HEAVY METALS CONCENTRATIONS IN CATFISH *(CLARIUS GARIEPINUS)* FROM THREE DIFFERENT FARMS IN SARAWAK, MALAYSIA

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ABSTRACT: *In Malaysia, aquaculture industry has increased significantly in number due to the demand of freshwater fish by the consumers. This study was conducted to determine the accumulation of some heavy metals such as Manganese (Mn), Zinc (Zn) and Lead (Pb) in liver, gills and muscles of Catfish (Clarius gariepinus) from three different farms in Sarawak, Malaysia. The fish farming cage system include polytanks, concrete tanks and earthen pond. This study showed the current condition of catfish from aquaculture farms in Sarawak, Malaysia. The samples were digested in concentrated* HNO_3 *and* H_2O_2 *and subjected to flame atomic absorption spectrometer for heavy metals analysis. There were differences in the concentrations of studied heavy metals between different organs and between different farms. The study revealed that the highest concentration of Zn and Pb were obtained in liver of catfish from three different farms. The highest Zn and Pb level were found in liver of catfish from Farm C (earthen pond) with mean concentration 156.6000 and 55.6500 mg/Kg respectively. High concentration Mn was found in gill of catfish from all studied farm whereas muscle showed the lowest concentration of all studied metals. This study revealed that high metal concentration in catfish tissues was from Farm C which is earthen pond. Based on the Malaysian Food Regulation, level of Zn in muscle of catfish did not exceed the permissible limit set by Malaysian Food Regulation whereas level of Pb exceeds the permissible limits set by Malaysian Food Regulation, WHO and Chinese national standards. Meanwhile, the level of Mn concentration in the fish muscles from all farms, exceed the permissible limit set by WHO. Therefore, the consumption of catfish by human should be monitored to avoid health problems caused by heavy metals. The finding indicates that catfish (Clarius gariepinus) is very prone to heavy metal accumulation.*

Keywords: Heavy Metals, Catfish, Fish Farm

1. INTRODUCTION

Heavy metal is a toxicant, commonly found in aquatic environment. In an aquatic environment, toxic metals naturally presence at low level, but level of heavy metals have increased caused by anthropogenic pollutants, [1]. Heavy metals may enter into aquatic ecosystem through various sources either natural or anthropogenic sources. Heavy metals content in upper member of the food web can reach values many times higher compared to those found in aquatic environment or in sediments. Contamination of heavy metal in the region is a significant issue due to the health of the aquatic animals and in turn, health of seafood consumers, [2].

Fish species has been used as bio-indicators in many studies to study heavy metal pollution in the environment, [3,4]. Fish is considered an important bio indicator to monitor level of pollutants in aquatic ecosystems, due to they are at high trophic level and essential source of protein in human diet, [4]. These bio indicators are testified in the form of their changes such as biochemical, physiological or behavioral due to exposure and bioavailability of pollutants that are presence in the surrounding environment, [5]. Monitoring contamination of fish tissue allows us to identify any toxic contaminants in fish that may be dangerous to consumers, and proper action can be taken to protect public health and the environment, [6]. Therefore, it is significant to study the bioaccumulation of heavy metals in fish at various fish organs to ensure that high heavy metals content are not transmitted to human through consumption of fish, [7]. The objectives of this study were to determine the concentration of manganese (Mn), zinc (Zn) and lead (Pb) in major organs of catfish, to compare heavy metal content in major organs of catfish (*Clarius gariepinus*) from three local farmed fish pond and to assess the current aquaculture catfish condition which can lead to two conclusions either the catfish from aquaculture contains unacceptable level of heavy metal or catfish are safe for human consumption.

2. METHODOLOGY

2.1. STUDY AREA AND SAMPLING

Catfish (*Clarius gariepinus*) of about the same weight and length were collected from three different aquaculture pond in Sarawak, Malaysia. Each sampling site has different rearing system. The catfish was grown in poly tanks in Farm A, concrete tanks in Farm B and earthen ponds in Farm C. Total length and total weight of catfish *(Clarius gariepinus)* were from 36 to 40 cm and from 300 to 350 g, respectively. Catfish collected during sampling period was individually wrapped with plastic bag, sealed and labeled. Then, all samples were preserved immediately on ice and transported to the laboratory on the same day.

2.2. HEAVY METAL ANALYSIS

Catfish was rinsed with ultrapure water before dissection. They were dissected by using stainless steel scalpels and Teflon forceps. Liver, gill and muscle were removed from fish body and dried in an oven at 70 ^{0}C until a constant weight was obtained. The dried samples were then grounded into powder by using mortar and pestle.

Approximately, 0.1 gram of dried fish tissue sample was placed into conical flask. Then, 6 ml of 65 % nitric acid (HNO_3), and 2 ml of 35 % hydrogen peroxide (H_2O_2) were added for acid digestion. The sample was heated on hot plate until the sample solution became clear. Then, the sample solution was allowed to cool at room temperature and was filtered by using filter paper. The filtrate was then transferred into a 25 ml volumetric flask and diluted to the mark with ultrapure water. Each sample was prepared for three replicates, [8]. Blank samples were also processed in the same way to avoid possible contamination during analysis. Samples were subjected to flame atomic absorption spectrometer (AA-7000, SHIMADZU) to analyze for Mn, Zn and Pb. Stock standard solutions (1000 mg/L) of each element were used to prepare calibration solutions to obtain calibration curves.

To avoid any possible contamination, all the glassware were kept in 10 % nitric acid (HNO_3) solution for overnight, rinsed with ultrapure water and air dried before use. The accuracy of the analysis was checked with blanks and quality control samples made up of standard solutions. Recovery studies of metal determination were conducted to demonstrate the efficiency of the method and to determine the accuracy of applied analytical procedures. The percentage recoveries were 99.44 % for Mn, 105.17 % for Zn and 98.24 % for Pb. The results were recorded as means ± standard deviation.

3. RESULTS

The concentration of Manganese (Mn), Zinc (Zn) and Lead (Pb) in organs of catfish *(Clarius gariepinus)* from three different farms were summarized in Table 1.

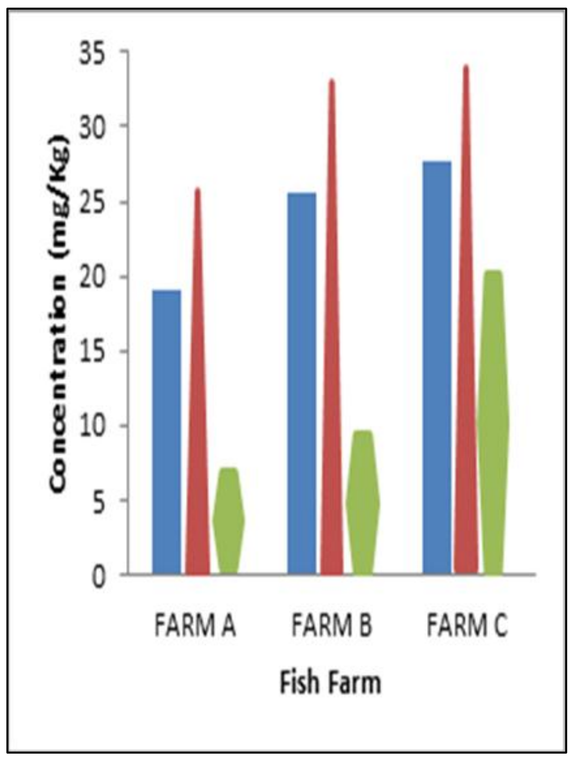
Table 1: Mean concentration of Mn, Zn and Pb (mg/Kg) with standard deviations (SD) in liver, gill and muscle of Catfish (*Clarius gariepinus*) from different fish farm, Sarawak, Malaysia.

Types of Farm	Tissue	Elements (mg/Kg)		
		Mn	*Zn*	*Pb*
FARM A (Polytanks)	Liver	19.0562 ± 1.4312	102.2000 ± 2.3062	18.1000 ± 4.0029
	Gill	25.3937 ± 2.4437	94.9000 ± 2.3250	16.7500 ± 5.7750
	Muscle	6.4000 ± 0.5405	35.2500 ± 1.6437	9.4500 ± 1.1149
FARM B (Concrete tanks)	Liver	25.5250 ± 0.7125	135.5250 ± 6.0875	34.5500 ± 5.2480
	Gill	32.1562 ± 0.2437	119.2500 ± 0.6703	33.8750 ± 7.2221
	Muscle	9.2200 ± 0.2550	81.7000 ± 1.2312	31.7750 ± 8.1876
FARM C (Earthen pond)	Liver	27.6875 ± 0.1000	156.6000 ± 1.5062	55.6500 ± 4.8495
	Gill	33.1000 ± 0.2375	95.3750 ± 1.4862	40.0500 ± 9.2513
	Muscle	19.6312 ± 0.1062	38.4250 ± 1.5000	17.5000 ± 8.2057

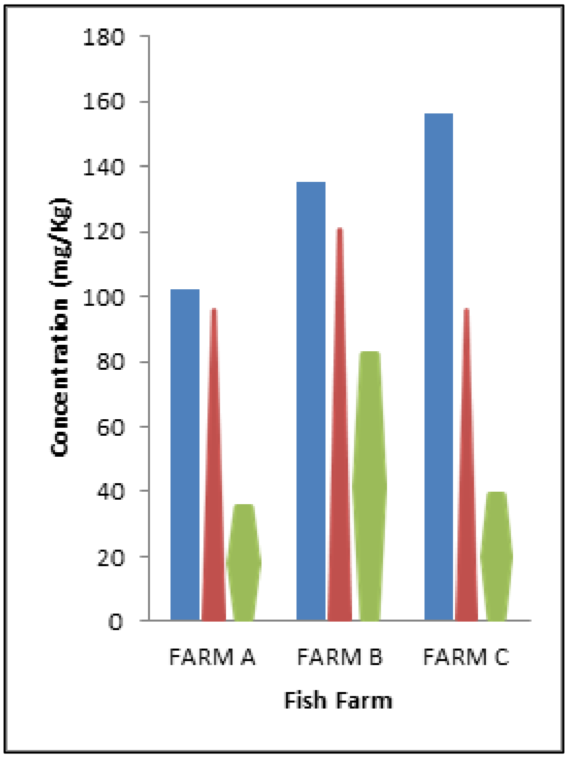
Although samples were grouped into approximately similar length and weight, concentration of heavy metal in their organs were varies significantly between farms. The ranges for mean concentration of Mn, Zn and Pb in catfish from different farmed fish pond were 6.4000-33.1000, 35.2500-156.6000 and 9.4500-55.6500 (mg/Kg) (DW) respectively.

The concentration of Mn in fishes were in the ranged of 6.4000 to 33.1000 mg/kg. The highest concentration of Mn was observed in the gill of catfish from Farm C which was from earthen pond with mean concentration of 33.1000 ± 0.2375 mg/Kg. The lowest concentration of Mn was found in muscle of catfish from Farm A (Polytanks) with mean concentration of 6.4000 ± 0.5405 mg/Kg. The findings showed that the highest concentration of Mn was found in gill of catfish from all three studied fish farms compare to other organs. The concentrations sequence of Mn were observed the highest in gill, followed by liver and the lowest in muscle as shown in Figure 1(a).

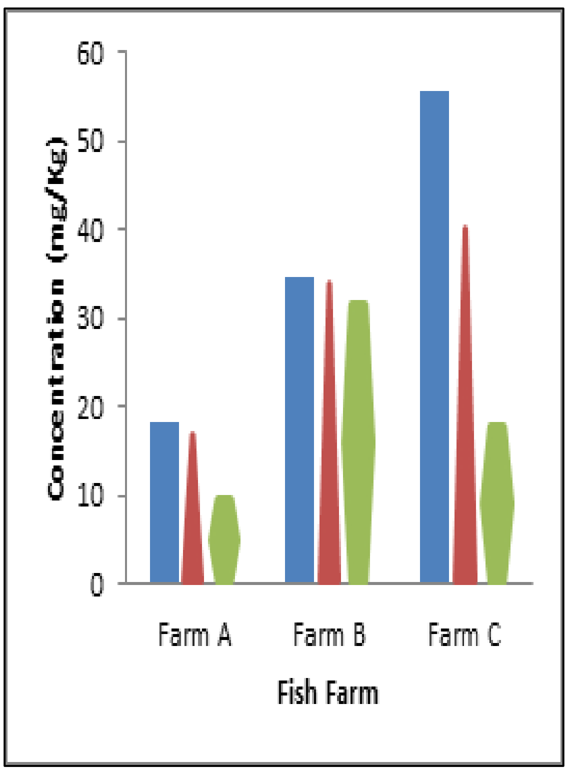
Meanwhile, the concentration of Zn was found in the range of 35.2500 to 156.6000 mg/Kg. The highest concentration of Zn was recorded in liver of *Clarius gariepinus* from Farm C which was from earthen pond with mean concentration of 156.6000 ± 1.5062 mg/Kg. The minimum concentration of Zn was observed in muscle of catfish from Farm A (Polytanks) with mean concentration of 35.2500 ± 1.6437 mg/Kg. In gill of *Clarius gariepinus*, high level of Zn was found in catfish from Farm B (Concrete tanks), followed by Farm C (Earthen pond) and the lowest from Farm A (Polytanks) with mean concentration of 119.2500, 95.3750 and 94.9000 mg/Kg respectively. The data showed that, the fish liver accumulated the highest concentration of Zn, followed by gill and muscles accumulated the lowest as shown in Figure 1(b).



(a) Manganese, Mn



(b) Zinc, Zn



(c) Lead, Pb

Figure 1: Distribution of the concentration (mg/Kg) of (a) Mn, (b) Zn and (c) Pb in different organs of (*Clarius gariepinus*) from different fish farm in Sarawak, Malaysia.

The concentration of Pb in this study varied from 9.4500 to 55.6500 mg/Kg. The highest concentration of Pb was observed in the liver of catfish from Farm C which was from earthen pond with mean concentration of 55.6500 ± 4.8495 mg/Kg. The lowest concentration of Pb was found in muscle of catfish from Farm A (Polytanks) with mean concentration of 9.4500 ± 1.1149 mg/Kg. There was no significant difference in concentration of Pb in catfish from Farm B (concrete tanks) in liver, gill and muscle with mean concentration of 34.5500, 33.8750 and 31.7750 mg/Kg respectively. The concentrations sequence of Pb found in all test organs used in this study were as shown in Figure 1(c).

In this study, the highest concentration of the studied elements in catfish tissues was Zinc (Zn), followed by Lead (Pb) and the lowest was Manganese (Mn). Among the three different fish farms, the highest Mn, Zn and Pb content were found in catfish from Farm C which was from earthen pond and followed by catfish from B which was from concrete tank. The lowest concentration of all studied metals was found in catfish from Farm A which was from polytanks.

4. DISCUSSIONS

4.1. HEAVY METAL LEVELS IN *CLARIUS GARIEPINUS* ORGANS AND COMPARISON WITH LITERATURE DATA

The highest concentration of Zn and Pb level were found in liver of catfish from Farm C with mean concentration 156.6000 and 55.6500 mg/Kg respectively as shown in Table 2. The result is expected as the liver has higher tendency for heavy metal bioaccumulation due to the presence of metallothioneins binding proteins and free protein-thiol group content that forms strong fixation with heavy metals, [7]. Since liver is highly active in the uptake, storage and detoxification of metals, it is considered as potential biomonitoring for metal pollution in the water due to the concentration of metal in liver are proportional to those that are present in the environment, [9].

The highest concentration of Mn was found in gill of catfish from all three studied fish farms as shown in Figure 1. The highest Mn level was found in gill of catfish from Farm C with mean level of 33.1000 mg/Kg. This agrees with other study who recorded the highest Mn concentration in gill than other organs of fish, [10]. Fish gills are exposed directly to surrounding water body. Fish gills consist of filaments and lamellae that provide large surface are for further facilitates the adsorption of metals onto the surface of gills during process of osmoregulation and respiration, [7]. Heavy metal concentration in fish gills can represent the immediate concentration of metals in the water in which the fish live, [11,12]. All studied metal were found the lowest in the muscle of studied catfish. This agrees with other studies whose recorded the lowest metal concentration was found in muscle of fish compare to other organs, [10,13]. Low concentration of metals in fish muscle compared to other organs may reflect the presence of low binding proteins in the muscle, [9].

The findings of the present study indicate that the examined fish were contaminated by Pb. Lead is an ubiquitous pollutant, and it may enter into the fish farm from various ways. For Farm A and Farm B, which are polytanks and concrete tanks respectively, the source of Pb may from poor conditions of fish farms and fish feeding that most likely has been contaminated by Pb. These agreed with other findings that show that high level of Pb was detected in catfish tissues fed with chicken waste, [14]. For Farm C which is rural pond also known as earthen pond, Pb may enter into the fish farm through runoff of Pb waste water from neighboring industries.

Table 2: Comparison of Heavy Metals Concentration in Fish with Literature Data and Food Safety Guidelines.

Area	*Organ*	Element (mg/Kg)			*Ref.*
		Mn	*Zn*	*Pb*	
Farm A (Poly tanks)	Liver	19.0562	102.2000	18.1000	This Study
	Gill	25.3937	94.9000	16.7500	
	Muscle	6.4000	35.2500	9.4500	
Farm B (Concrete tanks)	Liver	25.5250	135.5250	34.5500	This Study
	Gill	32.1562	119.2500	33.875	
	Muscle	9.2200	81.7000	31.7750	
Farm C (earthen pond)	Liver	27.6875	156.6000	55.6500	This Study
	Gill	33.1000	95.3750	40.0500	
	Muscle	19.6312	38.4250	17.5000	
Swamp Eel *(M. albus)* (Kelantan Malaysia)	Liver	-	94.9200	29.5500	Sow et (al. (2012
	Gill	-	98.3300	68.6900	
	Muscle	-	59.3100	22.7300	
Wels Catfish *(S. glanis)* (Danube River, Serbia)	Liver	4.5700	93.1400	0.0670	Jovičić et al., (2015)
	Gill	14.9800	80.4200	0.2360	
	Muscle	0.6700	19.6200	0.0060	
Malaysian Food Regulation		-	100	2	MFR (1985); Sow et al., (2012)
Chinese National Standard		-	-	0.5	Zhang et al., (2014)
WHO		0.5	-	2	Shaapera et al., (2013); Yap et al., (2015)

-: Not Studied; MFR: Malaysian Food Regulation

The high concentration of Mn, Zn and Pb in fish from all three studied fish farms might due to the use of chemicals in fish farming. Chemical additives are commonly used in aquaculture industry to promote growth, and other purposes such as health management, manipulation of reproduction, weed control and so on, [15]. Another factor that contributes to high level of metal in fish is unhygienic conditions of pond and tanks and types of fish feeding, [14]. This study revealed that high metal concentration in catfish tissues was found in Farm C which is an earthen pond. Earthen pond has bigger potential for heavy metal pollution due to water runoff and leachate from neighboring industries namely farmland. Runoffs from municipal activities such as illegal waste dumping area near the fish pond also contribute to high heavy metal content in fish farm. Therefore, the high metal content in all three studied farm catfish shows that catfish (*Clarius gariepinus*) tolerates harsh environmental conditions. This agrees with other studies that show that catfish grows well in harsh environment and poor water quality, [12,14,16].

4.2. COMPARISON WITH FOOD SAFETY GUIDELINES

When comparing the data with the permissible limit set by food safety guideline, the level of Pb in all parts of studied catfish exceeds the permissible limit set by Malaysian Food Regulation, WHO and Chinese national standards. The level of Pb in swamp eel *(Monopterus albus)* collected from paddy field in Kelantan also showed high level of Pb in liver, gill and muscle, which were 29.5500, 68.6900 and 22.7300 mg/Kg respectively and exceeds the permissible limit of food safety guidelines, [13]. Meanwhile, the level of Mn concentration in the fish muscles of all farms, exceed the permissible limit set by WHO [17]. When comparing the data with the value set by Malaysian Food Regulation, the level of Zn concentration in the fish muscles from all farms, did not exceed the permissible limit. On the other hand, concentration level of Zn in liver and gill exceed the permissible limit. The recommended limit of Zn proposed by Malaysian Food Regulation is 100 mg/Kg [18]. In this study, the fish muscle was the main organ focused since it is the major contribution in human diet. When comparing with food safety guidelines, some metals such as zinc were presented at safe concentration in muscle of catfish; other heavy metals such as lead and manganese were presented and exceed the permissible limit. High levels of heavy metals may cause toxic effects on humans. Hence, the consumption of catfish muscle should be monitored to avoid any serious health problems causes by heavy metals.

5. CONCLUSIONS

It can be concluded that, different fish organs showed significant difference in accumulation of heavy metals which reflects that different organs of fish have different metabolic level on heavy metals. In this study, the highest concentration of studied elements in catfish tissues was zinc (Zn), followed by lead (Pb) and the lowest was manganese (Mn). Among the three fish farms, the highest concentration of all studied metals was found in catfish from Farm C which was from earthen pond. The level of Zn in muscle of fish is lower than the permissible limit set by Malaysian Food Regulation. The level of Pb exceeds the permissible limits set by Malaysian Food Regulation, WHO and Chinese national standards, whereas the level of Mn concentration in the fish muscle exceed the permissible limit set by WHO. Therefore, the consumption of this fish should be monitored to avoid any serious health problems causes by heavy metals. In conclusion, catfish *(Clarius gariepinus)* is prone to heavy metals accumulations.

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