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Heavy Metal Accumulation in Commercially Important Fishes of South West Malaysian Coast

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ABSTRACT
Now-a-days, bioaccumulation of toxic metals in aquatic animals causes serious threats to the human health when they are consumed. Thus the detection of toxic elemental concentration in aquatic flora and fauna has attracted various researches to determine their toxic levels in organism's edible parts. Upon considering this issue, the accumulation of some heavy metals such as Mercury (Hg), Arsenic (As), Lead (Pb) and Zinc (Zn) in Muscle and gill tissues were determined in commercially important fishes. Five species (*Nemipterus japonicas, Chirocentrus dorab, Lutjanus sebae, Otolithes ruber* and *Pampus argenteus*) were collected from south west coast of Malaysia, covering 3 states (Johor, Melaka and Negeri Sembilan). Metal concentration was determined using Inductively Coupled Plasma Mass Spectrometer (ICP-MS). In general, higher metal accumulation was detected in gill tissues than the muscle tissue of selected fishes while Hg concentration was higher in muscle tissues except in *Pampus argenteus*. Similar observation was noted in As and Pb accumulation in *N. japonicus* and *L. sebae*, respectively. Hg and As concentration was higher in *P. argenteus* muscle and gill tissues on the other hand higher Pb and Zn level was noted in Muscle tissues *L. sebae*. Higher concentration of Pb and Zn were detected in gill tissues of *L. sebae* and *O. ruber*, respectively. There was no species specific differences in metal accumulation were noted ($p<0.05$). It was also observed that essential metal level in fish samples were greater than non-essential toxic metals. The metal concentrations found in this study were lower than the national and international standard maximum permissible limits for human consumption. Therefore, no public health problem would be raised in the consumption of these fishes.

Key words: Metal accumulation, biomagnification, mercury concentration, ICP-MS analysis, estimation of weekly intake (EWI)

INTRODUCTION
The contamination of fresh and marine waters with a wide range of pollutants has become a matter of concern over the last few decades (Vutukuru, 2005; Dirilgen, 2001). The natural aquatic systems may extensively be contaminated with heavy metals released form domestic, industrial and other anthropogenic activities (Velez and Montoro, 1998; Mohammad and Hossam, 2007). It is well documented that heavy metal contamination could have devastating effects on the ecological balance of the recipient environment via altering the diversity of aquatic organisms

(Farombi *et al*., 2007; Vosyliene and Jankaite, 2006; Ashraf, 2005; Javed, 2005) especially to the fish community (Olaifa *et al*., 2004). These metals could reach food chain through various biochemical processes such as bioconcentration, bioaccumulation and ultimately biomagnified in various trophic levels and eventually threaten the health of humans by seafood consumption (Etesin and Benson, 2007; Kudirat, 2008; Lakshmanan *et al*., 2009).

The natural concentrations of these metals in sea water are very low and hence the risk of contamination in living tissue is high, when the organisms started accumulating more amount of metals than the level of its excretion. These heavy metals, being conservative in nature have the maximum probability of biomagnification, when they are transferred to the human beings through the various members of different trophic levels in the marine food chain (Giarratano *et al*., 2007; Adefemi *et al*., 2008). The studies carried out on various fishes have shown that heavy metals may alter the physiological activities and biochemical parameters both in tissues and in blood (Basha and Rani, 2003).

Fisheries is one of the most important food production sectors in supplying protein to the human population. According to Food and Agriculture Organisation (FAO), world fisheries production in 2008 touched 146 million tonnes and a record value of 103 billion USD. Due to the increasing health consciousness of the consumers, seafood demands increase drastically during the past decade. The ever growing human population has pressurized on the sustainability of the fish population in the sea. The issues of over harvesting, global warming, pollution and fisheries stock management continue haunting the industry. FAO (2008) reported that if the present situation does not improved, 40 million tonnes of seafood shortage would be expected in 2030. Upon considering these issues, present research was initiated to determine the bioaccumulation level of hazardous metals in some commercially important fishes from South West coast of Peninsular Malaysia.

MATERIALS AND METHODS

Five fish species were purchased from local fish markets (LKIM) from 3 states (Johor, Melaka and Negeri sembilan) during 2008 (Fig. 1). Prior to the sampling, information on the fishing ground, time of fishing and the type of fishing gears and crafts used were noted from fisher men to make sure that the samples were collected from the respective sampling sites. Standard length and weight of the fishes documented prior to freezing. Fish samples were identified taxonomically using standard reference sources (www.fishbase.org).

Prior to analysis, fish samples were cleaned with running tape water and thawed at room temperature and gill and dorsal muscle tissues of selected fishes were excised using sterile scissors and transferred to the clean petridish. The tissues were dried in oven for three days at 60°C. The desired constant dry weight (0.5 g) of each sample was obtained after three days of drying process.

Acid digestion and ICP-MS analysis: Acid digestion method was performed to digest the samples which involved heating of 0.5 g of dried tissues of crabs in Teflon beaker with mixed concentrated acids (Hydrogen Peroxide (H_2O_2), Nitric acid (HNO_3), hydrochloric acid (HCl)) and sulphuric acid (H_2SO_4) in the ratio of 1:1 (Kamaruzzaman *et al*., 2007). After the digestion process hundred times dilution was performed using Mili-Q water then the samples were analyzed using Inductively Coupled Plasma Spectrophotometer (ICP-MS). The values of the heavy metal concentrations in the tissues were calculated based on dry weights as this discounts the variability due to inner parts differences in the moisture content of organisms. International certified standards (DORM-2) by National Research Council of Canada and a blank in replicates were used to control the accurateness of this procedure and percentage of recovery was between 95-105%.

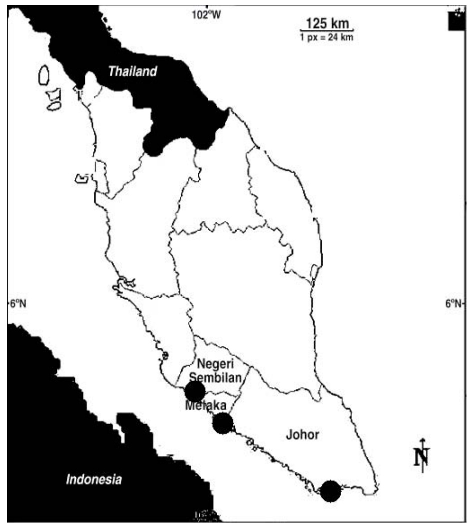

Fig. 1: Location of sampling sites (Kamaruzzaman *et al.*, 2010)

Data analysis: Analysis of Variance (ANOVA) statistical test was performed to check the significance in bioaccumulation of metals in different body parts. Weekly intake of metal level in fishes was determined using formula by Cardoso *et al.* (2010).

RESULTS AND DISCUSSION

The data obtained from the present study revealed that higher metal accumulation occurs in gill tissues compared to the muscle tissues of fishes (Table 1). This observation might be directly due to the respiratory mechanisms of fishes. Similar observation was noted by Playle (1998) who reported that during the respiratory process, the constant exposure of gills to the ambient water and the consecutive filtering action for oxygen intake might have enhanced the metal concentration in gill tissues. It was also reported that in general, the metal concentrations were lowest in muscle and did not exceed the established quality standards for fish (Alam *et al.*, 2002). Present study also showed that the concentration of essential metal (Zn) level in fish samples were greater than non-essential toxic metals (Pb, As and Hg) (Table 1). Similar observation was reported by various studies (Kamaruzzaman *et al.*, 2008, 2010). Due to the crucial role played by the essential metals (Fe, Zn and Cu) as precursors in most of the enzymatic activities, they are carefully regulated by the physiological mechanisms in most organisms and thus the knowledge of their concentrations in fish is important in terms of their management and for human consumption. *Otolithes ruber* accumulated higher amount of Zn in gills (10.517±1.273 $\mu g\ g^{-1}$) and tissues (5.870±1.967 $\mu g\ g^{-1}$) followed by *Lutjanus sebae* (Gill = 10.296±1.605 $\mu g\ g^{-1}$, tissue = 5.518±1.318 $\mu g\ g^{-1}$) and *Chirocentrus dorab* (gills = 10.212±3.578 $\mu g\ g^{-1}$). The lowest concentration of Zn was detected in *Pampus argenteus* (gill = 9.854±2.317 $\mu g\ g^{-1}$, muscle = 3.752±0.373 $\mu g\ g^{-1}$) followed by *Nemipterus japonicus* (gill = 8.722±1.663 $\mu g\ g^{-1}$, muscle = 2.327±1.066 $\mu g\ g^{-1}$) (Table 1). Similar observation was reported by Lakshmanan *et al.* (2009) who postulated that accumulation of metal in different species is the function of their respective membrane permeability and enzyme system which is highly species specifics and because of this fact different metals accumulated in different orders in different fish samples. The high Zn concentration in fishes in

Table 1: Trace metal concentrations and related statistical parameter for various samples of fish

Metal concentration (DW μg g^{-1})	Certified value	Analysis value	Recovery percentage	Tissue sample	*Nemipterus japonicus*	*Chirocentrus dorab*	*Lutjanus sebae*	*Otolithes ruber*	*Pampus argenteus*
Hg	4.64±0.26	4.59±0.11	98.92	Muscle	0.012±0.008	0.017±0.015	0.015±0.001	0.017±0.003	0.019±0.007
				Gill	0.006±0.003	0.016±0.009	0.011±0.005	0.007±0.002	0.033±0.014
As	18±1.1	18.68±0.37	103.78	Muscle	0.014±0.005	0.006±0.001	0.011±0.003	0.004±0.001	0.025±0.009
				Gill	0.013±0.002	0.017±0.003	0.023±0.005	0.013±0.004	0.035±0.007
Pb	0.065±0.007	0.062±0.039	95.38	Muscle	0.055±0.053	0.037±0.026	0.263±0.072	0.036±0.046	0.17±0.087
				Gill	0.119±0.066	0.106±0.063	0.193±0.067	0.101±0.04	0.192±0.054
Zn	25.6±2.3	24.73±0.4	96.6%	Muscle	2.327±1.066	3.316±1.283	5.518±1.318	5.870±1.967	3.752±0.373
				Gill	8.722±1.663	10.212±3.578	10.296±1.605	10.517±1.273	9.854±2.317
No. of samples					37	25	35	30	35
Length range (cm)					28.1-31.4	44-54.5	23-24.5	33.3-42.6	17.5-25.2
Weight range (g)					244-281	588-592	135-137	420-531	277-279

Value are Mean±SD. Recovery test results of the analysis of standard reference material Dog fish muscle (DORM-2) at 95% confident limit

the present study might also because Zn is a necessary element for embryo development and is important to reproductive organs as reported by Carpene *et al.* (1994) and El-Sherif *et al.* (2009).

L. sebae accumulated higher amount of Pb in gills (0.193±0.067 μg g^{-1}) and tissues (0.263±0.072 μg g^{-1}) followed by *P. argenteus* (Gill = 0.192±0.054 μg g^{-1}, tissue = 0.17±0.087 μg g^{-1}) and *N. japonicus* (gills = 0.119±0.066 μg g^{-1}, tissues = 0.055±0.053 μg g^{-1}). The lowest concentration of Pb was detected in *C. dorab* (gill = 0.106±0.063 μg g^{-1}, muscle = 0.037±0.026 μg g^{-1}) followed by *O. ruber* (gill = 0.101±0.04 μg g^{-1}, muscle = 0.036±0.046 μg g^{-1}) (Table 1). It was reported that Pb is a cumulative toxin. In human beings, it binds with SH group of proteins, apart from that, Pb damages blood circulation, central nervous system, liver and kidneys (Ekong *et al.*, 2006). Hence, knowledge on Pb accumulation is highly essential for the utilization of fishes for safer human consumption. Even though, the bioavailability of Pb in marine environment is low, their constant bioaccumulation by aquatic organisms especially fishes would cause serious threats to human health when they are consumed.

P. argenteus accumulated higher amount of As in gills (0.035±0.007 μg g^{-1}) followed by *L. sebae* (0.023±0.005 μg g^{-1}) and *C. dorab* (0.017±0.003 μg g^{-1}). The lowest concentration of As was detected in *N. japonicas* and *O. ruber* gills (0.013±0.004 μg g^{-1}). Higher concentration of As in muscle tissue was detected in *P. argenteus* (0.025±0.009 μg g^{-1}) followed by *N. japonicas* (0.014±0.005 μg g^{-1}), *L. sebae* (0.011±0.003 μg g^{-1}), *C. dorab* (0.006±0.001 μg g^{-1}) and *O. ruber* (0.004±0.001 μg g^{-1}) (Table 1). It is evident from previous study that long term exposure to As would cause lesions and gill damage (Bols *et al.*, 2001). It is known that As tend to accumulate in different body organs and are dangerous to fishes (Celino *et al.*, 2008) and hence detailed investigation would reveal their rate of bioaccumulation in fish samples.

P. argenteus accumulated higher amount of Hg in gills (0.033±0.014 μg g^{-1}) followed by *C. dorab* (0.016±0.009 μg g^{-1}) and *L. sebae* (0.011±0.005 μg g^{-1}). The lowest concentration of Hg was detected in *O. ruber* (0.007±0.002 μg g^{-1}) and *N. japonicus* gills (0.006±0.003 μg g^{-1}). Higher concentration of Hg in muscle tissue was detected in *P. argenteus* (0.019±0.007 μg g^{-1}) followed by *O. ruber* (0.017±0.003 μg g^{-1}), *C. dorab* (0.017±0.015 μg g^{-1}), *L. sebae* (0.015±0.001 μg g^{-1}) and *N. japonicus* (0.012±0.008 μg g^{-1}) (Table 1). The observed different concentrations of mercury in fishes might be due to the nature of fish habitats. As the species studied were all demersal fishes, various agricultural and industrial activities near by the fishing ground would have enhanced Hg levels in sediments which inturn expressed in tissue samples. Similar observation was reported by

Table 2: Estimated Weekly Intake (EWI) of metals such as Zn, Pb, As and Hg from sampled fishes with reference to international standards

	Heavy metal concentration in selected fishes (µg)			
Samples	Zn	Pb	As	Hg
Nemipterus japonicus	3.618	0.069	0.001	0.021
Chirocentrus dorab	5.061	0.035	0.001	0.013
Lutjanus sebae	6.104	0.05	<0.001	0.013
Otolithes ruber	5.590	0.24	0.016	0.018
Pampus argenteus	4.445	0.152	0.061	0.024
Tolerable weekly intake (µg) by WHO/FAO	25	50.00	0.015	0.043

Table 3: Mean concentration of metals in fishes from South West Zone and comparison with national and International standards

Metals	Hg	As	Pb	Zn	References
Present study	0.031±0.019	0.032±0.007	0.256±0.004	14.077±1.121	
Malaysia	0.5	0.5	2	100	MRF (1985)
Singapore	0.5	2	2	-	FRS (1990)
Australia	2	0.5	0.5	-	FSA (2002)
WHO	0.14	0.5	1.5	150	WHO (1990)

Lawrence and Mason (2001) and Mason *et al.* (2000). The detected significant relationship between fish size and Hg bioaccumulation ($p<0.05$) demonstrated the positive linear relationship between total Hg concentration and fish length. Khaniki *et al.* (2005) suggested that mercury concentration in fish ultimately determined by methyl mercury accumulation at the base of the food chain. World Health Organization (1990) reported that methyl mercury concentration in fish can be up to 100000 higher than its water concentration level. Upon considering the lethal effects of mercury on the normal physiologic condition of the fishes, its route of exposure to the aquatic organisms should be noted. The source of the mercury in the sampling sites might be from the industrial discharges from nearby area. Similar observation was noted by Lindeberg *et al.* (2007) who reported that Hg enters surface waters via industrial waste discharges and atmospheric route. Except with food, Hg might have entered fish body directly through skin and gills in the present study. Similar observation was also reported by Celechovska *et al.* (2007) who observed a part of Hg builds itself into a feeding chain and ultimately reach the higher trophic level and can also enter the fish body via skin and gills tissues during respiration.

Heavy metals have the tendency to accumulate in various organs of marine organisms, especially fish which in turn may enter into the human metabolism through consumption causing serious health hazards (Bravo *et al.*, 2010). Thus weekly intake of selected metals were calculated which showed all the metals gets accumulated by selected fishes lower than the international standard levels ($p<0.05$) except Arsenic (As) intake by *P. argenteus* (0.061) (Table 2). The detected concentration of Zn, Pb and Hg in the selected commercial fishes probably may not cause serious ill effects in human when the fishes are consumed. However, the slight increase in As level in *P. argenteus* should be revalidated with species specific study for the proper managemental steps. It was also observed that *P. argenteus* accumulated more amount of toxic metals than the selected fishes which was probably due to its feeding behaviour.

Significantly the detected mean concentration of metals in fishes were lower than the maximum permissible limits ($p<0.05$) of national and international standards and thus the selected commercial fishes from south west coast of peninsular malaysia could be used for human consumption (Table 3).

CONCLUSION

Present study clearly showed the lower toxicity of various metal concentrations in commercially important fishes from SW Malaysian coast. Hence it is evident that the fishes caught from south west Malaysian coast would not cause acute toxicity in human when it is consumed. But the long term monitoring program of metal bioaccumulation in fishes would be valuable and provide useful information for the assessment of the potential health risks of metals in Malaysian residents. Calculation of Permissible Tolerable Weekly Intake (PTWI) of the heavy metal concentration in the fish demonstrated that among all the heavy metal tested, Arsenic (As) was predicted to exceed the Malaysian permissible concentration limit. Due to the letahal toxic nature of Arsenic in human detailed study should be initiated to validate this observation which could ultimately helpful in utilization of commercial fishes from South west Malaysian coast for human consumption.

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