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Toxicity of Heavy Metals in *Meretrix meretrix* and *Anadara granosa* from Balok River at Kuantan Pahang, Malaysia

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ABSTRACT

A study was conducted to determine the concentrations of heavy metals Aluminium (Al), Iron (Fe), Cadmium (Cd) and Lead (Pb) in the soft tissues molluscs collected from Balok River at Kuantan, Pahang. Balok River was located near the Kuantan Port. Kuantan port was temporary storage of bauxite mining prior to being exported to China for mineral processing. Sampling was carried out during monsoon season and dry season. The molluscs were collected using digger and hand picking. Soft tissues of molluscs was digest by using Acid Digestion method and analyze using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Fe, Al, Cd and Pb in water sample are natural level in Class of River based on National Water Quality Standard from Department of Environment (DOE), 2014**.** Mean concentration of Al, Fe, Cd and Pb (mg/kg dry weight) during monsoon and dry season in the soft tissue of *Meretrix meretrix* were 52.3000±8.6000, 104.3222±17.2529, 0.0349±0.0012, and 0.2701±0.0602 respectively while for *Anadara granosa* were 43.8845±26.9467, 115.4073±54.8871, 0.1592±0.2670, and 0.2056±0.1165 respectively. The toxicity of metals in the soft tissue flowed in both *Meretrix meretrix* and *Anadara granosa* were in order of Fe>Al>Pb>Cd. All heavy metals concentration was lower in the soft tissues of both *Meretrix meretrix* and *Anadara* compares to the previous studies. The concentration of all heavy metals in molluscs was below the permissible limit according to Malaysia Food Act (MFA, 1983), World Health Organization (WHO, 1982) and Food and Drug Administration (FDA, 2001).

Keywords: Balok River, Heavy Metals, Mollusc, *Meretrix meretrix*, *Anadara granosa*

ABSTRAK

Satu kajian telah dijalankan untuk menentukan kepekatan logam Aluminium (Al), Ferum (Fe), Kadmium (Cd) dan Plumbum (Pb) dalam tisu lembut moluska yang dikutip dari Sungai Balok di Kuantan, Pahang. Sungai Balok terletak berdekatan dengan pelabuhan Kuantan. Pelabuhan Kuantan merupakan tempat penyimpanan sementara bauksit sebelum di eksport ke China untuk pemprosesan mineral. Pensampelan dijalankan semasa musim tengkujuh dan musim kering. Moluska dikutip menggunakan penggali dan tangan. Tisu lembut dicernakan menggunakan asid dan analisis logam ditentukan dengan menggunakan Spekrometer Jisim Plasma Gandingan Aruhan (ICP-MS). Keputusan mendapati bahawa kepekatan (ppm) Fe, Al, Cd dan Pb dalam sampel air adalah dalam peringkat semulajadi berdasarkan Indeks Kualiti Air Negara daripada Jabatan Alam Sekitar (DOE), 2014. Kepekatan purata Al, Fe, Cd dan Pb (mg/kg) pada musim kering dan tengkujuh dalam tisu lembut *Meretrix meretrix* adalah masing-masing pada kepekatan 52.3000±8.6000, 104.3222±17.2529, 0.0349±0.0012 dan 0.2701±0.0602 manakala untuk *Anadara granosa* adalah masing-masing pada kepekatan 43.8845±26.9467, 115.4073±54.8871, 0.1592±0.2670, dan 0.2056±0.1165. Ketoksikan logam dalam tisu lembut yang mengalir di kedua *Meretrix meretrix* dan *Anadara granosa* ialah pada turutan Fe> Al> Pb> Cd. Semua kepekatan logam berat lebih rendah dalam tisu lembut *Meretrix meretrix* dan *Anadara granosa* berbanding dengan kajian terdahulu. Kepekatan semua logam berat di moluska adalah di bawah had yang dibenarkan mengikut Akta Makanan Malaysia (MFA, 1983), Pertubuhan Kesihatan Sedunia (WHO, 1982) dan Pengakuan Makanan dan Dadah (FDA, 2001).

Kata Kunci: Sungai Balok, Logam Berat, Moluska, *Meretrix meretrix*, *Anadara granosa*

INTRODUCTION

Due to rapid urbanisation, most of the industrial areas in Kuantan are built close to major roads or river areas, where they are expose to many potential pollution sources, including industrial emissions. The rapid growth of industrial activities in Kuantan causes an increasing production and usage of toxic chemicals such as heavy metals. Today, heavy metals pollution has been controversial issue due to serious pollutants in our natural environment. Public water supplies and fishery sources were threated due to heavy metals

pollution into the rivers. Besides that, heavy metals cannot be destroyed through biological degradation and have a tendency to accumulate in the aquatic environment that will hazard to humans that consumed aquatic products as sources of food (Kalay et al., 1999; Aderinola et al., 2009).

Elements like Na, K, and Ca are essential for maintaining important cellular functioning (Farkas et al., 2000). Metals like Zn, Cu, Fe, and Mn are also important elements for normal cellular function, but in higher consumptions, they are toxic to the cells. These metals occur naturally in the environment but the concentration will increased due to human activities. There are several metals that cause toxic effects even at low levels under certain conditions such as As, Cd, Cu, Hg, Pb, and Zn (Cohen et al., 2001).

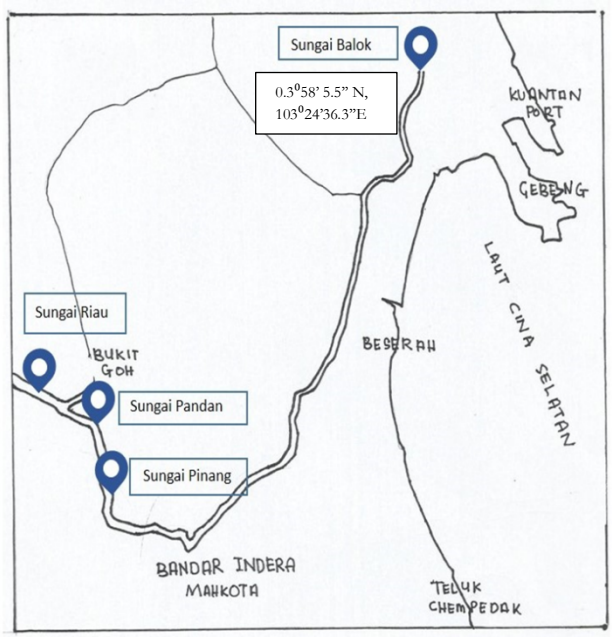
Molluscs such as bivalves are act as bioindicators of heavy metals pollution because they can collect these elements with time integrated indication of environmental contamination. Mollusca have very slow enzyme activity systems that capable of metabolizing persistent organic pollutants compare to fish and crustacean. Therefore, a mollusc is more accurate to use as bioindicator for heavy metal contaminations. However, bioaccumulation of pollutants in other aquatic species such as fish and crustacean also important to determine different content of trace metal from biomagnifications of food chains, metabolic capability and feeding habits (Asuquo et al., 2004).

Terra et al. (2008) reported that heavy metal pollution in rivers gives threat to public water supplies and also to consumer of fishery sources. Aluminium (Al), Iron (Fe), Manganese (Mn), Cadmium (Cd), Nickel (Ni), Lead (Pb), Copper (Cu) and Arsenic (As) are heavy metal that can cause toxicity to human. The anthropogenic activity by human such as industrial processes, domestic waste, agricultural activities and emissions from factory are causing the increased on concentration of heavy metals deposited into the rivers. These metals will accumulate by aquatic organisms in the river through respiration and breathing and can cause several health effects to human, if consumed. Heavy metals accumulation in fish is one of the biggest issues because many fish species were consumed by the population especially those who live near rivers (Yilmaz et al., 2003). The heavy metals can be neurotoxic, carcinogenic, mutagenic and teratogenic to human if the heavy metals were consumed above the recommended limit. General symptoms of human that cause by metal poisoning such as vomiting, convulsions, paralysis, ataxia, hemoglubinuria, gastrointestinal disorder, diarrhea, stomatitis, tremor, depression and pneumonia (McCluggage 1991). This study was focused on concentration analysis of heavy metals, namely Aluminium (Al), Iron (Fe), Cadmium (Cd), and Lead (Pb) in tissue of *Meretrix meretrix* and *Anadara granosa* colleted from Balok River at Kuantan, Pahang.

MATERIALS AND METHODS

Site Description

All the samples were collected from Balok River (0.3°58' 5.5" N, 103°24'36.3"E) which located close to the Gebeng industrial site and commercial beach area in Kuantan. The river flows pass through the wide industrial facilities which include steel industries, palm oil mills, pipe coating and others. Besides that, these three rivers are located near Kuantan Port. Kuantan port is temporary storage of bauxite mining prior to being exported to China for mineral processing (Yaakub *et al.,* 2017). The Kuantan Port Industrial Area located within the vicinity of the port is made up of companies that have leased or rented land from the port for their business activities. The Gebeng Industrial Estate has made it a world-class petrochemical zone covering an area of 8,600 hectares. The Gebeng Industrial Estate is a thriving hub of commercial activity where numerous multinational corporations in the petrochemical sector are head-quartered.



Mollusc Collections

Sampling for molluscs was carried out during the wet season (February 2017) and dry season (May 2017). Mollusc specimens were collected at river banks. These two species of mollusc (bivalve and clam) were commonly found at the river banks of Balok River. Bivalve and clam were collected using hand picker and digger. Samples were placed in an ice box. Upon arrival at the laboratory, the samples were stored in -20°C until further analysis. Samples were identified to species. Weight (g) and length (cm) of shell of the captured moluscs were recorded (APHA, 2005).

Digestion Method

Samples were thawed to room temperatures and were cleaned with tap water and soaked in boiling water for approximately 3 min. Tissues samples of the molluscs were removed from the shell, washed with deionized water and weighted. The tissues were oven-dried at 100°C for 24 hours until they achieved constant weight. Samples were then put into desiccator before the dry weights of the samples were taken. Acid digestion method was used based on the Association of Official Analytical Chemists (AOAC), (1984). Each sample of dried tissues was put in the digestion tube where 10 mL of 69% Nitric acid were added and left overnight. The samples were then digested in block thermostat at 100°C for 2 hours before were left to cool. After that, 2 mL of 30% hydrogen peroxide (H_2O_2) was added and heated again for 1 hour until clear solution was formed. Then, the digestion tubes were allowed to cool before the solutions were filtered through filter paper into a 25 mL volumetric flask. Deionized water was top-up until the volume reached 25 mL. The concentrations of heavy metals were determined using Inductively Couple Plasma – Mass Spectrometry (ICP-MS).

RESULTS AND DISCUSSION

A total of 40 molluscs from two different species were collected during the two sampling periods and were tabulated in the Table 1 below:

Table 1 Distribution of molluscs caught in two sampling period in monsoon season (S1) and dry seasons (S2)

Species name (n)	Common name	S1	S2
Meretrix meretrix (20)	Kepah	+	+
Anadara granosa (20)	Kerang	+	+

Note: + present, (n) number of individual mollusc collect

All molluscs were collected at river bank from Balok River during monsoon season and dry season. During the sampling in both monsoon and dry season, molluscs that were collected at Balok River identified as *Anadara granosa* and *Meretrix meretrix*. Tissues from both specimens were used to determine metal concentration by acid digestion method. The concentration of heavy metals Aluminium (Al), Iron (Fe), Cadmium (Cd), and Lead (Pb) were detected in the soft tissues of molluscs samples collected from Balok River and are summarized in the table below:

Table 2 Mean heavy metals (Al, Cd, Pb, and Fe) concentration (mg/kg ± SD) in soft tissues of molluscs collected from Balok River, Kuantan

Species name	Mean Heavy Metal Concentration (mg/kg dry weight)			
	Al	**Cd**	**Pb**	**Fe**
		Monsoon Season		
Meretrix meretrix	52.3000±8.6000	0.0349±0.0012	0.2701±0.0602	104.3222±17.2529
Anadara granosa	34.5095±17.6493	0.2478±0.3799	0.1820±0.1065	99.7678±37.8954
		Dry Season		
Meretrix meretrix	41.1152±14.7039	0.0522±0.02683	0.2092±0.1019	127.4686±96.6581
Anadara granosa	53.2595±33.8882	0.0705±0.0318	0.2292±0.1373	131.0469±70.2928

From Table 2, the metal concentrations (mg/kg dry weight) of Al, Cd, Pb and Fe in the soft tissues of *Meretrix meretrix* during monsoon season ranged from 52.3000±8.6000, 0.0349±0.0012, 0.2701±0.0602, and 104.3222±17.2529 mg/kg respectively while 34.5095±17.6493, 0.2478+0.3799, 0.1820+0.1065, and 99.7678+37.8954 mg/kg respectively in soft tissue of *Anadara granosa*. From these results, a metals concentration is higher in soft tissue of *Meretrix meretrix* compare to *Anadara granosa*. These indicate that *Meretrix meretrix* contained the highest metals in the soft tissue and mean that Meretrix *meretrix* accumulate more metal than *Anadara granosa* during monsoon season. During dry season, the metal concentrations (mg/kg dry weight) of Al, Cd, Pb and Fe in the soft tissue of *Meretrix meretrix* ranged from 41.1152±14.7039, 0.0522±0.02683, 0.2092±0.1019 and 127.4686±96.6581 mg/kg respectively while 53.2595±33.8882, 0.0705±0.0318, 0.2292±0.1373, and 131.0469±70.2928 mg/kg respectively in soft tissue of *Anadara granosa*. From these results, a metals concentration is lower in soft tissue of *Meretrix meretrix* compare to that of *Anadara granosa*. These indicate that *Anadara granosa* contained higher metals in their soft tissue. This also mean that *Anadara granosa* accumulate more metal than *Meretrix meretrix* during dry season. Based on the soft tissues of both *Meretrix meretrix* and *Anadara granosa*, the metal abundance were in order of Fe> Al> Cd> Pb. From this result, it was showed that concentration of essential metals was higher in the soft tissue that the non-essential metals. Besides that, the value concentration of all

metals during monsoon and dry season not much difference except Fe that higher during dry season. This might be due to Fe is not diluted with rain water and cause the molluscs absorb and accumulate more Fe compare to monsoon season.

Table 3 Comparison of different heavy metals (Al, Cd, Pb, and Fe) concentrations (mg/kg dry weight) in soft tissue of Molluscs collected from Balok River with other several organizations and previous study

Species name	**Mean Heavy Metal Concentration (mg/kg dry weight)**			
	Al	**Cd**	**Pb**	**Fe**
Present Study				
Meretrix meretrix	45.5891±12.8110	0.0453±0.0212	0.2335±0.1497	118.2101±70.0466
Anadara granosa	43.8845±26.9467	0.1592±0.2670	0.2056±0.1165	115.4073±54.8871
Permissible limit in molluscs based on several organizations				
WHO, 1982	-	10	5-30	-
FDA, 2001	-	0.2	1.5	-
MFR, 1985	-	1	2	-
Previous Study				
Pekan, Pahang (Yunus *et al.,* 2011)	-	0.3	0.47	576.45
Kuala Perlis (Lias *et al.,* 2013)	-	4.4	-	-
Tanjung lumpur, Pahang (Yunus *et al.,* 2010)	-	0.67	1.61	415.2

Based on Table 3, the mean concentration of Cd in soft tissue of both *Meretrix meretrix* and *Anadara granosa* in present study are 0.0453±0.0212 and 0.1592±0.2670 mg/kg respectively while the mean concentration of previous study Yunus et al. (2011), Lias et al. (2013) and Yunus et al. *(*2010) are 0.3, 4.4 and 0.67 mg/kg respectively. This result showed, all heavy metals concentration was lower in the soft tissues of both Meretrix *meretrix* and *Anadara granosa* compares to the previous studies (Yunus et al., 2011, Lias et al., 2013, *Yunus* et al., 2010). In addition, mean concentration of Pb and Fe in soft tissue of both *Meretrix meretrix* and *Anadara granosa* in present study are 118.2101±70.0466 and 115.4073±54.8871mg/kg respectively while the mean concentration of previous study Yunus et al. (2011) and Yunus et al. *(*2010) are 576.45 and 412.5mg/kg respectively. This result also showed the mean concentration of Pb and Fe in present study is lower than previous study. The order of metal accumulation in the soft tissue *Meretrix meretrix* and *Anadara granosa* collected from Balok river was in order of Fe> Al> Cd> Pb.

The limits of heavy metal concentration in molluscs have been set to safeguard public health. Malaysia, for example, has set maximum limits of contamination for Cd and Pb based on permissible limits recommended by the Malaysia Food Act (MFA, 1983). In this study, the mean concentration heavy metal were also compared with permissible levels others organization such as the World Health Organization (WHO, 1982) and Food and Drug Admistration (FDA, 2001). This study showed the Cd and Pb concentration in soft tissue of mollusc collected form Balok River was below below the permissible limit of World Health Organization (WHO, 1982), Malaysia Food Act (MFA, 1983) and Food and Drug Admistration (FDA, 2001). The result obtained in present study proves that the mollusc was safe for human consumption. Permissible limit for Al and Fe was not found for this study. However, from this study, we can observe high concentration of Al and Fe in the soft tissue of *Meretrix meretrix* and *Anadara granosa.* This clearly showed that both mollusc species are tendency of organisms in accumulating higher concentration of Al and Fe. Higher concentration of Al and Fe collected from Balok River might be due to various anthropogenic sources and industrial effluent surrounding the river. In addition, high concentration of Fe might be due to it act as essential metal in maintaining the proper physiological functions of organism. This are prove by previous study by Kanakaraju et al.(2008a) assumed that Fe plays an important role as an essential element in all living systems from invertebrates to human, hence they tend to accumulate high concentration of Fe from the surrounding environment. Furthermore, this occurrence also indices the natural capacity to regulate and accumulate elevated concentration of Fe in molluscs (Kanakaraju et al., 2008b).

Table 4 Concentration of Heavy Metals (Al, Cd, Pb, and Fe) in Water and Class of River based on National Water Quality Standard from Department of Environment (DOE), 2014

Type of Heavy Metals	Value (ppm)	Class of River (DOE,2014)
Al	0.0131±0.0167	
Cd	0.0001±0.0000	All Natural Level
Pb	0.0001±0.0000	
Fe	0.2027±0.0179	

From Table 4, concentrations of Al, Cd, Pb and Fe in water samples were at 0.0131±0.0167, 0.0001±0.0000, 0.0001±0.0000, and 0.2027±0.0179 ppm respectively. The order of metal in the water from Balok river was Fe> Al> Cd> Pb. From this result, the heavy metal Fe has higher concentrations value compare to others. However, based on National Water Quality Standard from Department of Environment, all heavy metals Al, Cd, Pb and Fe is at natural level and safe to be consume. Based on Hadibarata et al. (2012) said that leaching process from mineral-bearing rock and soil causes increasing heavy metals in water. Water quality data have been obtained from studies previously conducted in this area. According to Ab Wahab et al. (2016), Pb was determined in year 2014, while for year 2015, Pb and Cd was determined. The concentration of Pb during 2014 is 0.221ppm while the concentration of Pb and Cd during 2015 are 0.0006 and 0.0002 respectively which high than present study. However, concentration of Pb and Cd during 2015 still under natural level based on National Water Quality Standard from Department of Environment. During 2014, the concentration of Pb is excess the natural level might be due to leaded petrol spill from fishing boats and from lorry that flow into the river. This statement are supported in study by Kamaruzzaman et al. (2011) by mentioning that the potential source of lead contamination was might be occur due to leaded petrol, industrial effluents and residential sewage.

CONCLUSION

This study shows that the order of metal in the water, *Meretrix meretrix* and *Anadara granosa* from Balok river was Fe> Al> Cd> Pb. The highest heavy metal accumulate in *Meretrix meretrix* and *Anadara granosa* are Fe which are 118.2101±70.0466 and 115.4073±54.8871 respectively. However, all heavy metals Al, Cd, Pb, and Fe in the molluscs and water samples during monsoon and dry seasson was below the permissible limit of heavy metals recommended by Malaysia Food Act (MFA, 1983), World Health Organization (WHO, 1982) and Food and Drug Admistration (FDA, 2001). Thus, the molluscs collected from Balok River are considered safe to be consumed. In addition, by using *Meretrix meretrix* and *Anadara granosa* as a biomonitoring agent, the contamination of Al, Cd, Pb and Fe in this present study area was found not be seriously accumulates heavy metals in the soft tissue. However, a regular monitoring of heavy metals concentration in water and molluscs are needed to minimize heavy metals toxicity in freshwater ecosystem.

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