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Heavy Metals and Polycyclic Aromatic Hydrocarbons (PAHs) Concentration in Mud Crab (*Scylla Serrata*) from UMT Mangrove, Terengganu, Malaysia

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ABSTRACT

A study was carried out to determine the bioaccumulation of heavy metals (Cu, Zn, Cd and Pb) in different body parts (muscle, gills, stomach and carapace) and Polycyclic Aromatic Hydrocarbons (PAHs) in muscle of mud crabs (Scylla serrata) collected from mangrove area of University Malaysia Terengganu. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was used to determine the concentration of heavy metals accumulation in mud crabs. This study revealed that Cu and Zn content in muscle part of mud crab were also higher than the maximum permitted level which is only 100 µg/g and 150 µg/g, respectively. Besides, the concentration of Cu, Zn and Cd were increasing with the increase in length of mud crabs while decrease for Pb. This might due to the different in feeding habits and ecological needs metabolisms of young and adult mud crab. The concentration of Cu, Zn and Pb were exceed permissible limit that has set by FAO/WHO while Cd was below the permissible limit. The concentration of Zn, Cd and Cu were above the Provisional Tolerable Weekly Intake (PTWI) value that set by FAO/WHO while Pb was below the value. Besides, the concentration of PAHs accumulated in muscle was detected using Gas Chromatography – Mass Spectrometry (GC-MS). PAHs compounds detected were Dibenzo (a,h) Anthracene, Benzo (a) Pyrene, Benzo (k) Fluoranthene, Fluoranthene, Chrysene, Phenanthrene, Acenaphylene, Ideno (1,2,3-cd) Pyrene, Benzo (g,h,i) Perylene and Benzo (a) Anthracene. The highest concentration of total PAHs was detected in muscle of M2 (1.026 µg/g) while the lowest was in M3 (0.026 µg/g). According to safe consumption guide USEPA, the dosage of PAHs mud crabs may like to cause adverse health problem to human.

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INTRODUCTION

Mud crab (Scylla serrata) is a mangrove or green crab that commonly found in sheltered waters especially in estuaries and mangrove mud flat area of the littoral throughout the Indo-Pacific region. Nowadays, mud crabs was become one of the important fishery commodities in Southeast Asian countries [16]. The crabs stock in the wild is shrinking as throughout the Indo-Pacific region due to they are big in size, delicious and easy to catch. Malaysia is one of the 23 countries have high demand on mud crabs [8]. This famous seafood with high commercial value was widely used in aquaculture practices.

Some heavy metals are essential elements for live and play an important role in some biochemical mechanisms in organisms to support proper growth, development and physiology [32]. However, these heavy metals could become toxic to living organisms if the concentration levels are higher than necessary. High heavy metal concentration can deteriorate the biochemical processes and give adverse health effects to living organisms include human [22]. Previous studies reported heavy metal can increase morbidity and mortality in human being if they exposure to heavy metals continuously [1]. However, heavy metals are still increasing in particular parts of the world [12].

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human being if they exposure to heavy metals continuously [1]. However, heavy metals are still increasing in particular parts of the world [12].

Polycyclic aromatic hydrocarbons (PAHs) normally can be found in urbanized coastal areas around the world. PAHs can easily accumulate in sediments and organisms that and difficult to eliminate them [20]. If we consume some foods that contain PAHs, we will increase the chance to get cancers such as lung, skin and bladder cancers because of the toxicity of PAHs [23]. Some researches has reported that PAHs has high carcinogenic potential to humans [6]. Other than cancers, PAHs also will cause eye irritation, nausea, vomiting, diarrhea and confusion to human being [18].

Mud crabs have become one of the important fishery commodities in Southeast Asian countries. They are very famous seafood with high demand throughout the year. Unfortunately, their feeding behaviour cause high heavy metals accumulate in their body that can lead to death [14]. So, the assessmet on concentration of heavy metal that bioaccumulated in mud crabs is important. Meanwhile, burrowing crustaceans such as crab can be easily exposed to PAHs if the sediment they live contaminated by oil. Crustaceans are less ability to excrete PAHs from their body compared with fish (NOAA, 2010). So, mud crab consumers will get information on the safety level of heavy metals concentration that present in the mud crabs body and the health risk will face by consumers consuming them.

Experimentation:

The study area was located at mangrove area in University Malaysia Terengganu, about 20km to southern of the capital state of Terengganu, Kuala Terengganu (Figure 1). There are many mangrove areas surrounding UMT campus. The study area is located in the wet tropics where high precipitation during monsoon season which heavy rainfall recorded in the month of November and ends in January [15].

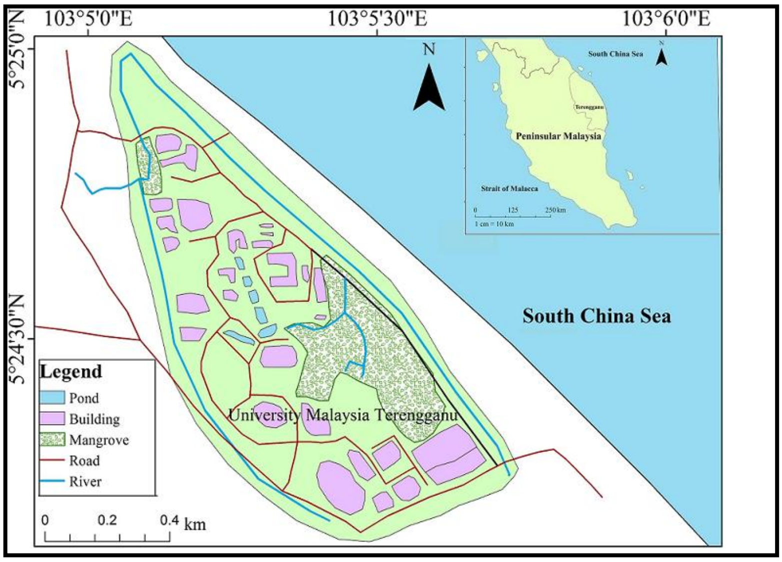

Fig. 1: Sampling stations at UMT enclosed lagoon, Terengganu.

Mud crabs were caught by using crab traps. 10 crab traps will be set in the sampling area. The catchment of samples was conducted before and after spring tides because the catch rates appear to be highest during this period [9]. Neap tides are not suitable to catch mud crabs due to the slow water movement during neap tide cannot spread out the smell of the bait and the crabbing area will be too shallow to access safety [10]. Besides that, samples collection was carried out during high tide. This is because mud crabs will dig into the mud at low tide and move to search their food during high tide [2]. The samples were collected on the next day after the crab traps has been set and identify by using a standard references [17]. After that, the samples were preserved in freezer at -20oC.

In laboratory, weight and size measurement of samples were measured. Before analysis, crab samples were washed with distilled water. After that, the crab samples were dissected in laminar flow bench by using a pair of sterile stainless steel scalpels and Teflon forceps. The dissected samples were replicated and transferred to Petri dishes. Then, samples for heavy metals determination were dried in an oven at 60 oC until they have a constant weight [14]. Meanwhile, samples for PAHs analysis were dried by using freeze drier.

For heavy metal analysis, mortar and pestle were used to homogenize dried samples. For PAHs analysis, the crab's muscles were homogenized by wrap in the aluminium foil and pounded it on the hard surface gently in order to smash the tissues into small pieces and mixed

0.05g of dried samples was weighed and digests with Suprapur nitric acid (HNO3) in a Teflon beaker and heat it. The reagent blank and standard was processed simultaneously. The container was covered and placed in a stainless steel bomb, which was then sealed with a screw closure to avoid any acid leakage and was placed in the oven. The oven temperature was kept for 6 hours at 150°C. The solution was then transferred into a centrifuge tube which has already diluted with Mili Q water after cooling. The values of heavy metals in fish samples were measured by using Inductively Coupled Plasma Mass Spectrometry (ICPMS). 3 replicates were carried out by using the external calibration method [27].

Soxhlet extraction was used for the hydrocarbon extraction by referring to the USEPA standard method 3540. Before carry out the extraction, 10g of the homogenized samples were mixed with 10g of anhydrous sodium sulphate (Na2SO4) that has been heated in the oven at 130°C for 4 hours to remove moisture by using stainless steel spatula. The samples were transferred into a Soxhlet extraction thimble and extracted by mixed with 200 mL solvent mixture of dichloromethane: hexane (1:1) for 4 to 5 cycles per hour. Each sample was extracted for 24 hours. After that, the extracted solution was further concentrated to volume approximately 1 mL by using rotary evaporator with the water bath at 30°C with rotation speed of 35 rpm (revolution per minute). The concentrated sample was then transfer into GC vial and blow to dryness for preservation by using nitrogen blow. All of the GC vial will be screw–capped and sealed with parafilm for the further analysis. These combined elutes were concentrated by using the nitrogen blow technique before inject into a gas chromatograph mass spectrometry (GC/MS) for PAHs analysis. Internal standard mixture and external standard mixture were used in PAHs recovery test.

RESULTS AND DISCUSSION

A total of 25 mud crabs were caught and analysed in this study. All the mud crab was weighted and measured their length and width. The biggest mud crab was measured 137mm in length, 96.5mm in width and 471g while the smallest crab was measured 68.2mm in length, 47.6mm in width and 69.5g in weight.

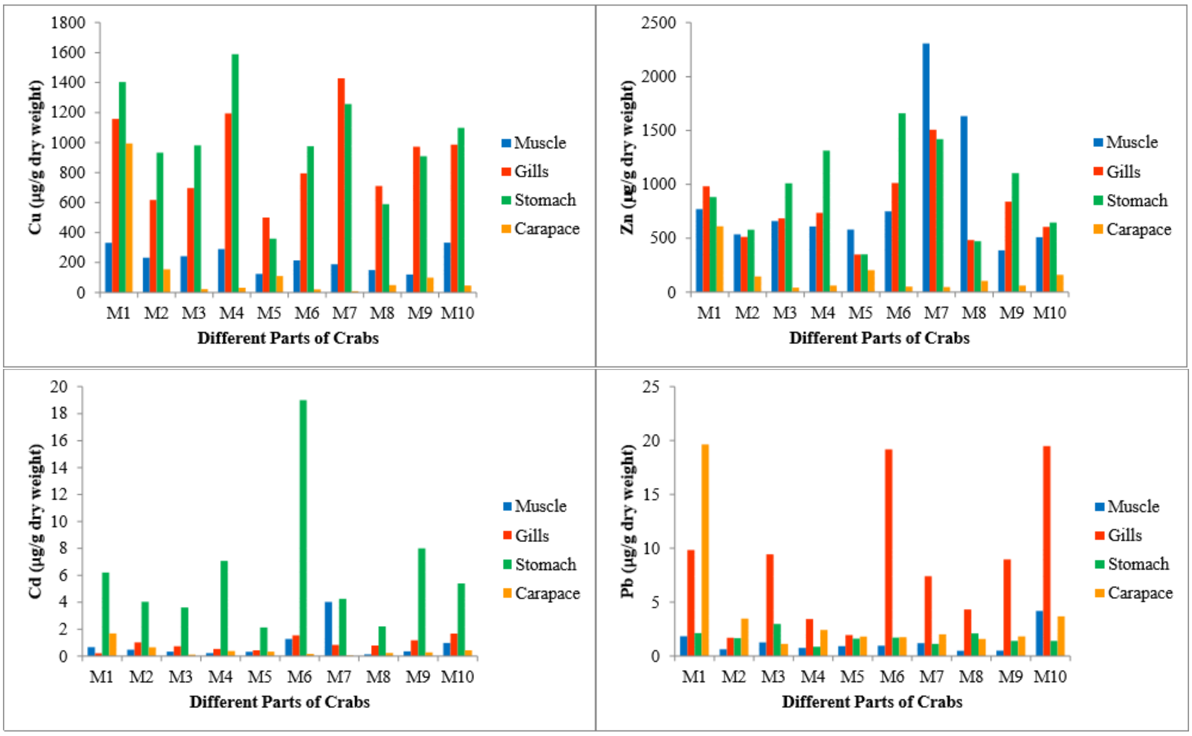

Fig. 2: The concentration of Cu, Zn, Cd and Pb (µg/g dry weight) in different parts of mud crabs

Figure 2 shows the concentration of heavy metals studied in different part of mud crabs collected in UMT mangrove area. In this study, the average concentration of Cu was highest in the stomach (1009 µg/g) compare to the muscle (222 µg/g), gills (906 µg/g) and carapace (153 µg/g). Meanwhile, stomach tissue also recorded the high concentration of Zn (944 µg/g), followed by muscle (874 µg/g) and gills (771 µg/g) but lower in the carapace which is only (149 µg/g). The concentration of Cu and Zn were higher compare to other metals in all body parts. This is because Cu and Zn are essential trace metals in crabs [14]. High concentration of Cu can be found in the haemolymph of crabs which is needed for oxygen transport [29]. Besides, one of the main probabilities that Cu accumulated in crabs is because of its feeding habits. Although there is no clear evidence show the Cu dietary transfer, many present studies have stated that diet is the main route of Cu accumulation in aquatic animals [33]. The Zn concentration higher than other heavy metals might due to Zn played an important role as a precursor in most enzymatic activities. So, high concentration of Zn have high tendency to accumulate in all crab's body parts [16]. Zn that important for normal growth and development in crustaceans cause high concentration of Zn accumulated in crabs hepatopancreas. Hepatopancreas is one of the parts for crabs to effectively eliminate metals through normal physiological processes or store when metals are bioavailable.

On the other hand, stomach (6.20 µg/g) was accumulated the highest Cd concentration compared to others part, gills, stomach and carapace were 1.68 µg/g, 18.9 µg/g and 1.69 µg/g, respectively. However, Pb accumulation was highest in gills (8.57 µg/g) compare to carapace (3.93 µg/g), muscle (1.27 µg/g) and stomach (1.69 µg/g). In aquatic environment, Cd accumulation in shell fish is around 10 times higher than in finfish [30]. This might due to the high affinity of calcium content to these heavy metals [3]. However, the heavy metals accumulated in the carapace can be affected by the periodic molting of the mud crabs [14]. In this study, high concentration of Pb can be found in gills compared to other body parts. This phenomenon can consider normal because high levels of lead was determined in gills in the present study [31]. Higher amounts of Zn and Pb in gills suggest that metals are excreted more rapidly and reduce the body burden of these metals and suggest that Zn and Pb are not accumulated.

There are only 10 PAHs compounds found in present study which included Dibenzo (a,h) Anthracene, Benzo (a) Pyrene, Benzo (k) Fluoranthene, Fluoranthene, Chrysene, Phenanthrene, Acenaphylene, Ideno (1,2,3-cd) Pyrene, Benzo (g,h,i) Perylene and Benzo (a) Anthracene. There are only 2 PAHs compound can be found in first mud crab while 7 PAHs compounds found in the last two mud crabs. To determine the carcinogenic rick of total amount of PAHs in mud crabs, the concentration of each PAHs compound were multiplied with Toxicity Equivalency Factor (TEF) as shown in Table 1 [19]. After calculation, the result for all the PAHs compound was showed in Table 2. The total of the multiplication of the PAHs compound concentration with the TEF gives a BaP equivalent concentration (BaPeq) for each compound. When all the BaPeq of each compound summed up, it will give a carcinogenic Potency Equivalent Concentration (PEC) of all the PAHs (Nisbet and Rasmussen, 1992). The highest BaPeq in present study is Benzo (a) Pyrene (346.36 ng/g) while the lowest is Fluoranthene (0.00514ng/g).

Table 1: Toxicity Equivalency Factor (TEF) of the PAHs Compounds

PAHs Compounds	Toxicity Equivalency Factor (TEF)
Dibenzo (a,h) Anthracene	1
Benzo (a) Pyrene	1
Benzo (a) Anthracene	0.1
Benzo (b) Fluoranthene	0.1
Benzo (k) Fluoranthene	0.1
Ideno (1,2,3-cd) Pyrene	0.1
Anthracene	0.01
Benzo (g,h,i) Perylene	0.01
Chrysene	0.01
Acenaphthene	0.001
Acenaphylene	0.001
Fluoranthene	0.001
Fluorene	0.001
Phenanthrene	0.001
Pyrene	0.001

To assess the potential health risk to human, normally, concentrations of heavy metals in crab were used in Provisional Tolerable Weekly Intake (PTWI) introduced by FAO/WHO. The main purpose of PTWI is to estimate the risk of toxicity bring by heavy metals [24]. Table 3 below showed the estimated Malaysian weekly dietary intake of metals from fish by eating 1kg of seafood a week per capita.

To calculate the PTWI, the body weight recommended by U. S. EPA in 1989 is 70kg which is the average body weight for adult population. According to MANS (Malaysian Adults Nutritional Survey) conducted by Malaysia Ministry of Health in year 2003, the average weight data of Malaysia adult (18-59 years old) is 62.65kg. So, 63kg was use in the calculation of PTWI in this study.

From the result above, the concentration of Zn, Cu and Cd in mud crabs were higher than the PTWI per person. The concentration of Zn is 874 mg per kg, it is 2 times higher than the PTWI which is only maximum 441 mg in a week. Same things happened on Cd which is also 2 times higher than PTWI value in a week. This means those consumers who consume on the mud crabs from study area will adverse their health effect. Conversely, Pb and Fe do not exceed the PTWI value which is only 1.27 mg and 0.151% respectively.

In order to get the total BaP concentration in each mud crabs, the concentration of each BaP equivalent was summed up. The total BaP equivalent concentration of each mud crab was showed in Table 4. There are 4 mud crabs contained more than 100 ng/g from 10 mud crabs. The similarities between these 4 mud crabs is that all of them already have very high concentration of BaP before multiply with the TEF. The highest BaP equivalent concentration is 371.829 ng/g while the lowest is 3.284 ng/g. This result showed that the mud crabs that caught in study area might have very high cancer risk. This is because the maximum level of BaP in crustaceans only 5.0 ng/g only [28]. By referring to this data, the highest result that obtained in this study was 74 times higher than the maximum level. There are only 1 mud crabaccumulated the BaP concentration that lower that the maximum level. This means that there are 9 out of 10 mud crabs are higher than the maximum level. It is worth highlighting that human health is exposed to cancer risk through consuming those mud crabs.

Table 2: PAHs compound detected in samples and its potency equivalency concentration (PEC).

Sample	PAH compounds detected	Concentration (ng/g)	TEF	BaP Equivalent Concentration (ng/g)
M1	Benzo (a) Anthracene (BaA)	27.88	0.1	2.788
	Benzo (k) Fluoranthene (BkF)	61.30	0.1	6.130
M2	Fluoranthene (Fl)	360.16	0.001	0.36016
	Chrysene (Chrys)	76.32	0.01	0.7632
	Benzo (a) Anthracene (BaA)	135.36	0.1	13.536
	Benzo (k) Fluoranthene (BkF)	108.10	0.1	10.810
	Benzo (a) Pyrene (BaP)	346.36	1	346.36
M3	Fluoranthene (Fl)	9.77	0.001	0.00977
	Chrysene (Chrys)	11.54	0.01	0.1154
	DiBenzo (a,h) Anthracene (dBahA)	5.17	1	5.17
M4	Phenanthrene (Ph)	24.18	0.001	0.02418
	Fluoranthene (Fl)	5.27	0.001	0.00527
	Chrysene (Chrys)	7.38	0.01	0.0738
	Benzo (k) Fluoranthene (BkF)	57.27	0.1	5.727
	Benzo (a) Pyrene (BaP)	117.26	1	117.26
M5	Phenanthrene (Ph)	5.65	0.001	0.00565
	Fluoranthene (Fl)	6.60	0.001	0.0066
	Chrysene (Chrys)	15.05	0.01	0.1505
	Benzo (a) Anthracene (BaA)	12.12	0.1	1.212
	Benzo (k) Fluoranthene (BkF)	163.78	0.1	16.378
	Benzo (a) Pyrene (BaP)	302.77	1	302.77
M6	Acenaphylene (Acy)	36.52	0.001	0.03652
	Phenanthrene (Ph)	18.55	0.001	0.01855
	Fluoranthene (Fl)	8.21	0.001	0.00821
	Chrysene (Chrys)	12.22	0.01	0.1222
	Benzo (k) Fluoranthene (BkF)	51.92	0.1	5.192
M7	Chrysene (Chrys)	4.25	0.01	0.0425
	Benzo (k) Fluoranthene (BkF)	37.50	0.1	3.75
	Benzo (a) Pyrene (BaP)	69.85	1	69.85
	Ideno (1,2,3-cd) Pyrene (IP)	93.36	0.1	9.336
	DiBenzo (a,h) Anthracene (dBahA)	6.19	1	6.19
M8	Fluoranthene (Fl)	5.14	0.001	0.00514
	Chrysene (Chrys)	5.01	0.01	0.0501
	Benzo (k) Fluoranthene (BkF)	32.29	0.1	3.229
M9	Phenanthrene (Ph)	91.63	0.001	0.09163
	Fluoranthene (Fl)	181.65	0.001	0.18165
	Chrysene (Chrys)	259.03	0.01	2.5903
	Benzo (k) Fluoranthene (BkF)	38.94	0.1	3.894
	Benzo (a) Pyrene (BaP)	95.79	1	95.79
	DiBenzo (a,h) Anthracene (dBahA)	8.05	1	8.05
	Benzo (g,h,i) Perylene (BghiP)	3.80	0.01	0.038
M10	Phenanthrene (Ph)	7.61	0.001	0.00761
	Fluoranthene (Fl)	10.38	0.001	0.01038
	Chrysene (Chrys)	10.07	0.01	0.1007
	Benzo (k) Fluoranthene (BkF)	51.23	0.1	5.123
	Benzo (a) Pyrene (BaP)	173.36	1	173.36
	Ideno (1,2,3-cd) Pyrene (IP)	31.70	0.1	3.17
	Benzo (g,h,i) Perylene (BghiP)	1.78	0.01	0.0178

Table 3: Estimation weekly dietary intake of metals from seafood by eating 1.0 kg per capita

Mud Crabs Part	Heavy metals concentration (mg/kg)			
	Cu	Zn	Cd	Pb
Muscle	222	875	0.88	1.27
PTWI (mg/kg bw/week)	0.35-3.50	0.21-7.00	0.01	0.025
PTWI (mg/kg) for a 63 kg adult	22.1-221	13.7-441	0.44	1.58

Table 4: Total BaP equivalent concentration in each sample

Sample	Total BaP equivalent concentration (ng/g)
M1	8.92
M2	372
M3	5.30
M4	123
M5	320
M6	5.38
M7	89.2
M8	3.28
M9	111
M10	182

These carcinogenic PAHs compound will bring negative impacts to human health skin disorders, harmful development and reproductive effects and the most dangerous is that PAHs are likely cause cancer in humans. This is because BaP-DNA adducts will be formed when BaP and BaP metabolites bind to DNA. This combination will then changes or alter the DNA replication during cell division. When the DNA replication has been altered, this may cause increasing in the risk of various forms of cancers. Thus, BaP is classified as carcinogenic due to its mutagenic mode of action that cause tumor formation. Besides, genotoxic effects in large number of prokaryotic and mammalian cell assays were also cause by BaP [13].

In natural environment, PAHs can be exposure to human and environment in many ways. Most of the foods that human consume everyday such as vegetables, meat andfish contain PAHs when those foods cooking at high temperature such as charbroiling, grilling and frying. However, in present study, the samples were carried out experiment in fresh condition. This means that mud crabs were contaminated PAHs from the surrounding environment. The main sources of PAHs entering environment is through the release of petroleum product (petrogenic sources) or by combustion of organic matter pyrogenic sources [4]. For example, petrol oil will leak from vehicles and contaminated on the road. So, storm water play an important role to brings PAHs compound down to the river. Surface runoff will happen when the rain water fall down and flush into river with oily substances on the land [34].

The PAHs concentration in present study was very high. So, the PAHs compounds that present on the road probably will runoff into the sampling area in raining days. After PAHs reached in river or coastal area, most of the PAHs will contaminate in the surface sediment [35]. The result is the increase in exposure risk of high concentration of PAHs in benthic biota [21]. This might be another reason that mud crabs in present study contaminated high level of PAHs since mud crabs is one of the benthic biota.

Conclusion:

The concentration of heavy metals (Cd, Cu, Zn, Fe and Pb) was higher compare to the previous study from other regions. The heavy metals (Cu and Zn) that accumulated in muscle part were also higher than the maximum permitted level. The high level of heavy metals in crabs probably due to the wastewater runoff from terrestrial into mangrove area of UMT. Besides, the concentration of Cu, Zn, Cd and Fe were directly proportional to the length of mud crabs while inversely proportional for Pb. This might due to the feeding habits and ecological needs metabolisms of young and adult mud crab. The concentration of Cu, Zn and Pb were higher than permissible limit that has set by FAO/WHO while Cd was below the permissible limit. At the same time, the concentration of Zn, Cd and Cu were exceed the Provisional Tolerable Weekly Intake (PTWI) value that set by FAO/WHO while Pb and Fe were below the value. The mud crabs in mangrove area of UMT may not safe for consumption since most of the heavy metals are higher the PTWI. On the other hand, the highest total PAHs was 1026 ng/g while the lowest was 26.5 ng/g. The level of PAHs in this study also higher than the safety level. The main sources of PAHs in sampling area might from the oil substances runoff from land with the help of storm. The high heavy metals and PAHs in sampling area might adverse the health effect of consumer who consume on it.

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REFERENCES

[1] Adal, A., 2013. Heavy Metal Toxicity. Retrieved from http://emedicine.medscape.com/article/814960-overview.
[2] Bandoquillo, M.G., D.G. Fernandez and L.C. Darvin, 1996. The Crab Industry in The Philippines. Bulletin of the Philippine Council for Aquatic and Marine Research and Development of the Department of Science and Technology, Los Banos, Laguna, 1(3): 20.
[3] Du Preez, H.H., V.E. Steenkamp, H.J. Schoonbee, 1993. Bioaccumulation of Zinc and lead in selected tissues and organs of the freshwater crab, Potamonauteswarreni. Science of the Total Environment, Supplement, 134: 469-478.
[4] Eaganhouse, R.P., B.R.T. Simoneit, I.R. Kaplan, 1981. Extractable organic matter in urban stormwater runoff. 2. Molecular characterization. Environmental Science & Technology, 15: 315-326.
[5] Environment Protection Agency, U.S., 2005. Supplemental Guidance for Assessing Cancer Susceptibility from Early-Life Exposure to Carcinogens. Retrieved from http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=116283
[6] Environment Protection Agency, U.S., 2011. Benzo (a) pyrene. Retrieved from http://www.epa.gov/pbt/pubs/benzo.htm
[7] Evert, A., 2013. Iron in Diet. Retrieved from http://www.nytimes.com/health/guides/nutrition/iron-in-diet/overview.html
[8] Ferdoushi, Z., Z. Xiang-guo and M.R. Hasan, 2010. Mud crab (Scylla sp.) marketing system in Bangladesh. Asian Journal of Food and Agro-Industry, 3(2): 248-265.
[9] Fisheries Queensland, 2009. Fisheries Long Term Monitoring Program Sampling Protocol – Mud Crab (2008 onwards) Section 1.Department of Employment, Economic Development and Innovation, Brisbane, Australia.
[10] Grubert, M. and M. Phelan, 2007. So You Want to Catch Crabs? A guide to catching mud crabs in the Northern Territory. Retrieved from http://www.nt.gov.au/d/Content/ File/p/Fishnote/FN24.pdf
[11] Hoffman, E.J., G.L. Mills, J.S. Latimer, J.G. Quinn, 1984. Urban runoff as a source of polycyclic aromatic hydrocarbons to coastal waters. Environmental Science & Technology, 18: 580-587.
[12] Jarup, L., 2003. Hazards of heavy metal contamination. Journal of British Medical Bulletin, 68(1): 167-182.
[13] Juhasz, A.L., R. Naidu, 2000. Bioremediation of high molecular weight polycyclic aromatic hydrocarbons: a review of the microbial degradation of benzo[a]pyrene. International Biodeterioration& Biodegradation, 47: 57-88.
[14] Kamaruzzaman, B.Y., B. Akbar John, B.Z. Maryam, K.C.A. Jalal and S. Shahbuddin, 2012. Bioaccumulation of Heavy Metals (Cd, Pb, Cu and Zn) in Scylla serrata (Forsskal 1775) Collected from Sungai Penor, Pahang, Malaysia. Pertanika Journal of Tropical Agricultural Science, 35(1): 183–190.
[15] Kamaruzzaman, B.Y., M.C. Ong and K.C.A. Jalal, 2008. Levels of Copper, Zinc and Lead in Fishes of MengabangTelipot River, Terengganu, Malaysia. Journal of Biological Sciences, 8(7): 1181-1186.
[16] Kamaruzzaman, B.Y., M.C. Ong, K.C.A. Jalal, S. Shahbudin, O. Mohd Nor, 2009. Accumulation of Lead and Copper in Rhizophoraapiculata from Setiu mangrove forest, Terengganu. Malaysian Journal of Environmental Biology, 30(5): 821-824.
[17] Keenan, C.P., P.J.F. Davie and D.L. Mann, 1998. A revision of the genus ScylladeHaan, 1833 (Crustacea: Decapoda: Brachyura: Portunidae). The Raffles Bulletin of Zoology, 46(1): 217-245.
[18] Lah, K., 2011. Polycyclic Aromatic Hydrocarbon. Retrieved from http://toxipedia.org/display/toxipedia/Polycyclic+Aromatic+Hydrocarbons
[19] Malcom, H.M., S. Dobson, 1994. The calculation of an environmental assessment level (EAL) for atmospheric PAHs using relative potencies. Department of The Environment, London, UK, pp: 34-46.
[20] Meador, J.P., J.E. Stein, W.L. Reichert and U. Varanasi, 1995. Bioaccumulation of Polycyclic Aromatic Hydrocarbons by Marine Organisms.Reviews of Environmental Contamination and Toxicology, 143: 79-165.
[21] Mirza, R., I. Faghiri, E. Abedi, 2012. Contamination of Polycyclic Aromatic Hydrocarbons in Surface Sediments of Khure-Musa Estuarine, Persian Gulf. World Journal of Fish and Marine Sciences, 4(2): 136-141.
[22] Mudgal, V., N. Madaan, A. Mudgal, R.B. Singh and S. Mishra, 2010. Effect of Toxic Metals on Human Health. The Open Nutraceuticals Journal, 3: 94-99.
[23] Mumtaz, M. and J. George, 1995. Toxicological Profile for Polycyclic Aromatic Hydrocarbons. Retrieved from http://www.atsdr.cdc.gov/toxprofiles/tp69.pdf

[24] Ngassapa, F. N., O.C. Othman, E. Elisante, 2010. Urban Dietary Heavy Metal Intake From Protein Foods And Vegetables in Dar Es Salaam. Tanzania Journal of Science, 36: 85-94.
[25] Nisbet, I.C.T., J.B. Ramussen, 1992. Toxic equivalency factors (TEFs) for polycyclic aromatic hydrocarbon (PAHs). Regulatory Toxicology and Pharmacology, 16: 290-300.
[26] NOAA, 2010. Bioaccumulation of Oil Chemicals in Seafood. Retrieved from http://www.floridahealth.gov/chdokaloosa/Deepwater_Horizon/Media/May05/BioaccumulationDWH1Pager.pdf
[27] Ong, M.C., N.A.M. Shazili, D. Menier and A.W.M. Effendy, 2013. Levels of trace elements in tissue of Ostreaedulis and Crassostreagigas from Quiberon Bay, Brittany, France. Journal of Fisheries and Aquatic Science, 8(2): 378–387.
[28] Oosterom, J.V., S.C. King, A.Negri, C. Humphrey, J. Mondon, 2010. Investigation of the mud crab (Scylla serrata) as a potential bio-monitoring species for tropical coastal marine environments of Australia. Marine Pollution Bulletin, 60: 283-290.
[29] Phillips, DH., PS. Rainbow, 1993. Biomonitoring of trace aquatic contaminants. London: Chapman and Hall.
[30] Ploetz, D.M., B.E. Fitts, T.M. Rice, 2007. Differential Accumulation of Heavy Metals in Muscle and Liver of a Marine Fish, (King Mackerel, Scomberomorus cavalla Cuvier) from the Northern Gulf of Mexico, USA. Bulletin of Environmental Contamination and Toxicology, 78: 134-137.
[31] Selvi, K., M. Akbulut, H. Kaya, A. Tulgar, 2012. Lead Bioaccumulation in Gill, Muscle and Hepatopancreas Tissues ofMediterranean Green Crab, Carcinusaestuarii (Nardo, 1847). Spill Science & Technology Bulletin, 1: 11-14.
[32] Shanker, A.K., 2008. Mode of Action and Toxicity of Trace Elements. Trace Elements: Nutritional Benefits, Environmental Contamination, and Health, 21: 525-555.
[33] Sindaigaya, E., R.V. Cauwenbergh, H.Robberecht, H.Deelstra, 1994. Copper, Zinc, Manganese, Iron, Lead, cadmium, mercury and Arsenic in fish from Lake Tanganyika, Burundi. Science of the Total Environment, 144: 103-115.
[34] Stein, E.D., L.L. Tiefenthaler, K. Schiff, 2005. Watershed-Based Sources of Polycyclic Aromatic Hydrocarbons in Urban Storm Water. Environment Toxicology and Chemistry, 25(2): 373-385.
[35] Tam, N.F.Y., L. Ke, X.H. Wang, Y.S. Wong, 2001. Contamination of polycyclic aromatic hydrocarbons in surface sediments of mangrove swamp. Environmental Pollution, 114: 255-263.