ACCUMULATION OF TRACE METALS IN THE MUSCLE, LIVER AND GILLS TISSUES OF *ARIUS MACULATUS* AND *PENNAHIA ANEA* FISH SPECIES FROM COASTAL WATERS, PENINSULAR MALAYSIA

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ABSTRACT

Fishes are considered as good indicators of trace metal pollution in the aquatic ecosystem in addition to being a good source of protein. Thus, the concentration of trace metals (Fe, Zn, Al, As, Cd and Pb) in water and muscle, liver and gills tissues of two important marine fish species, *Arius maculatus* and *Pennahia anea* collected from Kapar and Mersing in Peninsular Malaysia, were examined. Generally, Fe has the highest concentrations in both water and the fish species. While, Cd in both coastal waters showed high levels exceeding the international standards. The metal level concentrations in the fish samples are in the descending order from livers > gills > muscles. Fortunately, level of these metal concentrations in fish has not exceeded the permitted level of Malaysian and international standards.

Keywords: Trace metal, marine fishes, Kapar and Mersing, Peninsular Malaysia.

INTRODUCTION:

It is well documented that trace metals contamination could have devastating effects on the ecological balance of the recipient environment via altering the diversity of aquatic organisms especially to the fish community (Kamaruzzaman, 2011).

Fish enjoys agood reputation as a nutritious and healthy food and therefore consumption of which is recommended because it is a good source of omega-3 fatty acids which have been associated with positive health impacts like their cardio-protective effects (**Castro-Gonzalez and Mendez-Armenta 2008).**

Although fish consumption provides many benefits for cardiovascular health, there is agrowing concern that metals accumulated in fish muscle tissues may represent ahigher health risk than ahealth benefit, especially for populations with high fish consumption rates (**Burger et al 2011).** For instance, metals like Fe and Zn are essential for fish metabolism while some others such as Al, Cd, As and Pb are readily transferred through food chains and they are harmful for human when ingested over along time period (**Çelik and Oehlenschlāger, 2007).**

The fish tissues most commonly studied are muscles, livers, and gills, due to their different roles in the bioaccumulation processes. Gills and liver of fish are chosen as target organ for assessing metal accumulation.

The concentrations of metals in gills reflect the concentrations of metals in waters where the fish species live, whereas the concentrations in liver represent storage of metals. Although muscle is not an active tissue for accumulating the trace metals (**Yilmaz, 2003**), the study of potential metal accumulation in the muscle because it is the edible part of the fish for humans. Moreover, fishes are at the end of the aquatic food chain and may accumulate metals and pass them on to human beings when consumed as food, causing chronic or acute diseases (**Burger and Gochfeld, 2007**).

The marine ecosystem of **Kapar** is stressed due to its location in the Strait of Malacca where many pollution sources spread around this area. The Straits of Malacca is subjected to a great variety of pollutants due to its strategic location as amajor international shipping lane and the concentration of agriculture, industrialization, and urbanization activities along the coast of Peninsular Malaysia (**Abdullah et al., 1999**).

Besides the mentioned pollution sources, an electric power station that uses coal and discharges the polluted, pre-used water into the surface water systems surrounding **Kapar** may contribute to the trace metal pollution of the marine ecosystem in this area. Moreover, **Kapar** has great importance for the local fishery industry.

Therefore, estimation of the selected metals in fish of the marine coastal water of **Kapar** is necessary to define the current trace metal levels in the fish as well as to monitor the trends of change in fish trace metal levels with time. However, evaluation of the levels of the same metals in Mersing allows for comparison between the two areas, particularly in terms of the kinds and effects of different pollution sources in the two areas.

In the present study, the concentration of selected metals: iron (Fe), zinc (Zn), aluminum (Al), arsenic (As), cadmium (Cd) and lead (Pb) were determined in water and (muscles, livers, and gills) of two marine fish species to assess the status of trace metal pollution in two coastal waters in Malaysia.

MATERIALS AND METHODS:

Water and fish samples (***Arius maculatus*** **and** ***Pennahia anea***) were collected at two different stations of coastal waters of Peninsular Malaysia in November 2009 (Fig.1).

First sampling station was **Kapar** (3˚11'54 N, 101˚32'66 E) located in Selangor on the west coast of Peninsular Malaysia near the **Sultan Salahuddin Abdul Aziz** Power Plant station. The second sampling station was Mersing (2˚ 25'60 N, 103˚49'60 E) in **Johor Bahru** on the west coast of Peninsular Malaysia. A bout 200 ml of water collected using automated water sampler (21 cc capacity). Samples were stored in amber-coloured polyethylene bottles pre-washed with 1 (N) HNO_3 and deionised water. Fresh fish samples of each species from each station were collected from local fishermen.

The samples were kept in acool box (4 C˚) and transported to the laboratory for metal analysis. Total length (cm) and weight (g) of the fish samples were measured before dissection. Muscle, liver and gills were taken and dried until constant weight was obtained. The dried samples were digested in triplicate in amicrowave oven digestive system (Start D Microwave Digestive System). Analytical blanks were run in the same way as the samples and determined using standard solutions prepared in the same acid matrix.

Figure 1 Locations of the sampling sites:

The concentrations of Fe, Zn, Al, As, Cd and Pb in water and two fish species were analyzed using inductively coupled plasma-mass spectrometry (**ICP-MS, Perkin Elmer, Model Elan 9000, USA)**.

The results were expressed in micrograms of metal per gram fish on a dry weight basis (µg/g dry weight).

The performance of the method was evaluated by analyzing a standard reference material of marine biota sample (SRM2976, freeze-dried mussel tissue, National Institute of Standards and Technology, USA).

Recoveries for all metals studied were between 83% and 109% of the certified value (Table 1).

Due to the lack of normal distribution of data, the log transformation was implemented for the normalization process.

To examine the vital differences in the concentrations of heavy metals in the two research sites, the t-test was conducted. Moreover to investigate the denoting dissimilarity of concentrations of trace metals among the three fish organs, the **Kruskal-Wallis** test was used.

For which a *P* value less than 0.05 was considered as suggestive of statistical significance. All statistical tests were performed with SPSS 2010.

Table 1: Measured and certified[(1)] values of elemental metal concentrations µg/g dry weight ±SD.

Metal	Certified value	Measured value	Recovery (%)
Fe	158 ± 8	144.6 ±7.1	92.6
Al	134 ± 34	128.2 ±12	96.8
Zn	137 ±13	115.2±10	84.4
As	13.3±1.8	14.5 ± 0.9	109
Cd	0.82±0.16	0.68 ± 0.02	83.7
Pb	1.19±0.18	1.03 ± 0.04	86.8

(1) Certified mussel standard reference material (SRM) 2976.

RESULTS AND DISCUSSION

Trace metals in water:

Table 2 shows the trace metal levels in the seawater samples from **Kapar** and **Mersing.** The maximum concentration of the metals in the water samples in the descending order were Fe > Al > As > Zn > Pb > Cd and Fe > As > Cd > Zn > Al > Pb from **Kapar** and **Mersing** respectively. Al, Zn and As were higher concentration in **Kapar,** in the contrary, Fe and Cd were higher in **Mersing.**

The results represent that all the metals concentrations were below the maximum acceptable concentration, except for Cd.

Table 2 Trace metal concentrations in Sea water from Kapar and Mersing (mg/L):

		Metals					
		Al	Fe	Zn	As	Pb	Cd
Kapar	Mean	0.045	0.33	0.021	0.036	0.010	0.01
	Max	0.046	0.34	0.033	0.040	0.014	0.011
	Min	0.044	0.32	0.015	0.020	0.020	0.010
Mersing	Mean	0.011	0.36	0.015	0.030	0.010	0.019
	Max	0.012	0.40	0.016	0.033	0.014	0.030
	Min	0.010	0.32	0.014	0.028	0.002	0.011
NWQSM*		0.5	1	0.4	0.1	0.5	0.01
WHO**		–	–	5	0.01	0.01	0.003

Trace metals in various organs in fish:

Levels of six metals in muscle, liver and gill tissues of two fish species are shown in Tables 3 and 4.

In general, the highest concentrations of Fe, Zn, Al, As and Pb were found in the liver tissues of both examined fish species. The concentrations of the studied metals decreased following the order Fe > Zn > Al > As > Pb > Cd in the two species. Iron exhibited the highest concentrations in all the examined organs of both species, followed by Zn. On the other hand, the levels of Pb and Cd were generally the lowest. Similar findings were reported by many researchers (Dural et al., 2007; Tepe et al., 2007 and Turkmen et al., 2008).

It is observed that Fe concentration was the highest in both species and both studied areas. In the present study, with the exception of Al, liver had significantly ($P \leq 0.05$) higher trace metal concentrations than gills and muscle. It is observed that, the mean concentrations of metals in the muscle, liver and gills of each fish species showed great variations, this may be related to the differences in ecological needs, swimming behaviors and the metabolic activities among different fish species.

The differences in metal concentrations of the tissues might be due to their capacity to induce metal-binding proteins such as metallothioneins. The present study showed that metal levels in liver and gills were highest in the studied species.

Its well known that large amount of metallothioneins induction occurs in the liver tissue of fish. The adsorption of metals on to gill surface could also be an important influence in total metal levels of the gill (Roesijadi and Robinson 1994).

The mean concentrations of Fe in the muscles of *A. maculatus* and *P. **anea*** and in **Kapar** were 53.84 µg/g and 34.91 µg/g, respectively. However, in **Mersing** the mean concentrations were 21.62 µg/g in *A. maculates* and 21.47µg/g in *P.* ***anea*.** It is revealed that Fe concentrations varied significantly ($P < 0.05$) between the two stations.

Higher Fe concentration in muscle of both species was found in the fish of **Kapar** than that of **Mersing. Kapar** area is impacted by various sources of pollution such as electrical power station, international shipping activities, and urban and agricultural activities, amongst others. Similarly, the concentrations of Fe in the liver tissues of ***P. anea* and *A. maculatus* in Kapar** were approximately 1976.0 µg/g and 1008.0 µg/g, respectively whereas in the same tissues of the fish from **Mersing** the concentrations were 526.0 µg/g and 924.6 µg/g, respectively. The levels of Fe in the muscles of Mediterranean Sea fish that were reported in the literature range from 59.6 and 73.4 µg/g (**Kalay and Canli, 2000**). The concentrations of Fe in the fish muscle were reported to have the range of 24.1–50.3 µg/g in Parangipettai Coast, India (Raja et al., 2009) and the range of 49.9–889 µg⁄g in the Turkish seas (Tepe et al., 2008).Therefore, the levels of Fe in fish muscles reported by the current study are generally in agreement with the literature.

According to the results obtained in Tables 3 and 4, concentrations of Zn in the livers of ***P. anea*** and ***A. maculatus*** collected from **Kapar and**

Mersing were 114.1 µg/g and 555.9 µg/g (Table 3), and 104.8 µg/g and 341.9 µg⁄g (Table 4), respectively. Generally, High concentrations of Zn were observed in the livers of ***A. maculatus*** in both studied areas. Different levels of the investigated metals were detected in different species and even within the same species. It is observed that Zn concentration was the highest after Fe in both species and studied areas. The mean concentrations of Zn in the muscle tissues of ***P. anea*** and *A.* ***maculatus*** collected from **Kapar** were around 26.3 µg/g and 51.0 µg/g, respectively.

Table 3: Trace metal concentrations (Mean ± SD) in muscle, liver and gills tissues of *A. maculatus* and *P. anea* fish from the coastal waters of Kapar, Malaysia:

Species	Organ	Metals					
		Fe	**Al**	**Zn**	**As**	**Cd**	**Pb**
Amaculatus	Muscle	53.84±5.1	7.24±0.0	50.99±5.34	12.58±0.8	0.088±0.01	0.12±0.01
	Liver	1007.1±11.7	28.38±1.92	550.89±6.8	14.17±1.03	1.01± 0.07	1.54±0.19
	Gills	805.6±3.38	538.6±3.48	840.89±5.2	13.59±0.69	0.048±0.01	2.03±0.05
P. anea	Muscle	21.62±4.7	3.00±0.11	26.32±1.6	3.28±0.65	0.048±0.01	0.13±0.004
	Liver	1975.0±38.9	13.72±1.12	114.11±2.2	11.75±0.13	0.694±0.05	0.57±0.03
	Gills	891.6±28.6	299.5±0.75	60.21±0.44	4.75±0.65	0.690±0.05	0.26±0.02
WHO*		50	−	150	0.02	0.2	0.2
FAO**		−	−	30−100	7.88[a]	0.2	0.5-0.6
MFR***		−	−	100	−	1	2

* WHO (1989)/** FAO (1992) / a FAO 1983 /***Malaysian Food regulation(1985)/No is the number fish samples.

However, in **Mersing** the respective concentrations were 18.1 µg/g and 25.4 µg/g. Higher Zn concentrations in the muscle tissues of both species were found in **Kapar** than in **Mersing.**

The observed differences can be explained by the fact that the concentrations of these metals depend to a great extent on species, sex, biological cycle, and on the part of the fish analyzed **(Tuzen, 2003).**

Moreover, ecological factors such as season, location/environment of development, nutrient availability, temperature and salinity of the water may contribute to variations in the metal concentrations in fish.

Ranges of Zn concentrations reported earlier in the muscles and livers of Malaysian marine fish were 15.4-60.1 µg/g and 27.1–95.3 µg/g, respectively **(Agusa et al., 2005**). Another study conducted in **Langkawi** Island showed that all species had higher concentrations of Zn than of other metals and that the concentrations in muscles ranged from 34.3 µg/g to 49.4 µg/g (**Irwandi and Farida., 2009**). Accordingly, the Zn concentrations in the fish muscles detected by the present study are similar to those reported by **Irwandi and Farida (2009).**

In the present study, Al concentrations were the highest in the gills and ranged from 13.7 µg/g in ***P. anea*** to 538.6 µg/g in ***A. maculatus*** in **Kapar** and from 91.0 µg/g in ***P. anea* to 850**.14µg/g in ***A. maculatus*** at **Mersing.** In *A. maculatus,* the Al concentration was 7.2 µg/g in muscle and 28.4 µg/g in liver at **Kapar** station, whereas it was 5.4 µg/g and 10.0 µg/g in the fish muscle and liver tissues, respectively at **Mersing** station.

On the other hand, the concentrations of Al in the muscle and liver tissues of ***P. anea*** was 3.0 µg/g and 13.7 µg/g, respectively in **Kapar**, while the respective concentrations in **Mersing** were 1.5 µg/g and 5.1 µg/g, respectively. The mean Al concentrations were higher in the three organs of fish species captured from **Kapar** than its concentration in the same organs of the fish species collected from of **Mersing** except gills of ***A. maculatus*** from **Mersing**.

Table 4: Trace metal concentrations (Mean ± SD) in muscle, liver and gills tissues of *A. maculatus* and *P. anea* fish species from the Mersing coastal waters, Malaysia:

Species	Organ	Metals					
		Fe	Al	Zn	As	Cd	Pb
A.maculatus	**Muscle**	34.91±1.74	5.44±1.25	25.39±0.71	14.2±2.34	0.02±0.03	0.2±0.02
	Liver	924.6±24.7	10.05±1.7	341.9±3.35	21.89±0.9	2.075±0.1	0.87±0.07
	Gills	822.76±9.9	850.1±7.1	246.55±7.4	7.65±0.1	0.02±0.01	0.24±0.02
P. anea	**Muscle**	21.47±1.86	1.46±13	18.1±0.79	3.76±0.2	0.023±0.3	0.17±0.05
	Liver	525.96±17	5.13±0.65	104.84±1.23	8.38±0.22	2.46±0.02	1.07±0.07
	Gills	461.4±8.6	91.03±2.7	66.24±1.3	4.88±0.13	0.05±0.01	1.96±0.16
WHO*		50	−	150	0.02	0.2	0.2
FAO**		−	−	30−100	7.88[a]	0.2	0.5-0.6
MFR***		−	−	100	−	1	2

* WHO (1989)/** FAO (1992) / a FAO 1983 /***Malaysian Food regulation (1985).

The Al concentrations were reported to fall within the range 1.50–4.50 µg/g in fish muscles from the Parangipettai Coast, **India (Raja et al., 2009).** On the other hand, the Al concentrations were reported earlier to fall in the range of 51.9–166.3 µg/g in muscles and in the range of 229.01–1412.7 µg/g in gills of Malaysian marine fish from **Kapar (Mohamed et al., 2006).**

As such, the Al concentrations observed in this study generally agree with the values reported in the literature.

Arsenic levels in the muscles of the analyzed fish ranged from 3.8 µg/g in ***P. anea* in Kapar** to 14.2 µg/g in ***A. maculatus*** in **Mersing.** As to fish livers, were ranged from 11.8 µg/g in ***P. anea*** from **Kapar** to 21.9 µg/g in *A. maculatus* from Mersing. As regards fish gills, As levels were ranged from 4.9 µg/g in *P. anea* from Mersing to 13.6 µg/g in *A. maculatus* from Kapar

(Tables 3 and 4). There are very few data about As levels in fish tissues from Malaysia against which to compare the levels of As found in this study.

According to published literature, ranges of As concentrations reported earlier in the muscles of Malaysian marine fish were 1.05–2.14 µg/g (Titik Budiatic, 2010).

Another study conducted showed that, As content of fish from India coastal waters was within the range of 0.01–0.63 µg/g (Aditi Deshpande et al., 2008) which are levels well below the As levels detected in fish tissues by the current study.

In the present study, Pb concentrations in muscles ranged from 0.1 µg/g in *P. anea* in Kapar to 0.2 µg/g in *A. maculatus* in Mersing while for livers the concentrations ranged from 1.1 µg/g in *P. anea* from Mersing to 1.5 µg/g in *A. maculatus* in Kapar. On the other hand, the concentration ranged in gills from 1.96 µg/g in *P. anea* in Mersing to 2.03 µg/g in *A. maculatus* in Kapar. Pb levels were reported earlier in the literature to fall in the range of 0.018–0.023 µg/g for muscles and 0.115–0.380 µg/g for livers of fish from Mersing, and in the range of 0.026–0.72 µg/g for muscles and 0.041–0.872 µg/g for livers of fish from Langkawi coastal waters of Malaysia (**Agusa et al., 2005**). Hence, the Pb concentrations reported herein comply with these ranges.

The results of this investingation showed Cd levels did not significantly vary, ($P \leq 0.05$), the highest concentrations were observed in the livers of the fish species from Mersing, were the mean Cd concentration were ranged from 2.075 µg/g for livers of ***A. maculatus*** to 2.458 µg/g for the same organs of *P. anea* from **Mersing** coastal water. While the highest levels of Cd in the muscles were recorded as 0.088 µg/g in muscles of ***A. maculatus*** from **Kapar**, in the contrary, the lowest value of Cd detected was 0.021 µg/g in the muscles of *P. anea* from Mersing. Cd levels were reported in the literature to fall in the range of 0.14–0.57 µg/kg and 0.15–0.52 µg/kg for muscles of *A. maculatus* and *P. anea* from the same studied area **Kapar (Mohamed et al., 2006).** Another study was conducted on commercial marine fish from Klang Valley, Malaysia, concluded that the mean Cd concentrations in the fish muscles ranged from 0.121 mg/kg to 1.594 mg/kg (Nor Hasyimah et al., 2011).

Furthermore, Irwandi and Farida 2009 investigated on marine fin fish captured from the coast of Langkawi Island in Malaysia and reported that the mean Cd concentrations in the fish muscles ranged from 0.30 µg/g to 0.90 µg/g. Compared with the literature from different Malaysian marine coastal waters, our results for Cd concentration is lower than that reported in the literature. Cadmium and Pb have higher tendencies to bioaccumulate in the fish liver tissues which are involve in the detoxification process.

The presence of free protein-thiol group content and metallothioneins binding proteins in the liver forms strong fixation with the trace metals **(Iwegbue, 2008).** Meanwhile fish liver acts as major site for homeostasis **(Reynders et al., 2006).**

The variability in trace metal levels in different species depends on feeding habits, ecological needs , metabolism, age, size , length of the fish and fish habitat (**Canli and Atli, 2003).**

Concentrations of trace metals detected in the muscle, gill and liver samples indicate different bioaccumulation potentials. Muscles seem to be a transitory tissue in the pathway of metal uptake and in metal storage, and liver appears to be the tissue specialized in metal storage and detoxification (**Kotze et al., 1999).**

The gills comprise the chief exposure tissue and early uptake site of the soluble, waterborne metals in which metal concentrations are the highest in the early stages of exposure before these metals are transported to other fish tissues (**Jezierska and witeska, 2001**).

Although human activity is concentrated in the west coast of Peninsular Malaysia compared with the east coast, there is heavy contamination with trace metals in the east coast.

The results of the present study suggest that some point sources of trace metal contamination are present in the east coast of Peninsular Malaysia in spite of the relatively low human activities there.

Conclusion:

The highest metal concentrations were found in the fish liver and gill tissues, while the muscles tended to accumulate relatively low metal levels. Generally, the Fe concentrations were the highest in water and all organs of the two studied species in both studied areas, except the muscles of ***P anea*** from Kapar which had higher Zn concentration, and the gills of ***A. maculatus*** from both Kapar and Mersing had higher Al and Zn levels, respectively. Moreover, the ***A. maculatus*** species had higher metal concentrations than the *P anea*. The mean concentrations of trace metals analyzed in the muscles of both species were lower than the permissible limits.

Moreover, Cd concentrations in water samples in both sites exceed the international standard.

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تراكم المعادن النادرة فى الأنسجة العضلية والكبدية والخيشومية لنوعى الأسماك المصادة من مياه Pennahia anea و Arius maculates السواحل الماليزية

فتحي الهاشمي بشير– قسم الانتاج الحيواني– كلية البيطرة والعلوم الزراعية – جامعة الزاوية–ليبيا

تعتبر الأسماك مؤشرا جيدا على تلوث النظام المائى بالمعادن النادرة بالإضافة إلى أنها مصدر هام للبروتين. لذلك تم إختبار تركيز كل من المعادن (الحديد – الزنك – الألمونيوم – الزرنيخ – الكاديوم – الرصاص) فى الماء وكل من الأنسجة العضلية والكبدية والخيشومية لنوعى الأسماك Arius maculates و Pennahia anea التى تم اصطيادها من منطقتي كابار ومرسنق التى تقع على السواحل الماليزية.

بشكل عام، كان لمعدن الحديد أعلى تركيز فى كل من الماء نوعي الاسماك التى تم دراستهما، بينما أظهر معدن الكاديوم فى كل من مياه سواحل المنطقتين مستويات عالية تفوق الحدود المسموح بها دوليا.

كانت مستويات تركيز المعادن فى عينات الأسماك فى ترتيب تنازلى من حيث التركيز من الكبد > الخياشيم > العضلات. ولحسن الحظ كان تركيز هذه المعادن المدروسة فى عينات الأسماك أقل من الحدود المسموح بها محليا فى ماليزيا وعالميا.