MALAYSIAN JOURNAL OF ANALYTICAL SCIENCES

Published by The Malaysian Analytical Sciences Society

ISSN 1394 - 2506

DETERMINATION OF HEAVY METALS AND RADIONUCLIDES IN *LIGUMIA RECTA* (BLACK SANDSHELL) FROM KAMPUNG GAJAH, PERAK

(Penentuan Logam Berat dan Radionuklid di dalam *Ligumia recta* (Kijing) dari Kampung Gajah, Perak)

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Received: 30 April 2015; Accepted: 4 July 2015

Abstract

The abandoned mining area is known to contain high concentration of heavy metals and radionuclides that can be harmful to human and the ecosystem itself. Mussel families are commonly used as bio monitoring tools to monitor the environmental pollution. Therefore, *Ligumia recta* (Black Sandshell), locally known as 'kijing' was used to act as biological indicators that help in monitoring the heavy metals and radionuclides level. This study was carried out in Kampung Gajah, Perak which known as ex-mining area. The objective of this study is to determine the concentrations of As, Cd, Pb, Hg, ^{238}U, ^{232}Th and ^{40}K in *Ligumia recta* collected from two ex-mining lakes; Tasik Kapal Tujuh and Tasik Air Hitam. The samples were divided into three categories based on size. The flesh were separated from the shell, oven dried, ground, sieved and pelletizing before measured using Energy Dispersive X-Ray Fluorescence Spectrometer (EDXRF). The results show that the concentrations of heavy metals and radionuclides in the two lakes have significant differences, however there are no significant differences between the size of the samples ($p>0.05$). In addition, the concentrations were found to be higher at Tasik Kapal Tujuh compared to Tasik Air Hitam. This may be due to the size of the lakes and the acidity of water in the lakes. From the concentrations of the heavy metals and radionuclides, the exposure ingestion dose rate, health risk index (HRI) and radiological health risk were calculated to monitor the safety level in the *Ligumia recta* for human consumption.

Keywords: *Ligumia recta*, heavy metals, radionuclides, ex-mining lakes, Kampung Gajah

Abstrak

Kawasan bekas perlombongan diketahui mengandungi kandungan logam berat dan radionuklid yang tinggi yang boleh membahayakan manusia dan ekosistem itu sendiri. Keluarga kepah biasanya digunakan sebagai alat pemantauan biologi untuk memantau pencemaran alam sekitar. Oleh itu, *Ligumia recta* (Black Sandshell), lebih dikenali sebagai kijing digunakan sebagai alat penunjuk biologi yang membantu untuk memantau tahap logam berat dan radionuklid. Kajian ini dijalankan di Kampung Gajah, Perak yang terkenal dengan kawasan bekas lombong. Objektif kajian ini adalah untuk menentukan kandungan As, Cd, Pb, Hg, ^{238}U, ^{232}Th dan ^{40}K di dalam *Ligumia recta* yang dikumpulkan daripada dua tasik bekas perlombongan; Tasik Kapal Tujuh dan Tasik Air Hitam. Sampel dibahagikan kepada tiga kategori berdasarkan saiz mereka. Isi kijing dikeluarkan daripada cengkerangnya kemudian dikeringkan di dalam ketuhar, dikisar, ditapis and dibentukkan dalam bentuk pellet sebelum diukur menggunakan Teknik Pendaflur Serakan Tenaga Sinaran X (EDXRF) Spektrometer. Hasil kajian menunjukkan kandungan logam berat dan radionuklid di dalam dua buah tasik itu mempunyai perbezaan yang ketara, bagaimanapun tiada perbezaan yang ketara di antara saiz sampel ($p>0.05$). Tambahan pula, kandungan di dalam Tasik Kapal Tujuh adalah lebih tinggi berbanding Tasik Air Hitam. Ini mungkin disebabkan saiz tasik dan keasidan air di dalam tasik itu. Kadar dos pengambilan pendedahan, indeks risiko kesihatan (HRI) dan risiko kesihatan radiologi telah dikira daripada kepekatan logam berat dan radionuklid untuk memantau tahap selamat di dalam *Ligumia recta* sebagai sumber makanan kepada manusia.

Kata kunci: *Ligumia recta*, logam berat, radionuklid, tasik bekas perlombongan, Kampung Gajah

Introduction

The development of tin mining industry in Malaya was started in 1848 in Larut, Perak by Long Ja'afar. Kinta valley in Perak was discovered as one of the largest tin deposits in Malaya apart from Selangor and once become the richest tin deposits in the world [1]. Kampung Gajah located in Kinta valley which has many former mining lakes which still used for agricultural activities and aquaculture economic activities. As a consequence of tin mining activities, the abandoned tin mining area is identified to contain high concentration of heavy metals and radionuclides that may pose health risk to human and the ecosystem itself. Heavy metals and radionuclides are not biodegradable in the environment even though the areas left for decades [2]. Agricultural activities such as aquaculture, oil palms estates, cattle and pig farming, poultry and orchards also contribute to the accumulation of hazardous chemicals in the environment especially in the aquatic ecosystem [3].

Ligumia recta locally known as 'kijing', is a mussels' family usually found in rivers, lakes and large streams in riffles or race ways with good current [4,5] and are found abundant in ex mining lakes in Kampung Gajah. Mussels' families are commonly known to accumulate high concentration of heavy metals in their tissues without harm itself [6]. Hence, *Ligumia recta* can pose health risk to human due to the accumulation of radionuclides and heavy metals in their tissues via food chain. *Ligumia recta* is a filter feeders animal which eat by filtering the solid material in water such as bacteria, protozoans, algae and other organic matter out of the water. They ingest the detritus materials contaminated with heavy metals and radionuclides in the water, hence threatening the health of those who consume them. *Ligumia recta* live in sand mud, firm sand or gravel. They have been recognized internationally as first-order biological indicators that help in monitoring the health of the environment or ecosystem [7]. The used of mussels as bio-indicators has been developed for years since 1975 by Goldberg in USA and later by Philips (1985) in Asian region [3]. This study embarked on the determination of heavy metals and radionuclides found in *Ligumia recta* from ex-mining lakes in Kampung Gajah, Perak, Malaysia. From that, the annual effective dose, internal hazard index, contaminant ingestion dose rate and health risk index can be estimated to access the health risk for the consumption of *Ligumia recta* to human.

Materials and Methods

Study Area

Samples were collected from Tasik Kapal Tujuh (N 04° 12.547', E 101°02.652') and Tasik Air Hitam (N 04° 13.178', E 101°02.852'), two ex-mining lakes in Kampung Gajah which located at Perak Tengah District. The Tasik Kapal Tujuh is bigger than Tasik Air Hitam and it is connected to Sungai Kampar on the west side and Tasik Air Hitam on the east side via water channel which allow the water to flow in and out in two ways under certain condition. Besides, there are oil palm plantations along east side of the lakes.

Sample Collection and Preparation

About 10 kg of *Ligumia recta* with various sizes were collected randomly from their habitat (where they are found abundantly) from each lakes in October 2012. The mussels were categorized to small, medium and large according to their length (0-5, 5-10 and 10-15 cm). The pictures of the *Ligumia recta* are shown in Figure 2. The soft tissues of categorized samples were separated from the shell using plastic knife to avoid metals contamination, pooled and washed with deionized water. The samples were air dried and then oven dried at 60-70°C until constant weight. To reduce the particle size and to ensure the homogeneity, the dried samples were ground using agate type planetary ball mill and sieved using 250 µm sieves. About 5 grams of powdered mussel sample (without the addition of a binder) were pressed at about 15 tons for 30 second using Fluxana HD electronic fusion machine to obtained cylindrical pellets. Then, the pellets were analyzed for heavy metals and radionuclides concentrations using Energy Dispersive X-Ray Fluorescence (EDXRF) technique.

Figure 1. Google map of Tasik Kapal Tujuh and Tasik Air Hitam [Source: Google Earth]

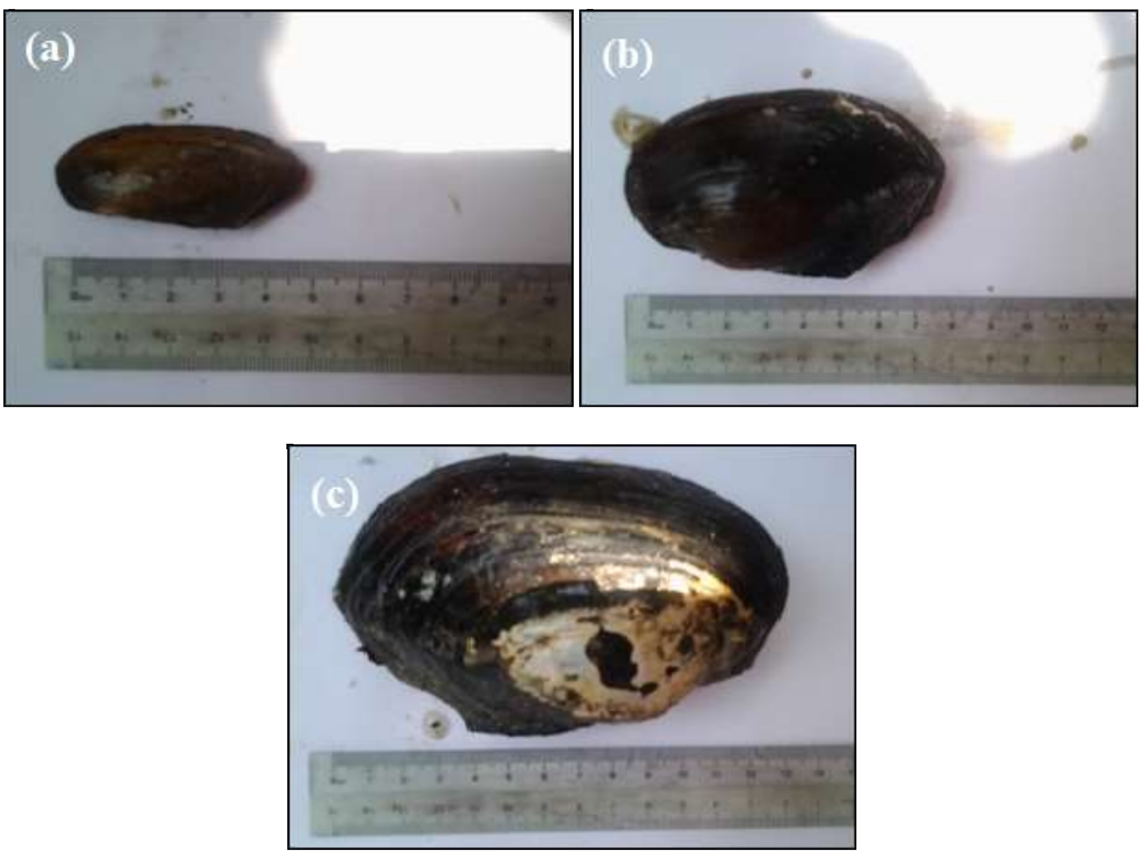

Figure 2. Picture of *Ligumia recta*, (a) small size (0-5 cm); (b) medium size (5-10 cm); (c) large size (10-15 cm)

Metal Analysis

Energy Dispersive X-Ray Fluorescence (EDXRF) Spectrometer is most widely used by researchers. X-Ray Fluorescence (XRF) is a multi-element and non-destructive technique which can simultaneously analyzed up to 30 elements on the filter medium at one time [8]. The measurement were performed using Si(Li) detector under 30 kV and 150 mA. Each of the elements was analyzed at 100 s using MiniPal/MiniMate software to determine the elemental concentration [9]. Generally, XRF quantitative analysis was carried out by the calibration curve method generated from many calibration standards. In order to achieve a good spread of data points over the range of each element to be determined, the standards must have similar matrices to the real samples. The standards were prepared by dropping about 0.5 mL of diluted elements standard solution onto a similar pellet size of cellulose filter. Then, the filter has been dried in oven at 60°C for 12 hours. The calibration curves for all elements have been constructed by using five different concentrations of elemental standards. The method was validated using SRM NIST 1566 b (oyster tissue). Meanwhile, the activity concentrations of radionuclides were calculated using conversion factors based on their concentrations measured by EDXRF.

Contaminant Ingestion Rate (IR)

The contaminant ingestion rates are used to estimate the rate of the metals being consumed by human through the consumption of mussel per body weight. The contaminant ingestion rates are calculated based on the equation 1 below [10,11]:

$$IR = (CC*CR*EF) / BW \quad (1)$$

where, CC is the contaminant concentration (mg/kg), CR is the mussel consumption rate (kg/day), EF is the exposure factor (days/year) and BW is the body weight (adult, kg).

Health Risk Index (HRI)

To evaluate the public risk assessment of mussel consumption, the health risk index was calculated. The health risk index is expressed in terms of contaminant ingestion rate to reference oral dose ratio. The equation 2 is shown below:

$$HRI = IR / RfD \quad (2)$$

where, IR is the contaminant ingestion rate and RfD is the reference oral dose.

Results and Discussion

The concentrations of arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg) in *Ligumia recta* are shown in Table 1. The concentrations of As, Cd, Pb and Hg in *Ligumia recta* from Tasik Kapal Tujuh are in the range of 23.26-33.88 mg/kg dry weight, 1.5266-2.2560 mg/kg dry weight, 0.5977-1.3677 mg/kg dry weight and 0.0302-0.0485 mg/kg dry weight, respectively. Meanwhile, the concentrations of As, Cd, Pb and Hg in *Ligumia recta* from Tasik Air Hitam are in the range of 26.28-36.52 mg/kg dry weight, 1.8548-2.3419 mg/kg dry weight, 0.4683-0.8399 mg/kg dry weight and 0.0350-0.0444 mg/kg dry weight, respectively. Ryan et al. [12] reported the concentrations of As, Cd and Pb in freshwater mussels of the upper South Alligator River, Australia were in the range of 2.9-4.2 mg/kg, 0.23-2.43 mg/kg and 1.67-4.92 mg/kg, respectively. These values were lower than the concentrations of metals in present study. In contrast, Lau et al. [13] reported the concentrations of As and Hg in freshwater mollusc of *Clithon sp. nr retropictus* were in the range of 9.17-39.0 mg/kg and 0.10-0.13 mg/kg, respectively. The concentrations of the metals in present study are in the range of the reported results by Lau et al. [13].

The concentrations of elements in this study are in dry weight basis, meanwhile the limit set by Malaysian Food Regulations 1985 and Laws of Hong Kong 1997 are in wet weight basis. The moisture content of the samples is expected to be 80% [14]. After conversion of the dry weight to wet weight basis, Cd and Hg from this study are lower than the limit set by Malaysian Food Regulations [15] meanwhile the concentrations of Pb are higher than the limit as shown in Table 1. Inorganic As is more highly toxic than organic As and it comprised in small amount which is less than 7% of the total As [16]. Limit set by Malaysia Food Regulation only specified value of inorganic As. Therefore, the measured values of As in this study are in the range of the given limit set in Malaysian Food

Regulation. However, concentrations of As, Cd, Pb and Hg in this study are lower than the limit set by Food Adulteration (Metallic Contamination) Regulations of the Public Health and Municipal Services Ordinance (PHMSO), Regulation 3 in Laws of Hong Kong [17]. Among the three size classes, the medium size class has the highest concentration of As than other two classes. Meanwhile, for Cd, Pb and Hg the concentrations are decreases from small size to the big size class. However, there are no significant difference between the sizes ($p>0.05$). Generally, Tasik Air Hitam has lower metals concentrations than Tasik Kapal Tujuh which may due to the acidity of the water in Tasik Air Hitam. The pH of the water in Tasik Air Hitam is in the range of 6.70-6.96 which slightly more acidic than Tasik Kapal Tujuh (6.98-7.10). The bioaccumulation of the metals in aquatic organisms were depends on the feeding habit, growth rate and age of the organism; while, the bioavailability of the metals were influence by the pH, hardness and acid-volatile sulphide of the water and sediment [13].

Table 1. Concentrations of arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg) in *Ligumia recta* from Tasik Kapal Tujuh and Tasik Air Hitam

Elements		**As**	**Cd**	**Pb**	**Hg**
Unit		**mg/kg**			
Kapal Tujuh	A	23.26±1.16	2.26±0.07	8.43±0.34	0.030±0.001
	B	33.88±1.69	1.57±0.05	11.69±0.47	0.048±0.002
	C	33.64±1.68	2.09±0.06	17.00±0.68	0.044±0.002
Air Hitam	A	29.98±1.49	2.34±0.07	9.52±0.38	0.044±0.002
	B	36.52±1.82	2.21±0.06	10.59±0.42	0.035±0.002
	C	26.28±1.31	1.85±0.05	11.69±0.47	0.043±0.002
Malaysian Food Regulations, 1985[a]		1.00*	2.00	1.50	0.50[#]
Food Adulteration (Metallic Contamination) Regulations, 1997[a]		10.0	2.0	6.0	0.5

Note: A- Small size, B- Medium size and C- Large size, [a] – wet weight basis, * - inorganic arsenic, [#] - methyl mercury

Table 2 shows the concentrations of potassium (K), uranium (U), thorium (Th) and activity concentrations of potassium-40 (^{40}K), uranium-238 (^{238}U) and thorium-232 (^{232}Th) in *Ligumia recta*. The activity concentrations are calculated based on the concentrations of the radionuclides. Potassium-40 is a naturally occurring isotope of potassium and it is occurring in small fraction (about 0.012%) in potassium. The concentrations of K in *Ligumia recta* from Tasik Kapal Tujuh and Tasik Air Hitam are in the range of 0.0868-0.0944 % and 0.0913-0.1806 %, respectively. The range of ^{40}K activity concentrations in Tasik Kapal Tujuh is from 28.28 to 55.92 Bq/kg and Tasik Air Hitam from 26.87 to 29.24 Bq/kg. The concentrations of U and activity concentrations of ^{238}U in *Ligumia recta* from Tasik Kapal Tujuh are in the range of 6.40-11.95 µg/kg and 0.08-0.15 Bq/kg, respectively. Meanwhile, the range of U concentrations and activity concentrations of ^{238}U in Tasik Air Hitam are 5.25-5.46 µg/kg and 0.06-0.07 Bq/kg. The concentrations of Th in *Ligumia recta* from Tasik Kapal Tujuh and Tasik Air Hitam are in the range of 45.56-168.23 µg/kg and 39.10-102.81 µg/kg, respectively. The range of ^{232}Th activity concentrations in Tasik Kapal Tujuh is from 0.19 to 0.68 Bq/kg and Tasik Air Hitam from 0.16 to 0.42 Bq/kg.

In the present results, the activity concentrations of ^{238}U are much lower than the reported results in mussels (*Mytilussp*) from Krka river estuary by Cuculić et al. [18] (12.1-19.4 Bq/kg). Ryan et al. reported the concentrations of U and the activity concentrations of ^{238}U in freshwater mussels of the upper South Alligator River, Australia were in the range of 0.12-1.00 mg/kg and 1.49-12.35 Bq/kg, respectively [12]. These values were much higher than the results from this study. The data in Table 1 for ^{238}U and ^{232}Th are also lower than the results by Alam et al. (2.8-3.6 Bq/kg and 2.6-3.2 Bq/kg, respectively) and the ^{40}K results are in the range of the reported results (23.1-80.0 Bq/kg) [7]. There are no limits set in the Malaysian Food Regulations (1985) for K, U and Th for bivalve mollusks. In Tasik Kapal Tujuh the concentrations of K and U for small size are the highest and for Th the highest concentration is big size class. Meanwhile, in Tasik Air Hitam, the medium size class has the highest concentration of K, U and Th than other two classes. However, there are no significant difference between the sizes and the two lakes ($p>0.05$).

Table 2. Concentrations of potassium (K), uranium (U), thorium (Th) and activity concentrations of potassium-40 (^{40}K), uranium-238 (^{238}U) and thorium-232 (^{232}Th)

Elements	Unit	Kapal Tujuh			Air Hitam		
		A	B	C	A	B	C
K	%	0.094±0.004	0.087±0.003	0.088±0.004	0.181±0.007	0.097±0.004	0.091±0.004
U	µg/kg	7.81±0.36	6.40±0.26	11.95±0.48	5.25±0.21	5.46±0.21	5.44±0.21
Th	µg/kg	45.56±3.58	66.26±4.64	168.23±11.78	39.10±2.73	54.17±3.79	102.81±7.20
^{40}K	Bq/kg	55.92±2.24	30.07±1.20	28.28±1.13	29.24±1.17	26.87±1.07	27.29±1.09
^{238}U	Bq/kg	0.096±0.004	0.079±0.003	0.148±0.004	0.065±0.002	0.067±0.003	0.067±0.003
^{232}Th	Bq/kg	0.19±0.01	0.27±0.02	0.68±0.05	0.16±0.01	0.22±0.02	0.42±0.03

Note: A- Small size, B- Medium size and C- Large size

Annual effective dose (AED) and internal hazard index (H_{in}) are calculated to evaluate the radiological risk for human consumption. Table 3 shows the annual effective dose and internal hazard index for *Ligumia recta* from Tasik Kapal Tujuh and Tasik Air Hitam. The annual effective dose is in the range of 0.0030-0.0057 mSv/yr, which below than the worldwide average of 0.3 mSv/yr for ingested food [19]. On the other hand, the internal hazard index is in the range of 0.0017-0.0050. Internal hazard index below than one indicating there are low radiological risk, meanwhile the internal hazard index more than one indicates high radiological risk. The internal hazard indexes from this study are below than one, means the radiological risk for the consumption of *Ligumia recta* are low.

Table 3. Annual effective dose (AED) and Internal hazard index (H_{in})

Samples		Annual effective dose (AED), mSv/yr	Internal hazard index (H_{in})
Kapal Tujuh	A	0.0057	0.0024
	B	0.0034	0.0024
	C	0.0041	0.0050
Air Hitam	A	0.0031	0.0017
	B	0.0030	0.0020
	C	0.0034	0.0031

Note: A- Small size, B- Medium size and C- Large size

The exposure ingestion dose rate is calculated based on the 70 kg adult person consumed 2.5 g of oyster per day in average of 52 days per year [10]. This value was used to calculate the health risk index. Table 4 shows the annual exposure ingestion dose rate of *Ligumia recta* from Tasik Kapal Tujuh and Tasik Air Hitam for all elements. Generally, all of the elements have exposure ingestion dose rate below than 1 mg/kg/yr except for K which in the range of 1.6117-3.3546 mg/kg/yr.

To evaluate the potential health risk for consuming the *Ligumia recta* to human, the health risk index (HRI) is calculated. It is the ratio of exposure ingestion dose rate to the referenced oral dose. The reference oral dose for As, Cd, Pb, Hg, U and Th are 3×10^{-4}, 0.001, 0.0036, 0.0003, 0.003 and 0.003 mg/kg/day, respectively (USEPA). Table 5 shows the HRI values for all elements in *Ligumia recta*. HRI less than one (HRI < 1) indicates that there are no potential health risk to human. On the other hand, HRI more than one (HRI > 1) indicating the human will have potential to adverse health effects. As calculated, all of the elements have HRI < 1 which means the consumption of *Ligumia recta* will not give potential adverse health effects to local people.

Table 4. Contaminant ingestion rate (mg/kg/yr)

Elements	**Kapal Tujuh**			**Air Hitam**		
	A	**B**	**C**	**A**	**B**	**C**
As	0.0432	0.0629	0.0625	0.0557	0.0678	0.0488
Cd	0.0042	0.0028	0.0039	0.0043	0.0041	0.0034
Pb	0.0025	0.0011	0.0012	0.0016	0.0012	0.0009
Hg	6.0×10^{-5}	9.0×10^{-5}	8.0×10^{-5}	8.0×10^{-5}	6.0×10^{-5}	8.0×10^{-5}
K	3.3546	1.8038	1.6964	1.7541	1.6117	1.6367
U	1.4×10^{-5}	1.2×10^{-5}	2.2×10^{-5}	1.0×10^{-5}	1.0×10^{-5}	1.0×10^{-5}
Th	8.0×10^{-5}	1.2×10^{-4}	3.1×10^{-4}	7.0×10^{-5}	1.0×10^{-4}	1.9×10^{-4}

Note: A- Small size, B- Medium size and C- Large size

Table 5. Health Risk Index (HRI)

Elements	**Kapal Tujuh**			**Air Hitam**		
	A	**B**	**C**	**A**	**B**	**C**
As	0.3946	0.5746	0.5706	0.5084	0.6193	0.4457
Cd	1.2×10^{-2}	7.8×10^{-3}	1.1×10^{-2}	1.2×10^{-2}	1.1×10^{-2}	9.4×10^{-3}
Pb	1.9×10^{-3}	8.0×10^{-4}	9.0×10^{-4}	1.2×10^{-3}	9.0×10^{-4}	7.0×10^{-4}
Hg	5.1×10^{-4}	8.2×10^{-4}	7.4×10^{-4}	7.5×10^{-4}	5.9×10^{-4}	7.3×10^{-4}
U	1.1×10^{-3}	7.8×10^{-3}	9.6×10^{-3}	5.7×10^{-3}	6.6×10^{-3}	8.7×10^{-3}
Th	2.0×10^{-5}	1.3×10^{-4}	1.5×10^{-4}	1.2×10^{-4}	1.2×10^{-4}	1.1×10^{-4}

Note: A- Small size, B- Medium size and C- Large size

Conclusion

The mussel family has been used for years in environmental monitoring. By using *Ligumia recta*, the contaminations of As, Cd, Pb, Hg, K, U and Th in Tasik Kapal Tujuh and Tasik Air Hitam were found not to be serious. The contaminations of these elements may be from the natural sources as they naturally occurring in the environments and anthropogenic sources such as used of fertilizers and pesticides from the surrounding plantations, soils run-off and the past mining activities.

Although some of the heavy metals have higher concentrations than the permissible limits, it will not give acute toxicity to human as the mussels are not regularly consumed. However, the risk is dependent on the amount of the mussels consumed; the higher the amount of *Ligumia recta* being consumed, the higher the risk to the human health. The annual effective dose and internal hazard index values indicates that the consumption of *Ligumia recta* has low radiological risk. Moreover, the HRI values show that the *Ligumia recta* are safe to consume by the local people.

Acknowledgement

The authors wish to thank the Faculty of Applied Sciences, Universiti Teknologi MARA and Institute of Sciences, Universiti Teknologi MARA for their support in finishing this study.

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