

(Pengumpulan Kepekatan Raksa (Hg) dan Metil Raksa (MeHg) dalam Hidupan Laut yang Terpilih dari Kawasan Pantai Manjung)

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Abstract

Level of mercury (Hg) and methyl mercury (MeHg) in marine ecosystem has been intensively studied as these toxic substances could be accumulated in the marine biota. This study is focusing on the Hg and MeHg content in marine biota in Manjung coastal area. This area has high potential being affected by rapid socio-economic development of Manjung area such as heavy industrial activities (coal fired power plant, iron foundries, port development and factories), agricultural runoff, waste and toxic discharge, quarries, housing constructions. It may has a potential risk when released into the atmosphere and dispersed on the surface of water and continue deposited at the bottom of the water and sediment and being absorbed by marine biota. The concentrations of Hg and MeHg in marine ecosystem can be adversely affect human health when it enters the food chain. In this study, five species of marine biota including *Johnius dussumieri* (Ikan Gelama), *Pseudorhombus malayanus* (Ikan Sebelah), *Arius maculatus* (Ikan Duri), *Portunus pelagicus* (Ketam Renjong) and *Charybdis natator* (Ketam Salib) were collected during rainy and dry seasons. Measurements were carried out using inductively coupled plasma mass spectrometry (ICP-MS) technique. The Hg concentrations for dry and rainy season are in the range 65.13-102.12 µg/kg and 75.75-106.10 µg/kg respectively, while for MeHg concentrations for dry and rainy seasons are in the range 4.35-6.26 µg/kg and 5.42-6.46 µg/kg, respectively. These results are below the limit set by Malaysia Food Act (1983). Generally, marine biota from the Manjung coastal area is safe to consume due to low value of ingestion dose rate and health risk index (HRI) for human health.

Keywords: mercury, methyl mercury, marine biota

Abstrak

Tahap kandungan raksa (Hg) dan metil raksa (MeHg) dalam ekosistem marin telah dikaji secara intensif sebagai bahan toksik yang berkemungkinan terkumpul dalam hidupan laut. Kajian ini memberi tumpuan kepada kandungan raksa (Hg) dan metil raksa (MeHg) dalam hidupan laut di kawasan perairan pantai Manjung. Kawasan Manjung ini terlibat dengan pembangunan sosio-ekonomi yang pesat seperti aktiviti berat (loji janakuasa arang batu, kilang besi, pelabuhan dan kilang-kilang), pengaliran air daripada pertanian, perlepasan toksik, kuari dan pembinaan perumahan. Ia berisiko apabila terdedah ke atmosfera dan tersebar di permukaan air dan terus disimpan di bahagian bawah badan air dan sedimen dan diserap oleh hidupan laut. Kepekatan raksa (Hg) dan metil raksa (MeHg) dalam ekosistem marin boleh menjejaskan kesihatan manusia apabila terdedah melalui rantai makanan. Dalam kajian ini, lima spesis hidupan laut iaitu Ikan Gelama (*Johnius dussumieri*), Ikan Sebelah (*Pseudorhombus malayanus*), Ikan Duri (*Arius maculatus*), Ketam Renjong (*Portunus pelagicus*) and Ketam Salib (*Charybdis natator*) telah diambil semasa musim panas dan hujan. Spektrometri jisim gandingan plasma teraruh (ICP -MS) teknik digunakan dalam pengukuran. Kepekatan Hg untuk musim panas dan hujan, masing-masing adalah dalam julat 65.13-102.12 μg/kg dan 75.75-106.10 μg/kg manakala,

kepekatan MeHg untuk musim panas dan hujan pula masing-masing dalam julat 4.35-6.26 µg/kg and 5.42-6.46 µg/kg. Keputusan kajian menunjukkan kepekatan Hg adalah di bawah had yang telah ditetapkan oleh Akta Makanan Malaysia (1983). Keseluruhannya, hidupan laut dari kawasan perairan pantai Manjung adalah selamat untuk dimakan disebabkan oleh kadar dos pengambilan dan indeks risiko kesihatan adalah rendah untuk kesihatan manusia.

Kata kunci: raksa, metil raksa, hidupan laut

Introduction

Manjung is an emerging district in southern west of Perak as a well developed and well planned administrative town, industrial, plantation and tourism. Rapid socio-economic developments along the coastline and population growth that make the level of toxic metals are potentially high. Hence, the toxic metals may pollute the Manjung and their coastal area. The industrial activities surrounding the Manjung area such as heavy industries (coal fired power plant, iron factories, port development and factories), agricultural runoff, waste and toxic discharge, quarries and housing constructions can be the contributors to the pollution in marine environment. Moreover, the aquaculture is the economic sources to local people surrounding Manjung area. Fish and fishery products industries from the coastal area may attribute the toxic metals into human body via food chain. However, they have several attractive areas encompass Pangkor Island, Teluk Rubiah, Teluk Batik, Teluk Nipah, Pantai Panjang and Pasir Bogak for tourism sector which can affect the natural environment.

Fish (marine biota) is an important source of protein for Malaysian population. The Malaysian per capita consumption of fish is 56 kg/person/year [1]. Fish consumption is the major route of mercury exposure to human and which often found in the form of methyl mercury [2]. Mercury enters the marine ecosystem in a variety of ways from various sources. They were released into the atmosphere and dispersed on the surface of water and continue deposited at the bottom of the water and sediment. Rivers carry the pollutants that containing toxic metal (dissolved, colloidal of or in particulate forms) to estuarine and finally to coastal. Later it is being absorbed by marine biota and it can be released back to the atmosphere by volatilization to adversely affect human health and the environment when exposed to human through food chain [3]. Moreover, linkage mechanism of sediment-water-marine biota provides information on final fate of the mercury (Hg) in the marine ecosystem in particular the input of the mercury (Hg) and methyl mercury (MeHg) into food chain.

Mercury is a shiny, silver-white metal and colorless and odorless in gas form. It is highly toxic element that is found in the environment originated from naturally and anthropogenic sources. It can be threat to the people's health and wildlife in environments. While, methyl mercury is formed from inorganic mercury by the action of anaerobic organisms through methylation process and this process converts inorganic mercury to methyl mercury in the natural environment especially in marine ecosystem. The effects of methyl mercury were first recognized since 1950's in Minamata, Japan. The toxicity fact of mercury is divided into two aspects that is first, mercury has no known metabolic function but when present in the body they disrupt normal cellular processes, leading to toxicity in a number of organs [4]. Secondly, the mercury accumulates in biological tissues by a process known as bioaccumulation. Bioaccumulation occurs when mercury once taken up into body is stored in particular organs and excreted more slowly than it is uptake [5]. The toxicity effect of mercury and especially methyl mercury form are adverse health effects including damage to the central nervous system (neurotoxicity) and on the brain which will affect the intellectual development in young children, digestive and immune system and lung, kidney, skin and eyes. Mercury will threaten the habitat of the marine ecosystem and lead to human health of the area. Identification of their sources may provide information on point source and lead to identifying the responsible parties for the pollution event and to regulate set limit the amount of mercury in fish is safe for human consumption. The objectives of this study are to determine mercury (Hg) and methyl mercury (MeHg) concentrations in marine biota species from Manjung coastal area and to assess health risk based on transfer factor (TF), ingestion dose rate (D) and health risk index (HRI) for human consumption.

Materials and Methods

Sample Collection

Marine biota samples were collected from Manjung coastal area in Perak which is located on the northern coast of Malaysia during dry (March to June 2013) and rainy (July to September 2013) seasons. Three fish species and two

crab species collected are *Pseudorhombus malayanus* (Ikan Sebelah), *Arius maculates* (Ikan Duri), *Johnius dussumieri* (Ikan Gelama), *Portunus pelagicus* (Ketam Renjong) and *Charybdis natator* (Ketam Salib). These species of marine biota are the most available at Manjung coastal area. Six seawater and marine sediment samples were collected along the coastal area at one kilometer from the beach. Seawater samples were collected at 10 to 20 cm depth using water sampler and kept in 10 L sterile polyethylene bottles during high tide, while marine sediment samples were collected using grab sampler. The sampling locations were determined using a global positioning system (GPS) and the locations are shown in Figure 1 and Table 1 respectively.

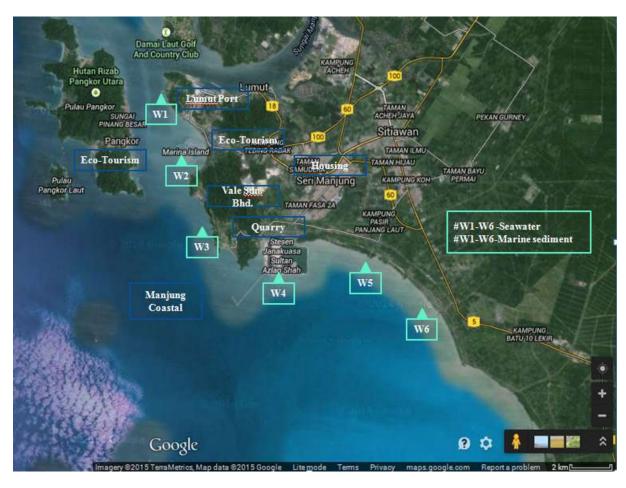


Figure 1. Location of sampling points along the Manjung coastal water area

Table 1. GPS location sampling points for seawater and marine sediment samples

Sampling Locations	Latitude	Longitude		
W1	N 04° 13.592'	E 100° 35.600'		
W2	N 04° 12.804'	E 100° 36.410'		
W3	N 04° 09.895'	E 100° 37.249'		
W4	N 04° 09.210'	E 100° 38.544'		
W5	N 04° 09.311'	E 100° 41.209'		
W6	N 04° 07.880'	E 100° 42.574'		

Sample Preparation

Flesh tissue of biota samples was obtained using plastic knife to avoid metals contaminations. The flesh tissue was then washed and cut into small pieces and air dried before continue dried in the oven at 60° C. Samples were ground and sieved (250 µm) to ensure sample transform to fine powder and homogenized. The 2 g of marine biota samples were digested using a total of 5 mL concentrated HNO₃ and 2 mL of H_2O_2 , heated at 60° C and until it become clear solution. The solutions were transferred to 25.0 mL volumetric flask and diluted with ultrapure water [3]. This method produces 97.6% of recovery for mercury (Hg) using Standard Reference Material (NIST 1566b Oyster tissue).

While for methyl mercury determination, 1.5 g of marine biota samples were digested using 10 mL of concentrated HCL in 50 mL separating funnel and shake for 5 minutes. Then, 20 mL of toluene was added to the solution and shake vigorously to mix and separated the organic layer which containing methyl mercury species. The extraction was repeated and combined both portions and centrifuged for 20 minutes at 2400 rpm. The solution was added 6 mL of L-Cysteine (1% solution) to strip methyl mercury from toluene and centrifuged for 20 minutes at 2400 rpm. Then, an aliquot of L-Cysteine extract was immediately analyzed with Inductively Coupled Plasma Mass Spectrometer (ICPMS) [6].

Sea water samples were acidified with 1% HNO₃ to below pH 2 to preserve and prevent chemical or biological reactions from other microorganism or foreign material. The samples were filtered through pre-weighed membrane filter paper with $0.45~\mu m$ pore size for the removal suspended solid particles. Samples were then prepared about 5 mL and markup with 1% HNO₃ in 50 mL and ready to analysis. However, marine sediment samples were oven dried at 60°C until constant weight, then samples were ground and sieved before be converted into 4.0~cm diameter pellet form using Fluxana HD Fusion Machine and ready to analysis [7].

Sample Analysis

Marine biota and sea water samples were measured using Inductively Coupled Plasma Mass Spectrometer (ICPMS) to determine the mercury concentrations. Samples were digested with 1% of HNO₃ as a blank solution, standards of mercury samples were performed directly under clean room conditions with inductively coupled plasma mass spectrometry (ICP-MS). While, marine sediment samples were measured directly using Energy Dispersive X-Ray Fluorescence (EDXRF).

Transfer Factor (TF)

Transfer factor of seawater- marine biota and marine sediment-marine biota was calculated using the following equations 1 and 2 [8]:

$$TF = C$$
 biota tissue (mg/kg)/ C seawater (mg/L) (1)

$$TF = C$$
 biota tissue $(mg/kg)/C$ marine sediment (mg/kg) (2)

where C is concentration of mercury. The transfer factor values are TF>1 indicates transfer and accumulation of mercury in marine biota from water to marine biota, while TF<1 indicate no accumulation.

Daily Exposure Ingestion dose (D)

Daily exposure ingestion dose is assessment of the human health risk from exposure ingestion of contaminated food requires information on the quantities of contaminated foodstuffs consumed for daily intake and the extent of contamination present in foodstuffs. Calculation using the following equation 3 [9]:

$$D = (C \times IR \times AF \times EF \times CF)/BW$$
(3)

where D is daily exposure ingestion dose (mg/kg/day), C is contaminant concentration (mg/kg), IR, intake rate of contaminated medium intake (mg/day) [1], AF, bioavailability factor (unitless), EF, exposure factor (unitless) The fish intake rate is a daily average, so the exposure factor is equal to 1, CF, conversion factor (10⁻⁶ kg/mg) based on suggestion by Agency for Toxic Substances and Disease Registry and BW, body weight (kg).

Health Risk Index (HRI)

Health risk index (HRI) is calculated using the following equation 4 [10 and 11], which important to assess the level of human health risk when expose to the heavy metals.

$$HRI = EDI (mg/kg/day)/RfD (mg/kg/day)$$
(4)

where EDI is estimated daily intake and RfD is Reference oral dose [12]. Based on HRI, the values of <1 means the exposed population is unlikely to experience obvious adverse effects and for HRI >1 means there is a chance of non-carcinogenic effects to the exposed population.

Results and Discussion

Table 2 shows the mercury and methyl mercury concentration in marine biota including Johnius dussumieri, Pseudorhombus malayanus, Arius maculates, Portunus pelagicus and Charybdis natator were collected during rainy and dry seasons. The mercury concentrations for dry season are in the range 65.13-102.12 µg/kg, while 75.75-106.10 μg/kg for rainy season. The highest mercury concentration is *Portunus pelagicus* for both seasons. This may due to the habitat of Portunus pelagicus live and feed at the bottom of sea, which nearly to the sediment where various kinds of toxic element deposited and accumulated [13]. The methyl mercury concentrations in marine biota for dry and rainy seasons are in the range of 4.35-6.26 µg/kg and 5.42-6.46 µg/kg, respectively. Arius maculatus shows the highest methyl mercury concentration for both seasons because of its habitat near the bottom of seabed and sediment which directly in contact with its skin and feed on bottom-living organism. The mercury concentrations in marine biota from Manjung coastal area determined are below the limit set in the Malaysia Food Act, (1983) (1000 µg/kg) [14]. Generally, mercury and methyl mercury concentrations in marine biota show no significant different for both seasons based on F test results which is F value is lower than F Critical Value (Hg 1.4<6.4) and (MeHg 2.8<6.4). The concentration of mercury will increase with the length, body size and age of marine biota for example older marine biota having higher concentration than younger marine biota [15 - 18]. Moreover, marine biota can absorb the bioavailability of mercury directly from the marine environment through the gill and skin or the ingestion of contaminated water and food [19, 20]. Table 2 shows the ratio of (MeHg/T-Hg) measured in marine biota for rainy and dry seasons. The ratios of (MeHg /T-Hg) in marine biota for dry and rainy seasons are in the range 5.28-9.61 % and 5.11-7.87 %, respectively. Generally, the ratios of (MeHg /T-Hg) in this study are below 10% which is common for methyl mercury content in marine biota.

Table 2. Total Mercury (THg), methyl mercury (MeHg) and ratio of MeHg/THg content in marine biota for dry and rainy seasons

Sample	THg (µg/kg)		MeHg (µg/kg)		MeHg/THg (%)	
	Dry Rainy season season		Dry season	Rainy season	Dry season	Rainy season
Johnius dussumieri	68.50±3.43	75.75±3.79	5.94±0.30	5.97±0.30	8.68±0.43	7.87±0.39
Pseudorhombus malayanus	76.50 ± 3.83	77.68±3.88	5.24 ± 0.26	5.51 ± 0.28	6.85 ± 0.34	7.10 ± 0.35
Arius maculates	65.13±3.26	82.74 ± 4.14	6.26 ± 0.31	6.46 ± 0.32	9.61±0.48	7.81 ± 0.39
Portunus pelagicus	102.12±5.11	106.10 ± 5.30	5.39 ± 0.27	5.42 ± 0.27	5.28 ± 0.26	5.11±0.26
Charybdis natator	71.50 ± 3.58	92.08 ± 4.60	4.35 ± 0.22	6.16±0.31	6.08 ± 0.30	6.69 ± 0.33

Table 3 shows comparison of mercury and methyl mercury concentration in marine biota from Manjung coastal area with other studies. Mercury concentrations in this study are in the range of reported results by Mukherjee et al. [21] in marine biota from Bay of Bengal, India, Maggi et al. [6] from Terra Nova Bay, Washington and Agusa et al. [23] from Cabang Tiga Kelantan, Kuala Terengganu, Mersing, Parit Jawa, Port Dickson and Langkawi. However, Maggi et al. [6] reported 62.3 μg/kg of methyl mercury concentration in their marine biota which is higher than the present

study. The results of mercury concentrations of this study are higher than reported by Kamaruzaman et al. [24] for demersal fish in Peninsular Malaysia, where demersal fish live on or near the seabed and feed on bottom living organisms and other fish.

Table 3.	Comparison of mercury and methyl mercury concentrations in marine biota from Manjung coastal area
	with other studies

References	Area	Marine Biota	Hg (µg/kg)	MeHg (μg/kg)
This study	Manjung coastal area	Demersal fish Shellfish	65.13-77.68 71.50-106.1	5.24-6.46 4.35-6.16
Mukherjee et al.[21]	Bay of Bengal, India	Marine fish	70-1600	-
Maggi et al. [6]	Terra Nova Bay, Washington	Marine fish	92.6	62.3
Agusa et al. [23]	Cabang Tiga Kelantan, Kuala Terengganu, Mersing, Parit Jawa, Port Dickson, and Langkawi	Marine fish	50-670	-
Kamaruzaman et al. [24]	Local LKIM fish market from Johor, Melaka, and Negeri Sembilan	Demersal fish	12-19	-

Table 4 shows the results of mercury concentrations in seawater and marine sediment. The mercury concentrations in seawater collected during high tide are in the range of 4.42 to 8.28 μ g/L which is below the limit set by Malaysian Marine Water Quality Criteria Standard (MMWMQS) Class 3: ports, oil and gas fields (50 μ g/L) [25] and comparable with the limit set by United States Environmental Protection Agency (0.3-15 μ g/L) [13]. The mercury (Hg) concentrations in marine sediment are in the range of 173.1-15.4 μ g/kg which is lower than United States Environmental Protection Agency [26] Screening Benchmark (130 μ g/kg), except for location W2. Generally, the results of mercury concentrations in seawater and marine sediment are correlated with Pearson coefficient value 0.9538. In term of geographical locations from W1 to W6 shows the decreasing trend from Lumut area to near Perak River estuary. Location W2 shows the highest mercury concentrations in marine sediment which may originate from Lumut port development in the north and iron industry, resort and residential activities in the south.

Table 4. Mercury concentrations in seawater and marine sediment

Sampling locations	Seawater (µg/L)	Marine sediment (μg/kg)		
W1	8.28±0.41	117.07±5.85		
W2	8.27 ± 0.41	173.1±8.66		
W3	5.11±0.26	27.6±1.38		
W4	5.31±0.26	26.0±1.30		
W5	4.37±0.22	15.4±0.77		
W6	4.42 ± 0.22	18.4±0.92		

Table 5 shows the transfer factor (TF) of seawater-marine biota and marine sediment-marine biota. Transfer factor explained the amount of the element expected to accumulate in a biota from the water and sediment when conditions are equilibrium [27]. Based on calculation (Equation 1 and 2) the results show that transfer factors from seawater-marine biota and marine sediment-marine biota are higher than one (TF>1), which indicated that the

transfer and accumulation of mercury in marine biota from seawater and marine sediment. The results also observed the transfer factor of sea water-marine biota are higher than marine sediment-marine biota. This might be due to the feeding behavior of biota which is filter feeder which the biota that feed by straining suspended matter and food particles from water [27, 28]. Generally, the transfer factor of demersal fish included [*Pseudorhombus malayanus*, *Arius maculates*, *Johnius dussumieri*], are lower than shellfish [*Portunus pelagicus* and *Charybdis natator*]. This might be due to the ability of demersal fish to absorb mercury mainly from through its skin as compared to shellfish as its live and feed on bottom of seabed and sediment. However, there is no significance difference for both seasons. The important factor of mercury accumulates in marine biota based on physiological processes which is influence the bioaccumulation including its bioavailability, uptake, distribution and elimination of pollutants, season, habitat, total organic carbon, biologic activity, pH, conductance, oxygen concentration, water temperature and water level and physiological different between different of fish species, migration from unpolluted areas to relatively polluted areas [21, 26, 27].

Table 5. Transfer factor of mercury for seawater-marine biota and marine sediment-marine biota

Sample	Seawater-	Marine Biota	Marine Sediment-Marine Biota		
	Dry Season Rainy Season		Dry Season	Rainy Season	
Johnius dussumieri	11.49	12.71	1.09	1.20	
Pseudorhombus malayanus	12.83	13.03	1.22	1.23	
Arius maculates	10.93	13.88	1.03	1.31	
Portunus pelagicus	17.13	17.80	1.62	1.69	
Charybdis natator	11.99	15.45	1.14	1.46	

Table 6 shows exposure ingestion dose rate in selected marine biota from Manjung coastal area which described the amount of element consumed in marine biota that an adult body weight can eat per day. The results of mercury ingestion dose rate are in the range $0.143-0.224\,\mu g/kg/day$ for dry season, while methyl mercury ingestion dose rate are in the range $0.166-0.233\,\mu g/kg/day$ for rainy season. However, for methyl mercury ingestion dose rate for dry and rainy seasons are in the range $0.00952-0.0137\,\mu g/kg/day$ and $0.0119-0.0142\,\mu g/kg/day$, respectively. All forms of mercury are poisonous and toxic to human health effect based on the amount and timing of exposure [29]. The main route of mercury and especially methyl mercury exposure from eating some types of biota based on the following factor are dose, duration, length of time since the exposure individual differences (routes of exposure, gender, age, genetic difference health of the person exposed and so on) [29, 30]. The mercury exposure ingestion dose rates in this study are below the limit, which the safe level of exposure to mercury that is $0.1\,\mu g/kg/day$ as set by United States Environmental Protection Agency [30, 31]. For methyl mercury exposure dose rates in marine biota show below the limit set by the various agencies, which the safe amount of fish for human consumption per body weight per day. For women, pregnant women and children the safe amount methyl mercury are in the range $0.1-0.3\,\mu g/kg/day$ [27, 32], adults are $0.47\mu g/kg/day$ and $0.23\,\mu g/kg/day$ [12] that is recommendation set as the limit of daily intake for mercury (Hg) in fish.

Table 7 shows health risk index (HRI) of mercury and methyl mercury in marine biota from Manjung coastal area, which is important to assess the level of human health risk when expose to the mercury and methyl mercury. Mercury entering into the marine ecosystem may pollute the natural environment and pose serious human health risks through food chain. The results of health risk index for mercury and methyl mercury in marine biota from Manjung area are less than one (HRI<1) in the range of 0.48-0.75 for dry season and 0.55-0.78 for rainy season and 0.10-0.11 for dry season and 0.12-0.14 for rainy season, respectively. This result indicates that the exposed population is unlikely to experience obvious adverse effects and which means there is no potential significant health risk associated with the consumption of marine biota from Manjung coastal area [27, 33, 34]. Therefore, marine

biota including Johnius dussumieri, Pseudorhombus malayanus, Arius maculates, Portunus pelagicus and Charybdis natator from Manjung coastal area are safe for human consumption.

Table 6	Exposure	ingestion	dose rate o	of mercury	and meths	d mercury	z in marine	hiota f	from Maniung area
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Element	Johnius dussumieri (µg/kg/day)	Pseudorhombus malayanus (µg/kg/day)	Arius maculates (μg/kg/day)	Portunus Pelagicus (µg/kg/day)	Charybdis natator (µg/kg/day)
Hg (dry season)	0.150	0.168	0.143	0.224	0.157
Hg (rainy season)	0.166	0.170	0.181	0.223	0.202
MeHg (dry season)	0.013	0.0115	0.0137	0.0118	0.00952
MeHg (rainy season)	0.0131	0.0121	0.0142	0.0119	0.0135

Table 7. Health risk index (HRI) of mercury and methyl mercury in marine biota from Manjung area

Element	Johnius dussumieri	Pseudorhombus malayanus	Arius maculates	Portunus pelagicus	Charybdis natator
Hg (dry season)	0.50	0.56	0.48	0.75	0.52
Hg (rainy season)	0.55	0.57	0.60	0.78	0.67
MeHg (dry season)	0.13	0.11	0.14	0.12	0.10
MeHg (rainy season)	0.13	0.12	0.14	0.12	0.13

Conclusion

The study found the concentrations of mercury and methyl mercury in selected marine biota from Manjung coastal area are below the limit set by Malaysian Food Act (1983) and comparable with the results by other studies. Transfer factor of seawater-marine biota shows higher than marine sediment-marine biota which indicated the transfer and accumulation of mercury in marine biota from seawater. However, no evidence of mercury and methyl mercury high input are observed in selected marine biota at Manjung coastal area and considered as safe to public for human consumption due to below the limit based on daily exposure ingestion dose (D) and support by Health Risk Index (HRI).

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