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Trace Metals in Muscle, Liver and Gill Tissues of Marine Fishes from Mersing, Eastern Coast of Peninsular Malaysia: Concentration and Assessment of Human Health Risk

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ABSTRACT

Trace metals (Cu, Zn, Cd and Pb) concentrations in muscles, livers and gills of three important marine fishes, Torpedo Scad (*Megalaspis cordyla*), Sea Catfish (*Arius thalassinus*) and Belangeri Croaker (*Johnius belangeri*) were studied using the Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The samples were collected from Mersing the eastern coastal waters of Peninsular Malaysia. The estimated ranges of Cu, Zn, Cd and Pb concentrations in the muscles, livers and gills of the three fish species were 1.51-3.48, 17.54-28.34, 0.02-0.12 and 0.12-0.15; 15.8-26.0, 80.58-365.1, 2.32-6.14 and 0.57-1.54; 3.04-5.51, 61.63-259.3, 0.03-0.12 and 0.14-2.03 μg g^{-1} dry weight, respectively. Metal concentrations in the edible parts of the fish were assessed for human consumption according to the Provisional Tolerable Weekly Intake (PTWI) and Provisional Tolerable Daily Intake (PTDI). Generally, levels of metal in muscles were lower than those in livers and gills. Zinc concentration was found to be the highest among the tested metals in all three species. The estimated weekly and daily intakes for the studied metals were far below the PTWI and PTDI limits. Present study reveals that consumption of these fishes from the study area does not pose a risk to human health.

Key words: Heavy metals, marine fish, Mersing, ICP-MS, provisional tolerable intakes

INTRODUCTION

Contamination and accumulation of an aquatic ecosystem by toxic metals has long since been a major environmental problem and is still growing at an alarming rate. Past episodes of trace metal contamination of the aquatic environment have increased the awareness about its toxicity (Martin-Diaz *et al*., 2005).

Among the different metals analyzed, cadmium (Cd) and lead (Pb) are non-essential and have no biological function. They are classified as chemical hazards and maximum residual levels have been prescribed for humans (Tort *et al*., 1987). Essential metals, such as copper (Cu) and zinc (Zn), are required in trace amounts for the smooth function of different biological systems and are essential for enzymatic activity and other biological processes (Hogstrand and Haux, 1991). The nutritional benefits of fish are important source of protein, minerals and vitamins. Worldwide, fish products represent only up to 10% of the human diet. However, they are the main uptake routes of metals into the human body (Ruelas-Inzunza *et al*., 2010). In addition, fish are a useful bioindicator for the determination of metal pollution in aquatic ecosystems (Ahmad and Shuhaimi-Othman, 2010). The specific advantages of fish as bioindicators are: (i) they are long-lived and integrate fluctuations of pollutants over time, (ii) they live in water, making a continuous monitoring of the presence of pollutants possible while also allowing for a spatial integration of pollutant data and (iii) they are easily sampled.

The concentrations of trace elements in fish organs are determined primarily by the level of pollution in their environment, notably in water and food (Farkas *et al*., 2003). Food ingestion is regarded as a more important source of trace metals in fish tissues if compared to water intake. Fish have a tendency to accumulate trace metals in a manner depending on their position in the food chain and their feeding habits. Top predators (piscivores fish) and species with high lipid contents have been shown to be the most sensitive indicators of environmental contamination with lipophilic compounds (De Pinho *et al*., 2002; Dusek *et al*., 2005).

The marine coastal water around Mersing, Johor located on the eastern coast of Peninsular Malaysia is a potential fish breeding ground and it is the main departures point for ferries to the nearby offshore islands such as Pulau Tioman and the more than 40 other islands in the Seribuat Archipelago. To date, varied levels of trace metals in fish samples in Malaysia were reported from various collection sites (Hajeb *et al*., 2009; Irwandi and Farida, 2009; Kamaruzzaman *et al*., 2010). However, there is a scarcity of reports on this similar study in literature for Mersing coastal waters.

In this study, we examined the total concentrations of trace metals Cu, Zn, Cd and Pb in the muscles, livers and gills of selected fish species from the coastal waters of Mersing, Malaysia. The daily and weekly intakes for studied metals were also estimated to evaluate the health risk to Malaysian people caused by the consumption of contaminated fish.

MATERIAL AND METHODS

Study area and fish sampling: Three selected fishes Torpedo Scad (*Megalaspis cordyla*), Sea Catfish (*Arius thalassinus*) and Belangeri's Croaker (*Johnius belangeri*) were collected during November 2009 from the local fishermen of Mersing (2°25'60N, 103°49'60E) the eastern coast of Peninsular Malaysia (Fig. 1). Samples were preserved in ice and transported to the laboratory. In the laboratory the weight and length of each fish were measured prior to harvesting the edible muscle, liver and gill samples. Samples were stored in (20°C) prior to analysis. The muscle, liver and gill samples were dried in an oven at 70°C until a constant weight was obtained.

Digestion procedures: The homogenized samples (muscle, liver and gill) were digested in triplicate in Teflon vessels with high purity 3 mL HNO_3 (65% v/v) and 1 mL H_2O_2 (30% v/v) and placed in a microwave oven digestive system (Start D Microwave Digestive System). After digestion, the samples were filtered through 0.45 μm Whatman filter paper (Whatman International Ltd., Cat) and transferred to a 50 mL volumetric flask and diluted to level with deionised water in the case of muscle and gill. However, in the case of liver tissues, the final dilution volume was 25 mL. The digested portion was then diluted to a final volume of 25 mL. A blank digest was performed in the same way. All metals were determined against aqueous standards. Digested samples were analyzed in triplicate for each metal.

Analytic procedures: The concentration of Cu, Zn, Cd and Pb were measured using Perkin Elmer Elan 9000 Inductively Coupled Plasma Mass Spectrometry (ICP-MS, USA) (Bashir *et al*., 2012). The ICP-MS is accepted as a fast, reliable means of multi-elemental analysis for a wide variety of

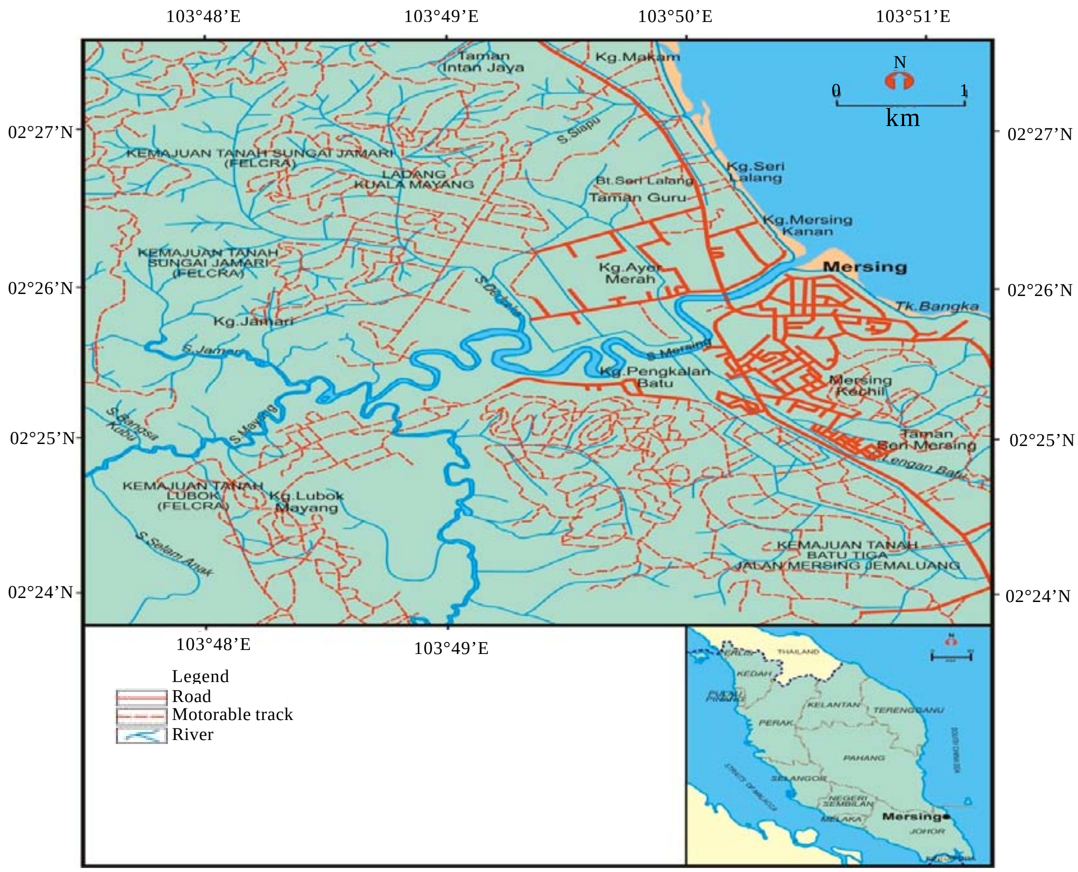

Fig. 1: Map showing the sampling site Mersing coastal waters, Malaysia

Table 1: The ICP-MS operating conditions and measurement parameters

Operating condition	Measurement parameters
RF Generator	40 MHz
RF Power	1000 W
Spray chamber	Ryton scott
Nebulizer	Cross-flow with Scott spray chamber
Plasma gas flow	15.0 L min^{-1}
Auxiliary gas flow	1.0 L min^{-1}
Nebulizer gas flow	0.60 L min^{-1}
Solution pump rate	1.5 mL min^{-1}
Sampler and skimmer cone	Nickel
Detector mode	Dual mode
Lens	Lens scan enabled
Scanning mode	Peak hopping
No. of sweeps/reading	20
No. of reading	3
No. of replicates	3

sample types (Date and Gray, 1988). The results are expressed in micrograms of metal per gram of dry fish (µg g^{-1}). The analytical conditions for the analysis of heavy metals using inductively coupled plasma mass spectrometry (ICP-MS) are described in Table 1.

Method validation: Precision and accuracy of analyses were determined by repeated analyses of biological Standard Reference Material (SRM) 2976 in muscle tissue. The results from the analysis of SRM were all within the 95% confidence limit.

Estimated weekly intake: The daily and weekly intakes for fish consumed by adults in the study areas (Mersing) were estimated. The Estimated Weekly Intake (EWI) values of metals by an adult (μg kg^{-1} b.wt.) were calculated using the average values of each fish species, i.e., EWI in μg kg^{-1} b.wt. = mean concentration of element (μg g^{-1} wet wt.) multiplied by the amount of fish consumed/week (g) and divided by the average weight of an individual (50 kg) (Ikem and Egiebor, 2005).

Statistical analysis: A logarithmic transformation was performed on the data to improve normality. One-way analysis of variance (ANOVA) and Duncan's test were used to assess whether heavy metal concentrations varied significantly between organs and species and possibilities of less than 0.05 ($p<0.05$) were considered statistically significant. All statistical analyses were performed using SPSS version 16.0 software.

RESULTS

The mean elemental concentrations (μg g^{-1} DW) in the muscles, livers and gill of the examined species, *M. cordyla*, *A. thalassinus* and *J. belangeri* from Mersing are summarized in Table 4. Generally, the highest concentrations of Cu, Zn, Cd and Pb were found in the liver. Differences in concentrations among different tissues were statistically significant for all assessed trace metals ($p<0.05$). The concentrations of the studied metals descended in the following order in the three species: Zn>Cu>Pb>Cd. The results from the analysis of biological Standard Reference Material (SRM) 2976 in muscle tissue were all within the 95% confidence limit. The recovery values for all metals ranged from 80 to 106% were obtained when compared to the certified values (Table 2).

Determination of detection limits: The detection limits (Table 3) for the methods were found to be (μg g^{-1} DW), Cu: 0.076, Zn: 1.42, Pb: 1.82 and Cd: 0.59. The method detection limits of all four metals, which are much lower than the recommended health-criteria values will enable the method to achieve accurate and precise measurements at the health-criteria levels in just one digestion.

Table 2: Reference concentration values of standard reference material (SRM) 2976 in mussel tissue

Elements (μg g^{-1} DW)	Certified values	Measured values	Recovery (%)
Cu	4.02±0.33	4.695±0.56	106
Zn	137±13	115.1±9.3	82
Cd	0.82±0.16	0.661±0.11	80
Pb	1.19±0.18	1.026±0.09	85

Table 3: Detection limits and FDA recommended health-criteria concentratious of metals

	Metals (μg g^{-1})			
	Cu	Zn	Cd	Pb
Detection limit (LOD)	0.076	1.42	0.59	1.82
FDA	120	480	4	2

*Health-criteria levels (Swami *et al*., 2001)

Table 4: Trace metal concentrations in fish organs from the coastal waters of Mersing, Malaysia

			Elements			
Fish species	N	Organ	Zn	Cu	Cd	Pb
		Muscle	28.34±4.20	2.02±0.15	0.03±0.01	0.12±0.01
A. thalassinus	10	Gill	259.3±9.10	3.15±0.07	0.03±0.01	2.03±0.05
		Liver	365.1±8.40	26.0±0.80	2.32±0.05	1.54±0.19
		Muscle	17.54±0.40	3.48±0.30	0.03±0.01	0.15±0.01
M. cordyla	10	Gill	61.63±0.71	5.51±0.40	0.12±0.01	0.14±0.01
		Liver	80.58±2.47	15.8±0.70	6.14±0.20	0.62±0.04
		Muscle	21.05±2.10	1.51±0.09	0.02±0.01	0.13±0.01
J. belangeri	10	Gill	70.18±9.40	3.04±0.50	0.04±0.03	0.26±0.04
		Liver	98.49±11.2	17.4±2.40	2.41±0.15	0.57±0.03
WHO (1989)			150	10	0.2	0.2
FAO (1992)			30-100	10	0.2	0.5-0.6
MFR (1985)			100	20	1	2

Values are Mean±SD

Trace metal concentrations in different fish organs: The results showed that, the highest concentration of Zn observed in the livers of *A. thalassinus*, 342.8±3.4 μg g^{-1} and its lowest levels were recorded in the livers of *M. cordyla*, 80.58±2.5 μg g^{-1} (Table 4). Moreover, the Zn concentrations in muscles of the different examined species were in the following order *A. thalassinus*>*M. cordyla*>*J. belangeri*. The highest mean concentration of Zn in the muscle tissues of *A. thalassinus* was 35.4±0.7 μg g^{-1} and the lowest levels of Zn in muscle were determined in *M. cordyla* 17.54±0.4 μg g^{-1}. The lowest mean Zn levels was in the gill of *J. belangeri*, 61.63±0.7 μg g^{-1}, whereas, the highest mean Zn concentration was in the gill of *A. thalassinus*, 249.7±6.5 μg g^{-1}. The maximum Cu levels among the examined fish species were 26.0±0.4, 17.4±0.4 and 15.8±0.4 μg g^{-1} in livers of *A. thalassinus*, *J. belangeri* and *M. cordyla*, respectively (Table 4).

According to our findings, the highest mean Cd concentrations were 6.14±0.2, 2.46±0.2 and 2.08±0.08 μg g^{-1} in the livers of *M. cordyla*, *J. belangeri* and *A. thalassinus*, respectively. Its lowest mean levels were 0.03±0.01 μg g^{-1} in the muscles of both *A. thalassinus* and *M. cordyla* and 0.02±0.01 μg g^{-1} in the muscles of *J. belangeri*.

The maximum Pb levels among the examined fish species were 2.03±0.01, 0.62±0.04 and 0.57±0.04 μg g^{-1} in the livers of *A. thalassinus*, *M. cordyla* and *J. belangeri*, respectively. Its minimum levels were 0.12±0.01 and 0.13±0.01 μg g^{-1} in the muscles of *A. thalassinus* and *J. belangeri*, respectively and 0.14±0.01 μg g^{-1} in the gill of *M. cordyla* (Table 4).

DISCUSSION

This study examined the concentrations of trace metals (Cu, Zn, Cd and Pb) in three commercially important fishes (*A. thalassinus*, *M. cordyla* and *J. belangeri)* from Mersing coastal waters Malaysia (Table 4). The concentrations of metals in muscle and gill reflect the concentrations of metals in the waters. Increased metal concentrations in the liver may represent storage of sequestered products in this organ (Hamilton and Mehrle, 1986). Fish are able to closely regulate their internal Cu concentrations. The values of Cu in fish samples obtained in the present study were also similar with those reported by Agusa *et al.* (2005) (1.59-2.94 and 1.05-2.87 μg g^{-1}) (Table 5) for fish sampled from two stations in Mersing and Port Dickson, on the eastern and western coastal waters of Peninsular Malaysia, respectively. On the other hand, the Cu

Table 5: Comparison of metal accumulation in fishes from different regions in Malaysia

		Metals (μg g^{-1} DW)				
Area	Tissue	Cu	Zn	Cd	Pb	References
Terengganu	Muscle	1.33-2.16	28.0-32.4	0.06-0.81	0.03-0.04	Agusa *et al.* (2005)
	Liver	9.64-17.1	119.0-151	3.43-12.6	0.09-0.36	
Mersing	Muscle	1.59-2.94	22.8-39.2	0.01-0.03	0.02-0.03	Agusa *et al.* (2005)
	Liver	2.62-23.8	27.1-158	0.19-4.82	0.04-0.67	
Port Dickson	Muscle	1.05-2.87	17.1-29.7	0.01-0.02	0.03-0.05	Agusa *et al.* (2005)
	Liver	7.60-25.3	86.5-179	1.57-11.3	0.04-0.67	
Langkawi	Muscle	1.31-3.48	17.9-33.1	0.02-0.20	0.03-0.07	Agusa *et al.* (2005)
	Liver	6.93-20.9	65.3-193	1.01-33.8	0.04-0.88	
Langkawi	Muscle	11.55±2.1	49.39±8.2	0.30±0.08	1.0±0.2	Irwandi and Farida (2009)*
Melaka	Muscle	-	2.33-5.87	-	0.04-0.26	Kamaruzzaman *et al.* (2010)
Klang valley	Muscle	-	-	0.12-0.86	0.62-1.42	Nor Hasyimah *et al.* (2011)*
	Liver	-	-	0.79-1.23	0.10-2.15	
	Gill	-	-	0.88-1.48	1.10-1.46	
Mersing	Muscle	-	18.1-25.4	0.023-0.03	0.17-0.2	Bashir *et al.* (2012)
	Liver	-	104.8-341.9	2.08-2.46	0.87-1.7	
	Gill	-	66.2-246.2	0.02-0.05	0.24-1.96	
Mersing	Muscle	1.51-3.48	17.5-28.3	0.02-0.03	0.12-0.15	Present study
	Liver	15.8-26.0	80.6-365.1	2.32-6.14	0.57-1.54	
	Gill	3.04-5.51	61.6-259.3	0.03-0.12	0.14-2.03	

*μg g^{-1} wet weight bases, -: Not studied

concentrations (11.55±2.1 μg g^{-1}) in fish muscles collected from Langkawi Island in an investigation conducted by Irwandi and Farida (2009) were higher than our present findings. Muscle tissues of the three species presented Cu levels far below the permissible level.

Mean Zn concentrations in the muscles were 28.34±4.2 μg g^{-1} dry wt. for *A. thalassinus*, 17.54±0.4 μg g^{-1} dry wt. for *M. cordyla* and 21.05±2.1 μg g^{-1} dry wt. for *J. belangeri*, respectively. However, Zn levels in liver of *A. thalassinus* were 365.1±8.4 μg g^{-1} dry wt. This high level of Zn in our study exceeded both Malaysian permissible concentrations (100 μg g^{-1}) and the FAO limit (30-100 μg g^{-1}). Moreover, mean Zn concentrations in the gill of *A. thalassinus* were 259.3±9.1 μg g^{-1} dry wt. The Cd levels (0.02±0.002 μg g^{-1} dry wt.) in this research were lower than previous estimated levels (0.06-0.81 and 0.30 μg g^{-1}) in fish collected from Terengganu and Langkawi (Agusa *et al.* 2005; Irwandi and Farida, 2009) (Table 5). The Cd levels in the fish from Mersing reported in the literature fall in the range of 0.19-4.82 and 0.01-0.05 μg g^{-1}. for the livers and muscles of *A. thalassinus*, respectively (Agusa *et al.*, 2005). Compared with the literature from different Malaysian marine coastal waters, our results for Cd concentration is almost similar to what has been reported (Table 5). The highest lead concentrations in fish muscles (0.62-1.42 μg g^{-1}) reported in the literature from Klang Valley (Nor Hasyimah *et al.*, 2011), were higher than the present study (Table 5). Pb concentrations reported in demersal fishes collected from Straits of Malacca was 1.3±0.01 μg g^{-1} of wet weight (Alina *et al.*, 2012). Another study showed that Pb levels were 0.12-0.24 μg g^{-1} for muscles and 0.014-0.023 μg g^{-1} for livers in fish from Mersing's coastal waters, Malaysia (Agusa *et al.*, 2005) (Table 5). This study's Pb concentrations were generally in agreement with what was previously reported.

Many studies have indicated that different concentrations of trace metals in different fish species might be a result of different ecological needs, metabolism and feeding patterns (Yilmaz, 2003). Moreover, metal accumulation may depend on seasonal variations

(Deram *et al*., 2006). Levels of the essential metals in the fish samples were higher than those of the non-essential metals. Among the four metals under study, Zn showed the highest level of accumulation. Similar results were observed in previous study (Etesin and Benson, 2007).

Accumulation of bioactive metals like Cu and Zn was actively controlled by the fish through different metabolic processes and the levels of accumulations are usually independent from ambient concentrations (Chatterjee *et al*., 2006). Cd and Pb have higher tendencies to bioaccumulate in the liver, which is involved in detoxification. The sequence of metal accumulation in various organs of fish was: liver>gill>muscle, which was observed in many studies (Dural *et al*., 2006; Storelli *et al*., 2006).

In the present study, the liver had significantly higher trace metal concentrations than the gills and muscles. The difference in accumulation potential between muscles, livers and gills can be explained by the activity of metallothioneins, proteins that are present in liver but not in the muscle, which have the ability to bind and accumulate certain heavy metals at a higher degree (Ploetz *et al*., 2007; Iwegbue, 2008). Meanwhile, the fish liver acts as major site for homeostasis (Reynders *et al*., 2006). Gills are an important route of metal uptake and thus metal levels in gill can be used to assess metal exposure (Catsiki and Strogyloudi, 1999).

Conversely, the lowest concentrations of metals were found in the muscle tissues. Muscle analyses are used to investigate the direct transference of heavy metals into human bodies, as the muscles are the main edible part of the fish and a major target of metal storage. Therefore, studying the level of metal accumulation constitutes a tool for environmental assessment and for determining public health risk (Reinfelder *et al*., 1998). The Cu, Zn, Cd and Pb levels in muscle were found to be lower than permissible limits reported by MFR (1985), WHO (1989) and FAO (1992).

Estimation of weekly intake (EWI): PTWI values for Zn, Cu, Cd and Pb are 2000, 2500, 7 and 25 μg kg^{-1} b.wt./week (FAO/WHO, 2004), respectively. Therefore, PTWI values of essential metals Zn and Cu for 50 kg are 100,000 and 125,000 μg week^{-1} and PTWI values for nonessential metals Cd and Pb for a 50 kg person are 350 and 1250 μg week^{-1}, respectively (Table 6). The average daily fish consumption in Malaysia is 160 g person^{-1} (FAO, 2005). The average consumption of the Malaysian population is equivalent to 1120 g/person/week. The Estimated Weekly Intake (EWI) values of metal by an adult (μg kg^{-1} b.wt.) were calculated using the average value of each fish species, i.e., EWI in μg kg^{-1} b.wt. = mean concentration of element (μg g^{-1} wet wt.) multiplied by the amount of fish consumed/week (g) and divided by the average weight of an individual (50 kg) (Ikem and Egiebor, 2005; Agusa *et al*., 2007).

Table 6: Estimated daily and weekly intake of trace metals from consumption of fish from Mersing, Malaysia

				A. thalassinus		*M. cordyla*		*J. belangeri*	
Metals	Std.PTWI	PTWI	PTDI	EWI	EDI	EWI	EDI	EWI	EDI
Zn	2,000	100,000	14285.7	1856.40	265.20	1571.50	224.50	1622.60	231.80
Cu	2,500	125,000	17857.1	226.20	32.32	389.80	55.70	169.10	24.16
Cd	7.0	350	50	2.24	0.32	3.36	0.48	1.61	0.32
Pb	25.0	1250	178.6	13.44	1.92	172.50	24.64	14.56	2.08

Std.PTWI: Standard Provisional permissible tolerable weekly intake (μg kg^{-1} b.wt./week), PTWI: Permissible tolerable weekly intake for 50 kg person (μg^{-1} week), PTDI: Permissible tolerable daily intake for 50 kg person (μg^{-1} day), EWI: Estimated weekly intake (μg), EDI: Estimated daily intake (μg) (FAO/WHO, 2004)

Thereafter, these calculated values were compared with those of Provisional Tolerable Weekly Intake (PTWI) values stipulated by the WHO as standard. It is clear that, calculated PTWI values of other metals are always below the standard value. The calculated PTWI values of *A. thalassinus, M. cordyla* and *J. belangeri* are marginally higher and thus, contribute significant amounts of metal through consumption. Standard PTWI values take into consideration the uptake of the toxic metals through all sources like air, water and other various types of foods. The antagonistic effect caused by the presence of other metals may reduce metal toxicity to a great extent. Therefore, without having knowledge of the cumulative effect of all metals comprising of both essential and non-essential elements on the degree of metal toxicity, no definitive conclusions could be made. However, the present study highlights a grave concern that these fishing areas are not at all free from trace metal contamination. Therefore, the trace element emission into coastal waters also needs to be examined for its impact on the ability of marine organisms to survive and reproduce.

CONCLUSIONS

The present study describes the concentrations of trace metals in different organs of three commercially important fishes (torpedo scad: *Megalaspis cordyla*, sea catfish: *Arius thalassinus* and Belangeri's croaker: *Johnius belangeri*) collected from Mersing coastal waters Malaysia. The descending order of metal levels obtained from the organs: liver, muscle and gill were Zn>Cu>Pb>Cd. Our findings also revealed that Zn concentrations were the highest in all organs of all examined species from the study area. The highest metal concentrations were found in the liver and gill, while the muscle tended to accumulate less metal. Moreover, Pb concentration in muscles and gill of the examined fishes were higher than the Cd levels. These results can also be used to understand the chemical quality of fish and to evaluate the possible risk associated with their consumption. The heavy-metal concentrations in the majority of the samples analyzed were well within the prescribed limits set by various authorities.

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