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# Contamination profile of heavy metals in marine fish and shellfish

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**ABSTRACT**

Rapid industrialization along with advanced agricultural activities led to the contamination in aquatic environment with heavy metals. Heavy metals ultimately pass into human body through having aquatic animals like fish, prawn and crab. In this study, accumulation of heavy metals (zinc, copper, iron, cadmium and lead) in various organs of four commonly consumed fish (*Euthynnus affinis*, *Pampus argenteus, Descapterus macrosoma,* and *Leiognathus daura*), prawn (*Fenneropenaeus indicus) and* crab *(Portunus pelagicus)* of Tok Bali Port, Kelantan, Malaysia were determined. Health risk was assessed using estimated daily intake and target hazard quotients. Although the concentrations of all the heavy metals in all fish, prawn and crab species were lower as per Malaysian Food Act, but the concentrations showed remarkable differences among the species and organs. The concentration of heavy metals in the gill was the highest of all fish species followed by in the liver and flesh. The total accumulation of heavy metals was maximum in *Euthynnus affinis* followed by *Leiognathus daura*, *Descapterus macrosoma* and *Pampus argenteus* of the fish species. However, the highest concentrations (µg/g) was for Zn (72.97±2.75), followed by Fe (4.309±0.68), Cd (1.189±0.78), Cu (1±0.87) and Pb (0.41±0.19) among all the heavy metal contents of fish. No significant variation ($P<0.05$) of the heavy metal concentration in prawn and crab species was observed. The investigation indicated that the fish, prawn and crab species of this port were safe for human consumption but the safe disposal of various wastes should be practiced to control the heavy metal accumulation in future.

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## INTRODUCTION

Fish is consumed all over the world for its nutritional value, along with its high quality proteins, great omega-3 fatty acid content, and low saturated fat content and due to having a good level of vitamin-B (Özden *et al.,* 2018; Hajeb *et al.,* 2009; Arulkumar *et al.*, 2017; Gu *et al.,* 2017). It is anticipated that fish contributes about 17% of animal protein and almost 6% of all protein consumed by human beings (Jos´e *et al.,* 2007). However fish become threat to human health because of heavy metals accumulation in marine and fresh water via various urbanization,

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agricultural and industrial activities (Li *et al.,* 2017; Martin *et al.,* 2015; Zhang *et al.,* 2011). Heavy metals like Cadmium (Cd), Chromium (Cr), Lead (Pb), Iron (Fe), Manganese (Mn), Copper (Cu), Nickel (Ni), Arsenic (As), Mercury (Hg) and Zinc (Zn) have high level of toxicity, and persistence capacity possessing potential for biomagnification, bioaccumulation and incorporation into the food chain after reaching a certain limit in the aquatic environment (Hosseini *et al.,* 2012; Zhang *et al.,* 2010; Ahmed *et al.,* 2015; Kibria *et al.,* 2016). Bioaccumulation and biomagnification denote the processes and pathways of heavy metal pollutants from one trophic level to others in the food-web (Part *et al.,* 1985). In the aquatic environment heavy metals are assimilated by fish, since fish is great bioaccumulator, assimilation can occur by ingestion, ion exchange across gills or membrane surface and adsorption by tissues of the fish (Ahmed *et al.,* 2014). Fish also have diverse bioaccumulation characteristics, and accumulation can occur in the head, liver, bones, kidney, stomach, heart, muscle, operculum, vertebrae and flesh of fish species *(Murtala et al., 2012; Zhao et al.,* 2012). It happens in different parts of different fish species due to the varying soluble nature of metals in water, bioavailability and the different habitats, life cycles, nature of feeding, ecology and physiological nature of fish (Perugini *et al.,* 2014; Anandkumar *et al.,* 2017). High bioaccumulation of heavy metals in the aquatic body can lead to genotoxic damage of aquatic species and high concentrations of heavy metals can have cytotoxic, mutagenic and genotoxic effects on fish species (Matos *et al.,* 2017). Consumption of fish with high level of heavy metal contamination can be a serious health hazard (Salamat *et al.,* 2015). Cd can be seriously injurious for health, causing kidney damage, respiratory dysfunction, renal and hepatic system damage, bone disease, hyper tension, poor reproductive capacity, tumors etc. due to its long term uptake by the human body (Satarug *et al.,* 2017; Lee *et al.,* 2011). The accumulation of Hg in the human body causes damage to distinct visual cortex areas, and neuronal loss of adult brain (Al-Busaidi *et al.,* 2011) and neural impairment in infants (Harada *et al.,* 1994). Pb can have a contribution to renal failure as well as liver damage in humans (Lee *et al.,* 2011). Long-term Pb consumption can cause mental retardation and even mortality of humans (Wani *et al.,* 2015). Non- essential heavy metals (Cd and Pb) even at trace amounts can exhibit extreme toxicity while essential metals (Cu, Zn and Fe) can also cause health problems due to overdosing. As heavy metals cannot be degraded they accumulate in aquatic organisms or animal and are deposited in sediments (Merian 1991, Linnik *et al.,* 2000; Gale *et al.,* 2004, Wei *et al.,* 2014), it is very essential to assess the health risk assessment of heavy metals in human health through consumption of aquatic animal. The sources of heavy metals in water-bodies include the paint on ships, diverse industrial processes e.g. stabilizers for PVC and agricultural activities such as the use of fungicides, pesticides etc. (Lee *et al.,* 2016). Where rapid industrialization and mechanized agricultural activities enhance the heavy metal pollution rate the environmental hazards to invertebrates, and vertebrates,- including fish and humans are increased (Bashir *et al.,* 2013; Yi *et al.,* 2017). Fast moving industrialization and economic development in Malaysia has led to the utilization of toxic chemicals including heavy metals, and has resulted in increasing water pollution in its coastal regions (Bashir *et al.,* 2013, Agusa *et al.,* 2005). Tok Bali Port is the largest port in Kelantan and makes a great economic contribution to Malaysia. However, the various shipping and transportation activities around this port may also contribute to heavy metal pollution in sea water and subsequent accumulation in commercially important marine fishes, prawn and crabs (Salleh and Halim 2018). Several studies in port city and coastal region around the world shows the presence of heavy metals in commercially important fish, prawn and crab species and their health effects ( Amisah *et al.,* 2011; Schintu *et al.,* 2016; Sany *et al.,* 2012; Nemr *et al.,* 2016; Sharif *et al.,* 1993; Velusamy *et al.,* 2014; Gu *et al.,* 2015; Sarkara *et al.,* 2016). However there is less data available about the presence of heavy metals in fish, prawn and crabs consumed around the Malaysia and consequently about their contribution to the dietary intake of the Malaysian population. Considering this situation it is important to determine the concentration of heavy metals in edible commercially important fish, prawn and crab species available in the east coast region of Tok Bali Port, Kelantan, Malaysia. In this study concentration of heavy metals (Zn, Cu, Fe, Cd and Pb) in various organs of four commonly consumed fish namely *Euthynnus affinis (*eastern little tuna), *Pampus argenteus* (silver Pomfret)*, Decapterus macrosoma* (Layang scad), and *Leiognathus daura* (blackfin ponyfish), one prawn species namely *Fenneropenaeus indicus* (Indian white prawn) and one crab species *Portunus pelagicus* (blue crab) were determined along with various health risk assessment indexes (EDI and THQ). The study has been carried out in Malaysia in 2015.

## MATERIALS AND METHODS

*Study area*

Tok Bali Port is the largest port in Kelantan, Malaysia and provides a major food source to the community of Kelantan. Tok Bali is located about 48 kilometer from Kota Bharu and is considered as a major player in marine services in the state of Kelantan. The shipping and transportation industry near to this port are major sources of water pollution. Greywater from ships also contributes to the remarkable pollution in this port. Fish, prawn and crab samples were collected from Tok Bali Port in Pasir Puteh, Kelantan with an exact location of $5^0$53’49.1”North and $102^0$29’06.8”Eeast in 2015. The sampling location is given in Fig. 1.

*Collection, sample preparation and heavy metals analysis*

Four fish species, namely, layang scad (n=10), estern little tuna (n=10), blackfin ponyfish (n=10), and silver pomfret (n=10) and two different species of shellfishes namely, indian white prawn (n=10), and blue crab (n=10) were collected in January, 2016. The size and weights of the collected fish and shellfish are given in Table 1. The samples were primarily retained in the zip-locked acid-washed polyethylene bags at -20 °C and finally transported to the laboratory for further processing. In the laboratory, after separating the gills, livers and muscles the sample tissue was homogenized by grinding. The homogenized sample was then freeze dried at (-10 °C) and ground to a fine powder. After adding 5 ml of concentrated nitric acid and 5 ml of hydrogen peroxide to the 5 g of dried sample in the digester and stored at room temperature for 24 h (Asegbeloyin *et al.,* 2010). Finally, the concentration of heavy metals were determined by atomic absorption spectrometer (AAS); Perkin-Elmer-3300 by using standard diluted sample solution. For each set of experiments blanks, certified reference materials (CRMs), and samples were run and corrections applied where necessary. All of the experiments were carried out in triplicate to eliminate any batch-specific errors and only average values were reported (Islam *et al .,* 2014). All laboratory equipment used was washed with

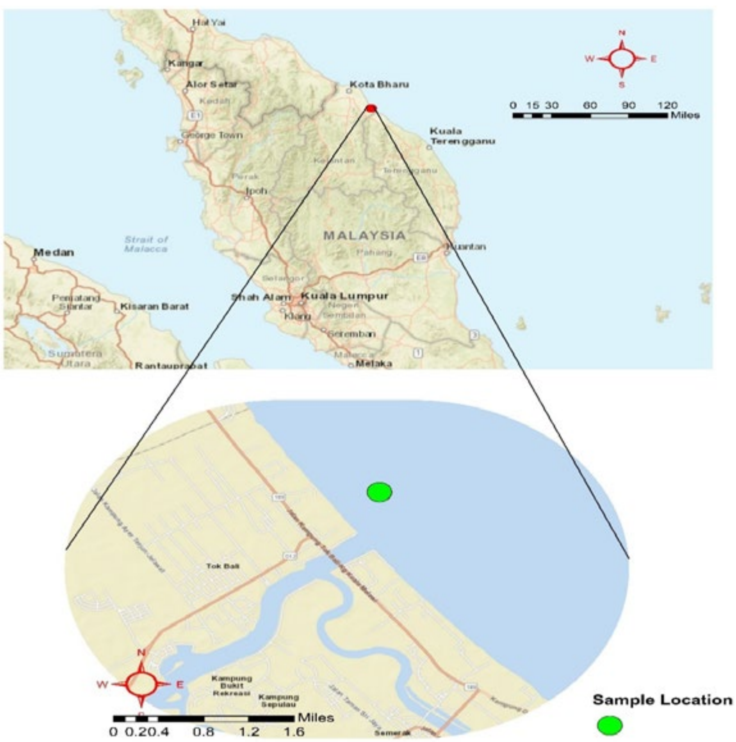


Fig. 1. Geographic location of the study area along with the sampling sites in Kelantan, Malaysia

Table 1. List of fish, prawn and crab species with length, weight and Fulton's condition factor (K) along with ecological characteristics

| English name | Scientific name | Habitat | Length (cm) | Weight (g) | K |
|---|---|---|---|---|---|
| Fish species | | | | | |
| Layang Scad | *Descapterus macrosoma* | Pelagic (reef slopes) | 15.5 | 57.5 | 1.54 |
| Estern little tuna | *Euthynnus affinis* | Epi-pelagic | 25.1 | 181.01 | 1.14 |
| Blackfin ponyfish | *Leiognathus daura* | Demersal and pelagic (water column) | 32.01 | 509.98 | 1.56 |
| Silver pomfret | *Pampus argenteus* | Benthopelagic | 23.5 | 164.05 | 1.26 |
| Prawn | | | | | |
| Indian white prawn | *Fenneropenaeus indicus* | Demersal | - | 200 | - |
| Crab | | | | | |
| Blue crab | *Portunus pelagicus* | Intertidal | - | 200 | - |

phosphate-free soap, double rinsed with distilled water and left in 10% $HNO_3$ for 24 h to prevent contaminations. Calibration, the use of blanks, spike samples, the performance characteristics of the procedure and the reporting of outcome were the significant aspect of QA and QC (Natural Resources Management and Environment Department, 1998) in the study. Recovery of all metals studied ranged from 90 to 98.6%.

*Statistical analysis*

Descriptive statistical analysis throughout the study was carried out by using Statistical Package for the Social Sciences (SPSS) 16 software. The statistical significance of the heavy metals concentrations within the same tissue and the heavy metals concentrations among the six different species were calculated by using the Tukey (Honestly Significant Difference) HSD test. Correlation values among heavy metals content among various organs and fish length were calculated by SPSS. Fulton condition factor (K) was calculated (Table 1) according to Eq. 1 (Htun-Han, 1978).

$$K = 100\left(\frac{W}{L^3}\right) \quad (1)$$

Where W is the weight of fish in gram (gm) and L is the length of fish in centimeter (cm).

*Health risk assessments*

The estimated daily intake of metals was estimated by using Eq. 2 (Onsanit *et al.,* 2010).

Estimated daily intake (EDI)=

$$\frac{metal\ concetation\left(\frac{ug}{g}w\ /w\right)\times consumtion\ rate\left(\frac{g}{d}\right)}{body\ weight\ (kg)} \quad (2)$$

Metals concentration in the fish, prawn and crab used in above equation, were the average maximum values of trace element concentrations in muscle. The body weights for children and adults were assumed to be 32.7 kilogram (kg) and 55.9 kg, respectively, the consumption rate was 50 g/day and 93 g/day for children and adults (Zhao *et al.,* 2012). The Tolerable intake was considered in this study to examine the EDI of metals in fishes, prawns and crabs. The tolerable intake for Zn, Cu, Fe Cd and Pb are 300-1000, 50-500, 80-100, 1 and 3.57 µg/kg/day, respectively as Eq. 3. (JECFA, 2009).

$$\text{Target hazardous quotient (THQ)} = \frac{EF\ X\ ED\ X\ FIR\ X\ C}{RfD\ X\ WAB\ X\ TA}10^{-3} \quad (3)$$

Here EF represents exposure frequency in day/year; ED represents exposure duration (70 years and 6 years for adult and children respectively); FIR (Food ingestion rate) represents the food ingestion rate (93 g/Day and 50 g/Day for adults and children); C is the measured concentration; RfD = reference dose (µg/g); WAB is average body weight in kg and TA is average exposure time for non-carcinogens (365 day/year X ED). There would be no obvious risk if the calculated THQ is-less than 1 (USEPA, 2011).

## RESULTS AND DISCUSSION

*Physical characteristics of fish, prawn and crab*

Table 1 shows the values of Fulton's condition factor calculated in this study for all analyzed samples. The value of "k" greater than one suggesting that well grown fish were collected and also indicating that the fish were in good condition (Nasri *et al.,* 2017).

*Comparisons of heavy metals in selected fish, prawn and crab species*

Age, diet, metabolism, size and length of the fish should be considered as the factors for analyzing the fish species of same coastal environment (Noelia *et al.,* 2017). The concentrations of five heavy metals (Zn, Cu, Fe, Cd and Pb) have been determined in the tissues (i.e. gill, liver and flesh) of four fish species, one prawn and one crab species in the coastal area of Tok Bali, Kelatan, Malaysia are listed in Table 2. The ranges of concentration of heavy metal content are as follows: 72.970 - 4.827 µg/g for Zn, 0.789 - 0.018 µg/g for Cu, 4.309 - 0.033 µg/g for Fe, 1.189 -0.003µg/g for Cd and 0.410 - 0.001 µg/g for Pb. The highest concentrations was for Zn, followed by Fe, Cd, Cu and Pb. The order of species according to the heavy metal accumulation is as follows: *Leiognathus daura> Euthynnus affinis> Descapterus macrosoma> Pampus argenteus* for Zn, *Euthynnus affinis> Pampus argenteus> Descapterus macrosoma>Leiognathus daura* for Cu, *Leiognathus daura> Euthynnus affinis> Descapterus macrosoma> Pampus argenteus* for Fe, *Euthynnus affinis> Leiognathus daura> Pampus argenteus> Descapterus macrosoma* for Cd and Pb, as seen in Table 2. In the case of prawn and crab species the highest amounts of Zn and Fe were found in *Fernneropenaeus indicus* 14.40 µg/g and 2.11µg/g respectively because *Fernneropenaeus indicus* (indian white prawn) are usually prefers mud or sandy mud at depths of 2 - 90 meters. The highest concentrations of Cu, Cd and Pb were 1.0 µg/g, 0.1µg/g and 0.02µg/g respectively and were found in *Portunus pelagicus*. Among all species of fish, prawn and crab, the hazardous heavy metal content (Pb and Cd) in shellfish species were the highest and were considered alarming. For other heavy metals (Zn, Fe and Cu) concentration were much lower for the fish species, which is also supported by other research (Losasso *et al.,* 2015). The assessment of heavy metals concentrations in fish muscle in different studies around the world is shown in Table 2. From Table 2, it is clear that the concentrations of Zn and Cu from the present study were higher than in the *Lates calcarifer* of Malaysia. The major hazardous heavy metals (Pb, Cd) concentration were lower in this study as compared to the mentioned species of different regions. The Cd content in *Fenneropenaeus indicus* of Tok Bali Port was much lower than in the species found in Gangha River, India.

*Comparisons of heavy metals in various organs of fish species*

The bioaccumulation of heavy metals in fish organs may differ due to the physiological differences in the function and structure of different tissues (Kotze, 1997). The gill tissue was selected for study because it is considered to be the organ most exposed to heavy metals while the liver tissue was selected because it is considered as the storage of heavy metals in the fish body and the muscle tissue was selected as it is the most popular the edible part that and hence as the main route of heavy metals exposure in humans (Agusa *et al.,* 2007; Taweel *et al.,* 2013).

*Gills*

The study found the highest concentration of Zn and Pb of 72.97 µg/g and 0.203 µg/g respectively were in the gills of *Leiognathus daura* species while the lowest concentrations of Zn and Pb, 0.099 µg/g and 0.014 µg/g respectively, were found in the gills

Table 2. Comparison of heavy metals in fish muscle tissue with other different studies in the world

| Species | Location | Heavy metals (µg/g) Zn | Cu | Fe | Cd | Pb | Reference |
|---|---|---|---|---|---|---|---|
| *Euthynnus affinis* | Malaysia | 62.43±7.01 | 0.705±0.2 | 2.125±0.28 | 0.007±0.008 | 0.002±0.001 | Present study |
| *Pampus argenteus* | Malaysia | 4.827±0.67 | 0.038±0.01 | 0.038±0.02 | 0.004±0.001 | 0.024±0.04 | Present study |
| *Descapterus macrosoma* | Malaysia | 5.286±1.08 | 0.064±0.03 | 1.288±0.36 | 0.003±0.002 | 0.001±0.001 | Present study |
| *Leiognathus daura* | Malaysia | 5.456±0.67 | 0.018±0.004 | 4.309±0.68 | 0.004±0.003 | 0.003±0.002 | Present study |
| *Pampus argenteus* | Hara Reserve , Iran | NA | NA | NA | 0.22±0.006 | 0.58±0.003 | Mohammadnabizadeh *et al.,* 2014 |
| *Euthynnus affinis* | Karachi Fish Harbour,Pakistan | NA | NA | 38.02±9.10 | NA | 0.46±0.14 | Ahmed *et al.,* 2015 |
| *Lates calcarifer* | Malaysia | 6.500±1.917 | 0.495±0.066 | 5.103±1.77 | 0.007±0.004 | 0.201±0.080 | Nasri *et al.,* 2017 |
| *Fenneropenaeus Indicus* | Malaysia | 14.4±1.58 | 0.34±0.39 | 2.11±0.87 | 0.04±0.03 | 0.008±0.01 | Present study |
| *Fenneropenaeus Indicus* | Gangha, India | 12-226 | 14-92.10 | NA | NA | 2.20-23.10 | Bhattacharyya *et al.,* 2013 |

*NA-Not available*

of *Decapterus macrosoma*. The highest concentration were observed in the gills because, as respiratory organ, the gills extracts heavy metals along with dissolved oxygen from the water during respiratory action as reported by various authors (Zhao *et al.,* 2012, Monday *et al.,* 2007; Bashir *et al.,* 2013). The maximum concentrations of Cu, Fe and Cd were observed to be 0.79 µg/g, 3.969 µg/g and 0.191 µg/g respectively in the gills of *Euthynnus affinis* while the lowest concentration of Cu was 0.099 µg/g in the gills of *Pampus argenteus* and, the lowest concentrations of Fe, Cd and Pb were 1.313 µg/g, 0.013 µg/g and 0.014 µg/g respectively in gill of *Descapterus macrosoma*. *Pampus argenteus* is a bottom feeding species while *Euthynnus affinis* takes food from the surface waters and *Decapterus macrosoma* is pelagic (being neither close to the bottom nor near the shore) schooling species which feeds on smaller planktonic invertebrates. Differences in the trophic level of a species' habitat show different characteristics of heavy metal accumulation in fish species where the organs of fish play vital roles in binding metals but the binding abilities varies from organ to organ *(Qiao-qiao et al.,* 2007; Gu *et al.,* 2015). As *Leiognathus daura* is a demersal (it lives on or near the bottom) species, there is higher bioavailability of Zn to the species because of the availability of a higher content of Zn in the sediment and water (Akbulut and Akbulut, 2010). Bottom level inhabiting fish have higher potentials for the uptake of heavy metals than top level inhabiting fish due to direct contact with the sediment (El-Moselhy *et al.,* 2014; Wei *et al.,* 2014, Gu, 2015). The present study found the highest concentration of Zn at 72.97 µg/g in *Leiognathus daura* species while there was less Zn in *Pampus argenteus and Descapterus macrosoma.* Although, heavy metal uptake depends on various factors such as age, geographical distribution, feeding nature, trophic level and species specific factors that varied from organ to organ (El-Moselhy *et al.,* 2014).

*Flesh*

The determination of heavy metals in fish flesh is important as it is directly consumed by humans. The maximum level of Fe detected was detected 4.32 µg/g in the flesh of *Leiognathus daura* and the lowest amount of 0.04 µg/g was in the flesh of *Pampus argenteus*. Although both *Leiognathus daura* (blackfin ponyfish) and *Pampus argenteus* (silver pomfret) are benthopelagic species, their feeding habits are different. The maximum concentrations of Zn, Cu and Cd were observed in the flesh of *Euthynnus affinis* where the concentrations were 62.430 µg/g, 0.705 µg/g and 0.007 µg/g respectively. The minimum concentrations of Zn, Cu and Cd were 4.827 µg/g, 0.018 µg/g and 0.003 µg/g in flesh of *Pampus argenteus, Leiognathus daura* and *Descapterus macrosoma* respectively. The maximum concentration of Pb (0.024 µg/g) in flesh of *Pampus argenteus* and the minimum concentration of Pb (0.001 µg/g) was in the flesh of *Descapterus macrosoma*. The flesh of all fish species contained low amounts of Pb. The concentration of Cd was also minimum in the flesh of all fish samples studied.

*Liver*

Higher accumulations of Zn, Fe Cd and Pb of 43.740 µg/g, 3.897 µg/g, 1.19 µg/g and 0.41µg/g respectively were found in the liver of *Euthynnus affinis* while the lower amounts of Cd and Pb were found to be 0.114 and 0.015 µg/g in the liver of *Descapterus macrosoma*. The lowest concentration of Fe was 0.77 µg/g in the liver of *Pampus argenteus* and the lowest concentration of Zn was 9.440 µg/g in the liver of *Leiognathus daura*. However, the highest concentration of Cu was 0.637 µg/g in the liver of *Pampus argenteus* and the lowest concentration was 0.216 µg/g in the liver of *Leiognathus daura*. The liver plays major role in metabolic processes and shows a tendency to accumulate metals at higher levels (Zhao *et al.,* 2012*)*, thus a higher concentration of Cd, Pb, Zn and Fe was observed in the liver of *Euthynnus affinis*. Generally, it is considered that the liver acts as an environmental indicator of water pollution because it accumulates various kinds of pollutants at higher levels than surrounding environment and plays an important role in contaminant storage, detoxification, redistribution, transformation and act as an active pathological site (Licata *et al.,* 2005; Ebrahimpour *et al.,* 2011).

*Relationships between inter metal levels in various organs and fish sizes*

The relationship between metal concentrations and fish length were determined and are shown in Table 4. From the correlation (Pearson Correlation) values (r) it is clear that there is a negative correlation between fish size and metal concentration in the various organs of fish. For instance, the Fe concentration in the gills of all the species has a strong negative correlation (r=-

0.99) with the fish length and the Cd concentration in the liver of all species is also strongly negatively correlated (r= - 0.99) with fish size. The decrease in metal concentration with fish size may be due to rapid tissue growth rather than the metal uptake rate (Merciai *et al.,* 2014). From the correlation value it is seen that the metal concentration in the gills of fish has a stronger negative correlation than other organs. This is because the respiratory rates of small fish are higher than larger fish, resulting in a higher quantity of respiratory water passing through the gills and therefore higher accumulation of metals (Merciai *et al.,* 2014). Usually *Euthynnus affinis* has an average length of 45 - 60 cm, but our collected samples had an average length of 25.1 cm, and due to this small size and associated higher respiratory rate its metal accumulation in the gill was higher as compared with other species. In the case of *Leiognathus daura* the higher metal concentrations in the gills (Table 3) was observed because it is a demersal fish which lives and feeds on or near the sea floors and there is always a strong possibility of heavy metal accumulation in sediment (Bergstad, 2009). Again the high metabolic rate and faster short-term uptake by smaller species are also responsible for the higher accumulation of heavy metals (Newman and Mitz, 1988; Newman and Mitz, 1989). In our study we observed a positive correlation of Pb concentration with fish size and a negative correlation of Pb content in fish muscle with Pb content in other organs of fish.

Table 3. Heavy metal concentration (μg/g) found in different selected fish, prawn and crab species

| Fish | Organ | Zn | Cu | Fe | Cd | Pb |
|---|---|---|---|---|---|---|
| *Euthynnus affinis* | gill | $69.2\pm9.31^{aA}$ | $0.789\pm0.16^{aB}$ | $3.969\pm1.52^{aB}$ | $0.191\pm0.15^{aB}$ | $0.134\pm0.06^{aB}$ |
| | liver | $43.74\pm8.45^{aA}$ | $0.445\pm0.28^{aB}$ | $3.897\pm1.55^{aB}$ | $1.189\pm0.78^{aB}$ | $0.41\pm0.19^{abB}$ |
| | flesh | $62.43\pm7.01^{bA}$ | $0.705\pm0.2^{aB}$ | $2.125\pm0.28^{aB}$ | $0.007\pm0.008^{aB}$ | $0.002\pm0.001^{bB}$ |
| *Pampus argenteus* | gill | $14.53\pm3.46^{aA}$ | $0.377\pm0.16^{aB}$ | $2.609\pm1.39^{aB}$ | $0.03\pm0.01^{aB}$ | $0.203\pm0.02^{aB}$ |
| | liver | $15.25\pm4.42^{abA}$ | $0.637\pm0.33^{aB}$ | $0.77\pm0.12^{abB}$ | $0.636\pm0.3^{bB}$ | $0.17\pm0.02^{aB}$ |
| | flesh | $4.827\pm0.67^{bA}$ | $0.038\pm0.01^{bB}$ | $0.038\pm0.02^{bB}$ | $0.004\pm0.001^{bB}$ | $0.024\pm0.04^{bB}$ |
| *Descapterus* | gill | $13.54\pm1.23^{aA}$ | $0.099\pm0.09^{aB}$ | $1.313\pm0.25^{aB}$ | $0.013\pm0.006^{aB}$ | $0.014\pm0.01^{aB}$ |
| *macrosoma* | liver | $23.69\pm2.75^{bA}$ | $0.218\pm0.18^{aB}$ | $1.99\pm0.33^{aB}$ | $0.114\pm0.11^{aB}$ | $0.015\pm0.009^{aB}$ |
| | flesh | $5.286\pm1.08^{cA}$ | $0.064\pm0.03^{aB}$ | $1.288\pm0.36^{aB}$ | $0.003\pm0.002^{aB}$ | $0.001\pm0.001^{aB}$ |
| *Leiognathus daura* | gill | $72.97\pm2.75^{aA}$ | $0.338\pm0.13^{aB}$ | $2.673\pm1.78^{aB}$ | $0.033\pm0.02^{aB}$ | $0.205\pm0.02^{aB}$ |
| | liver | $9.44\pm1.08^{bA}$ | $0.216\pm0.06^{abB}$ | $1.364\pm1.03^{aB}$ | $0.649\pm0.9^{bB}$ | $0.15\pm0.08^{aB}$ |
| | flesh | $5.456\pm0.67^{cA}$ | $0.018\pm0.004^{bB}$ | $4.309\pm0.68^{aB}$ | $0.004\pm0.003^{bB}$ | $0.003\pm0.002^{bB}$ |
| *Fenneropenaeus indicus* | | $14.4\pm1.58^{aA}$ | $0.34\pm0.39^{aB}$ | $2.11\pm0.87^{aB}$ | $0.04\pm0.03^{aB}$ | $0.008\pm0.01^{aB}$ |
| *Portunus pelagicus* | | $12.43\pm3.85^{aA}$ | $1\pm0.87^{aB}$ | $1.78\pm0.79^{aB}$ | $0.09\pm0.07^{aB}$ | $0.015\pm0.01^{aB}$ |
| WHO/FAO | | 150 | 10 | 43 | 0.2 | 1.5 |
| Malaysia Food Act | | 100 | 30 | NA | 1 | 2 |

Letter (A, B) in rows with different letter are significantly different (P<0.05)
Letter (a, b, c) in columns with different letter are significantly different (P<0.05)
WHO: World Health Organization; FAO: Food and Agriculture Organization (FAO, 1983; MFA, 1983).

Table 4. Relationships between inter metal levels in various organs and fish sizes

| | | Length | Gill | | | | | Liver | | | | | Flesh | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Zn | Cu | Fe | Cd | Pb | Zn | Cu | Fe | Cd | Pb | Zn | Cu | Fe | Cd | Pb |
| Length | | 1 | | | | | | | | | | | | | | | |
| Gill | Zn | -0.75 | 1 | | | | | | | | | | | | | | |
| | Cu | -0.98 | 0.63 | 1 | | | | | | | | | | | | | |
| | Fe | -0.99 | 0.71 | 0.98 | 1 | | | | | | | | | | | | |
| | Cd | -0.89 | 0.59 | 0.94 | 0.87 | 1 | | | | | | | | | | | |
| | Pb | -0.50 | 0.42 | 0.39 | 0.55 | 0.07 | 1 | | | | | | | | | | |
| Liver | Zn | -0.57 | 0.22 | 0.69 | 0.54 | 0.86 | -0.39 | 1 | | | | | | | | | |
| | Cu | -0.36 | -0.2 | 0.44 | 0.45 | 0.25 | 0.47 | 0.16 | 1 | | | | | | | | |
| | Fe | -0.63 | 0.49 | 0.69 | 0.59 | 0.89 | -0.35 | 0.94 | -0.1 | 1 | | | | | | | |
| | Cd | -0.99 | 0.69 | 0.98 | 0.99 | 0.88 | 0.54 | 0.55 | 0.44 | 0.59 | 1 | | | | | | |
| | Pb | -0.98 | 0.63 | 0.99 | 0.98 | 0.95 | 0.38 | 0.70 | 0.44 | 0.70 | 0.98 | 1 | | | | | |
| Flesh | Zn | -0.84 | 0.54 | 0.90 | 0.82 | 0.99 | -0.03 | 0.92 | 0.21 | 0.93 | 0.89 | 0.96 | 1 | | | | |
| | Cu | -0.81 | 0.50 | 0.88 | 0.78 | 0.98 | -0.08 | 0.94 | 0.21 | 0.94 | 0.72 | 0.89 | 0.99 | 1 | | | |
| | Fe | -0.28 | 0.84 | 0.11 | 0.21 | 0.11 | 0.24 | -0.1 | -0.6 | 0.16 | 0.2 | 0.11 | 0.08 | 0.03 | 1 | | |
| | Cd | -0.95 | o.64 | 0.98 | 0.94 | 0.99 | 0.24 | 0.79 | 0.34 | o.80 | 0.94 | 0.99 | 0.96 | 0.95 | 0.13 | 1 | |
| | Pb | 0.06 | -0.5 | -0.02 | 0.02 | -0.2 | 0.54 | -0.3 | 0.85 | -0.6 | 0.02 | 0.04 | -0.3 | -0.3 | -0.64 | -0.17 | 1 |

*Health risk assessment*

In this study both EDI and THQ were used to examine the health risk associated with the uptake of fish, prawns and crabs inhabiting in the Tok Bali coast in Kelantan, Malaysia. Health risk assessments were carried out independently for adults and children because children are considered to be more sensitive to the pollutants than adults (Shou Zhao *et al.,* 2012; Wang *et al.,* 2012).

*Estimated daily intake*

The EDI of heavy metals by human was evaluated according to the mean concentration of metals in aquatic organisms and the amount consumed amount. Table 5 shows the estimated daily intake of heavy metals from fish. The highest concentration of daily intake of Zn for adults was 103.86 µg/kg/day from *Euthynnus affinis* and the lowest intake was 8.03 µg/kg/day from *Pampus argenteus*. The maximum and minimum daily intake of Cu was 1.66 µg/kg/day from *Portunus pelagicus* and 0.03 µg/kg/day from *Leiognathus daura* respectively for adults while it was 1.52 and 0.03 µg/kg/day from *Portunus pelagicus* and *Leiognathus daura* respectively for children. The highest daily intake of Fe was estimated to be 7.17 µg/kg/day from *Leiognathus daura and lowest was 0.06* µg/kg/day from *Pampus argenteus* for adults. For children the highest daily intake estimated for Fe was 6.59 µg/kg/day from *Leiognathus daura* and the lowest was 0.06 µg/kg/day from *Pampus argenteus*. The daily intake of Cd of 0.15 µg/kg/day was the highest from *Portunus pelagius* and the lowest was 0.005 µg/kg/day from *Descapterus macrosoma* for adults. For children the highest daily intake was estimated to be 0.14 µg/kg/day from *Portunus pelagius* and the lowest was 0.005 µg/kg/day from *Descapterus macrosoma*. The maximum and minimum daily intake of Pb was 0.04 µg/kg/day from *Pampus argenteus* and 0.002 µg/kg/day from *Descapterus macrosoma* respectively for adults while it was 0.4 and 0.002 µg/kg/day from *Pampus argenteus* and *Descapterus macrosoma* respectively for children. All estimated daily intake values were lower than the standard estimated data (JECFA, 2009).

*Target hazard quotients*

All of the fish, prawn and crab species discussed in this study were easily available and commonly consumed by the people of Kelantan. The target hazard quotients is considered to recognize the non-carcinogenic health risks due to the pollutants exposure of population (Chien *et al.,* 2002; Yi *et al.,* 2017; Zhang *et al.,* 2017). In Fig. 2, there was no THQ greater than 1 through the consumption of sea food indicating that health risks associated with heavy metals exposure were insignificant. If the total target hazard quotient exceeds 1, it denotes a non-carcinogenic health risk from the accumulative effects of heavy metals among the exposed population (Yi *et al.,* 2017). The THQ of heavy metals from marine fishes, prawn and crab consumption follows the order Zn >Fe >Cd>Cu> Pb in case of adults while the THQ for children follows the order Zn >Fe > Pb>Cd>Cu. Table 5 shows the comparison of the estimated daily intake (µg/kg/day) of heavy metals from studied marine species with the recommended daily dietary allowances by Joint FAO/WHO Expert Committee on Food Additives (JECFA).

The total and individual THQ values of six metals in adults (TTHQ= 0.0012) were higher than those of

Table 5. Estimated daily intake (µg/kg/day) of heavy metals from fish

| Consumer | Species | Zn | Cu | Fe | Cd | Pb |
|---|---|---|---|---|---|---|
| Adult | *Euthynnus affinis* | 103.86 | 1.173 | 3.535 | 0.012 | 0.003 |
| | *Pampus argenteus* | 8.031 | 0.063 | 0.063 | 0.007 | 0.04 |
| | *Descapterus macrosoma* | 8.794 | 0.106 | 2.143 | 0.005 | 0.002 |
| | *Leiognathus daura* | 9.077 | 0.03 | 7.169 | 0.007 | 0.005 |
| | *Fenneropenaeus indicus* | 23.96 | 0.566 | 3.51 | 0.067 | 0.013 |
| | *Portunus pelagicus* | 20.68 | 1.664 | 2.96 | 0.15 | 0.025 |
| Children | *Euthynnus affinis* | 95.46 | 1.078 | 3.249 | 0.011 | 0.003 |
| | *Pampus argenteus* | 7.381 | 0.058 | 0.058 | 0.006 | 0.037 |
| | *Descapterus macrosoma* | 8.083 | 0.098 | 1.969 | 0.005 | 0.002 |
| | *Leiognathus daura* | 8.343 | 0.028 | 6.589 | 0.006 | 0.005 |
| | *Fenneropenaeus indicus* | 22.02 | 0.52 | 3.226 | 0.061 | 0.012 |
| | *Portunus pelagicus* | 19.01 | 1.529 | 2.722 | 0.138 | 0.023 |
| | Tolerable limit (µg/kg/day) | 300-1000 | 50-500 | 80-100 | 1 | 3.57 |

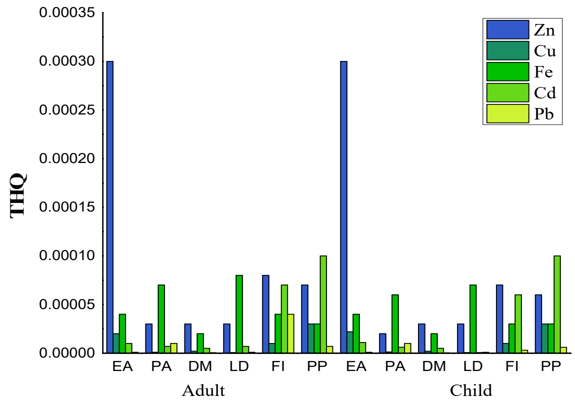


Fig. 2. Target hazardous quotient of heavy metals in fish and shellfish by adult and children (EA: *Euthynnus affinis*; PA: *Pampus argenteus*; DM: *Descapterus macrosoma;* LD: *Leiognathus daura;* FI: *Fenneropenaeus indicus;* PP: *Portunus pelagicus)*

children (TTHQ= 0.00103), implying that adults may suffer higher health risk (Zhao, 2012). Among all the heavy metal studied, marine fish contaminated by Zn, Fe and Cd posed relatively higher health risks, particularly for adults. Overall, the average EDI and THQ values for both adult and children were below harmful levels indicating that the consumption of both fish and shellfish around the Tok Bali Port of Kelantan, Malaysia would not result in health risks from the five studied metals.

## CONCLUSION

This study revealed that the levels of heavy metals in all samples were within the acceptable limit. However, the highest levels of both essential and hazardous heavy metals were found in the gills and liver of the fish species. Among the studied metals, Cd was found to be at the highest levels followed by Pb on the basis of permissible limits. Both the EDI and THQ values revealed that the studied fish, prawns and crabs species should not be considered as a threat to consumers. The data obtained from this study can be used as a safeguard for sustaining of both native and export market of fish industries in Malaysia. In view of importance of the fish nutrition, it is necessary to maintain regular biological monitoring of the Tok Bali Port waters and fish to ensure the continuing safety of seafood. Finally safe disposal of domestic wastes and industrial effluents should be ensured and these waste should be recycled if possible to avoid their accumulation in the marine environment.


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## CONFLICT OF INTEREST

The author declares that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been completely observed by the authors.

## ABBREVIATIONS

| | |
|---|---|
| *AAS* | Atomic Absorption Spectrometer |
| *WAB* | Average body weight |
| *Cd* | Cadmium |
| *⁰C* | Celsius |
| *cm* | Centimeter |
| *CRM* | Certified reference material |
| *Cu* | Copper |
| *DM* | *Descapterus macrosoma* |
| *Eq.* | Equation |
| *EDI* | Estimated daily intake |
| *EA* | Euthynnus affinis |
| *ED* | Exposure duration |
| *EF* | Exposure frequency |
| *FI* | Fenneropenaeus indicus |
| *Fig.* | Figure |
| *FAO* | Food and Agriculture Organization |
| *FIR* | Food ingestion rate |
| *K* | Fulton factor |
| *g* | Gram |
| *HSD* | Honestly Significant Difference |
| *Fe* | Iron |
| *JECFA* | Joint FAO/WHO Expert Committee on Food Additives |
| *kg* | Kilogram |
| *km* | Kilometer |

| | |
|---|---|
| *Pb* | Lead |
| *LD* | Leiognathus daura |
| *MFA* | Malaysia Food Act |
| *m* | Meter |
| *µg/g* | Microgram/gram |
| *µg/kg* | Microgram/kilogram |
| *µg/kg/day* | Microgram/kilogram/Day |
| *PA* | Pampus argenteus |
| *r* | Pearson correlation value |
| *%* | Percent |
| *PP* | Portunus pelagicus |
| *QA* | Quality assurance |
| *QC* | Quality control |
| *RfD* | Reference dose |
| *SPSS* | Statistical package for the social sciences |
| *TA* | Average exposure time for non-carcinogens |
| *THQs* | Target hazard quotients |
| *USEPA* | United States Environmental Protection Agency |
| *WHO* | World Health Organization |
| *Zn* | Zinc |

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