestion in a microwave oven (Milestone model Start D, Italy). The samples were digested by adding 3 ml of nitric acid (65%) and 1 ml hydrogen peroxide (35%) (Taghipoura and Aziz, 2010). Hydrogen peroxide was added to the nitric acid as it reduces nitrous vapors and accelerates the digestion of organic matters by raising the temperature (Dig-Acids, 2001). The microwave was adjusted for 20 min at 150℃ and left for 35 min to cool in the microwave until they reached room temperature. The samples were then transferred to clean volumetric flasks, and diluted to 50 ml for muscle and gill and 25 ml for liver samples with deionized water. Then the samples were filtered using Whatman filter paper (0.45 µm). Concentrations of Pb, Cd, Cr, Cu, Ni and Zn were then determined using an inductively coupled mass spectrometer (model ELAN 9000 Perkin Elmer ICP-MS, USA). The operational parameter settings were used in ELAN 9000 Perkin Elmer as shown in Table 1. All the plastics and glassware were washed in nitric acid for 15 min and rinsed with deionized water before being used.

Statistical analysis

Statistical analysis were done using a computer program SPSS version 15 and two way ANOVA, and the significance was reported at $P < 0.05$ levels.

RESULTS AND DISCUSSION

The concentration of heavy metals (µg/g dry tissues) in the liver, gills and muscles of the tilapia are summarized in Tables 2, 3 and 4, respectively. Pb, Cd, Zn, Ni, Cr and

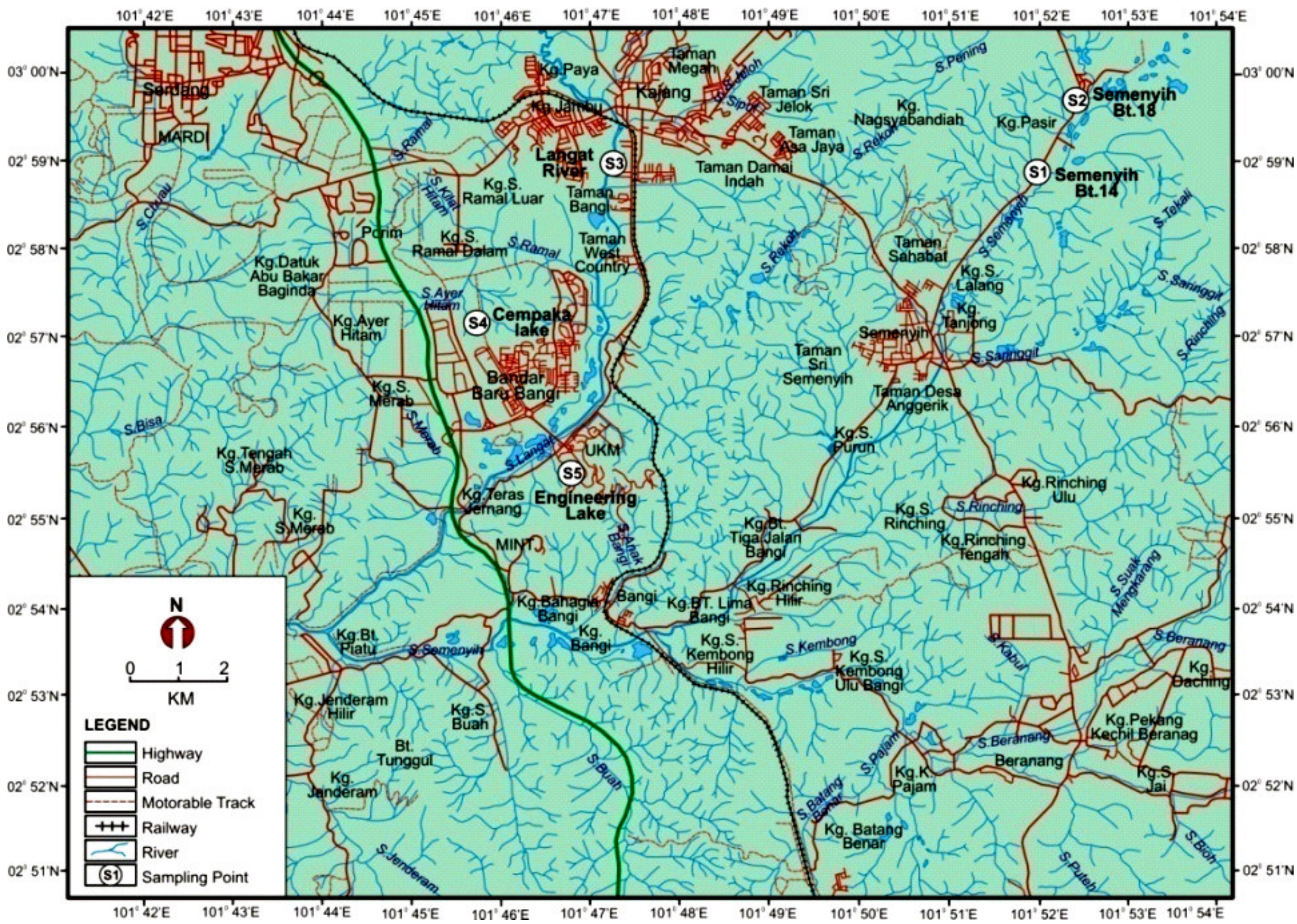

Figure 1. Location of sampling sites (site 1: Culture Pond A, Semenyih town; site 2: Culture Pond B, Semenyih town; site 3: Langat River, Bangi town; site 4: Cempaka Lake, Bangi town and site 5: Engineering Lake, Bangi town.

Table 1. Operational parameter settings used in ELAN 9000 Perkin Elmer.

Characteristic	Instrument condition
RF generator	40 MHz
RF power	1000 W
Spray chamber	Ryton Scott
Nebulizer	Cross-flow
Plasma gas flow	15.0 L/min
Auxiliary gas flow	1.0 L/min
Nebulizer gas flow	0.60 L/min
Sampler and skimmer cone	Nickel

Cu were detected in all the samples. The heavy metals levels in fish tissue from the river and lakes were higher than the culture pond locations, and they are significantly different. The distribution patterns of Pb, Cd, Zn, Ni, Cr and Cu in the three organs of the tilapia followed the order: liver > gills > muscles (Chi et al., 2007). Mean concentration in the muscle in Culture Pond A, Culture Pond B, Langat River, Cempaka Lake and Engineering Lake were as follows: Zn > Cr > Ni > Cu > Pb > Cd; Zn > Cr > Ni > Cu > Pb > Cd; Zn > Cu > Cr > Ni > Pb > Cd; Zn > Cr > Cu > Ni > Pb > Cd; Zn > Cr > Ni > Cu > Pb > Cd, respectively. Pb concentration in liver at Langat River was the highest (4.8 ± 0.84 μg/g) followed by Engineering Lake (3.28 ± 1.15 μg/g) and the lowest levels were at Culture Ponds A and B (0.54 ±.15 and 1.4 ± 0.40 μg/g), respectively. The Pb content seen in fish liver collected

Table 2. Heavy metals concentration (µg/g dry wt) in liver of the tilapia fish (*Oreochromis niloticus*) in five sites (mean value ± standard deviation).

Metal	Pond A	Pond B	Langat River	Cempaka Lake	Engineering Lake	Other study
Pb	0.54±0.1[a]	1.40 ±0.40[b]	4.80 ±0.84[c]	2.50 ±0.47[d]	3.28 ± 1.15[e]	0.60 µg/g[1]
Cd	0.09±0.0[a]	0.22 ± .06[b]	0.21 ± .04[b]	0.46 ± 0.13[c]	0.70 ± .17[d]	0.52 µg/g[1]
Zn	74 ± 22[a]	108 ± 13[b]	143± 9.80[c]	112 ± 42[d]	123 ± 40[e]	85 µg/g[1]
Ni	11 ± 1.30[a]	10 ± 1.14[a]	14 ± 2.30[b]	20.90±5.70[c]	16 ± 5.80[d]	-------------
Cr	18 ± 1.10[a]	18.50±1.1[a]	20.3±0.73[b]	20 ± 1[b]	20.60 ±0.88[b]	-------------
Cu	397 ±114[a]	434 ± 97[b]	329 ± 45[c]	449 ± 37[d]	332 ± 67[c]	3.90 µg/g[1]

Results show mean ± SD. Values in each row marked by the same superscript letter are not significantly different at P<0.05. The superscript numbers mean: 1 is Low et al. (2010).

Table 3. Heavy metal concentration (µg/g dry wt) in the gills of tilapia fish (*Oreochromis niloticus*) in five sites (mean value ± standard deviation).

Metal	Pond A	Pond B	Langat River	Cempaka Lake	Engineering Lake	Other study
Pb	0.23±0.0[a]	0.22 ±0.08[a]	1.50±0.23[b]	1.50 ±0.59[b]	1.10 ± 0.54[c]	1.3 µg/g[1]
Cd	0.03±0.0[a]	0.03 ± .01[a]	0.03 ±.01[b]	0.03 ±0.00[b]	0.04 ±.01[c]	0.08 µg/g[1]
Zn	79 ± 07a	71 ± 2.60[b]	82 ± 8.10[c]	92 ± 04[d]	87 ± 05[e]	70 µg/g[1]
Ni	3.50±0.4[a]	3.80 ± .27[b]	4.70± 0.67[c]	5.00 ± 0.53[d]	4.70 ± 0.37[c]	4.04 µg/g[2]
Cr	5.34±0.2[a]	5.46 ± 0.37[a]	4.86±0.20[b]	4.80 ±0 .49[b]	4.70 ± 0.31[b]	----------------
Cu	7.12±0.7[a]	6.18 ±1.55[b]	5.96 ±1.29[c]	7.25 ±2.78[d]	3.91 ±0.89[e]	1.6 µg/g[1]

Results show mean ± SD. Values in each row marked by the same superscript letter are not significantly different at P<0.05. The superscript numbers mean 1 is Low et al. (2010) and 2 Shen et al. (1998).

Table 4. Heavy metals concentration (µg/g dry wt) in muscles of tilapia fish (*Oreochromis niloticus*) in five sites (mean value ± standard deviation).

Metal	Pond A	Pond B	Langat River	Cempaka Lake	Engineering Lake	Other study	Limited level
Pb	0.11±0.01[a]	0.10 ±0.01[a]	0.18 ±0.04[b]	0.15 ±0.01[c]	0.14 ± 0.05[d]	0.09 µg/g[1]	6 µg/g[6]
Cd	0.01±0.00[a]	0.01 ± .00a	0.02 ± .00b	0.03 ±0.00c	0.03 ±.00c	0.02 µg/g[2]	0.2 µg/g[7]
Zn	31 ± 2.80[a]	29 ± 1.60[a]	33 ± 5.30[b]	37 ± 06[c]	45 ± 08[d]	70 µg/g[2]	100 µg/g[8]
Ni	2.80±0.25[a]	2.70 ± .17[a]	3.00 ±0.22[b]	3.20 ±0.33[c]	3.00 ± 0.43[b]	2.3 µg/g[3]	---
Cr	6.21±0.60[a]	6.10 ±0.29[a]	5.92 ±0.32[b]	6.00 ± .15[b]	5.70 ± 0.22[c]	30.08 µg/g[4]	50 µg/g[8]
Cu	2.65±0.76[a]	2.33 ±0.16[a]	5.47 ±0.49[b]	3.40 ±.74[c]	2.36 ±0.40[a]	3.06 µg/g[5]	30 µg/g[9]

Results show mean ± SD. Values in each row marked by the same superscript letter are not significantly different at P<0.05. The superscript numbers mean 1 is Low et al. (2010), 2 is Al-Weher (2008), 3 is Swaibuh Lwanga et al. (2003), 4 is Mansour and Sidky (2002), 5 is Al-Kahtani (2009), 6 is Kong (1987), 7 is Authority (1999), 8 is MFR (1985) and 9 is FAO/WHO (1987).

from Pond A (0.54 µg/g) is closer to the results reported by others (Low et al., 2010) which was 0.61 µg/g, while the results from Pond B was higher (1.4 µg/g). Also, the Pb concentration in the muscles was almost the same, except that Ponds A and B had the lowest concentration (0.11 and 0.10 µg/g, respectively) as compared to the study by Low et al. (2010) which showed that the concentration was 0.09 µg/g. The highest Cd level of the liver tissue was found in Engineering Lake (0.70 ± 0.17 µg/g) followed by Cempaka Lake (0.46 ± 0.13 µg/g), while Low et al. (2010) found 0.70 µg/g, whereas the highest Cd value in muscles tissue was 0.03, 0.03 and 0.02 µg/g in the fish that were taken from Cempaka Lake, Engineering Lake and Langat River, respectively which approximately is the same result (0.02 µg/g) with the one reported by Al-Weher (2008). The Zn and Ni concentration in all fish organs were the highest in the natural sites (Engineering Lake, Cempaka Lake and Langat River), while the lowest was in the cultured ponds as shown in Tables 2 to 4. The Cu concentration in fish muscles at Langat River was the highest (5.47 ± 0.49 µg/g) as compared to other sampling sites followed by

Cempaka Lake (3.4 ± 0.74 µg/g) which has similarity with the result recorded by Al-Kahtani (2009), which was 30 µg/g. In general, the heavy metal levels varied significantly in different tissues of tilapia fish and different sites, particularly between natural sites and cultured ponds. The concentration of heavy metals in fish collected from natural river and lakes is higher than that collected from cultured ponds due to the fact that natural water sources are more exposed to contamination more than controlled artificial ponds. The gills and liver were chosen as important organs for assessing metal accumulation. The levels of heavy metals in the gills reflect the concentrations of metals in the waters, where the fish live, while the concentrations of metals in liver represent storage of metals in the fish body (Roméo et al., 1999). The present study provides valuable information, and it shows that tilapia fish from different sites exhibited higher level of studied heavy metals which were detected in the liver and the lowest level was seen in muscle tissue. These results are consistent with what has been reported by others (Yilmaz et al., 2007). The concentration of heavy metals in all fish organs did not exceed that which is allowed for human consumption. All results were well below the limits for fish proposed by EU (2001), FAO/WHO (1987) and MFR (1985).

Conclusion

This study shows that the concentration of heavy metals in different tilapia fish organs is still below the allowable limits in both natural and cultured ponds sites. In the cultured ponds sites, there are clearly less heavy metals concentrations than in the natural sites. The higher level of these heavy metals is in the liver in all sites as compared to other organs. The level of metals in the edible organ (muscle) was lower than the acceptable value for human consumption from all locations mentioned in this study.

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