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LEVEL OF TOTAL ARSENIC AND MERCURY IN SELECTED BIVALVE SPECIES FROM MELAKA AND PAHANG STATES OF MALAYSIA

Munirah Abdul Zali[1]*, Nurul Fatehah Ibnul Kasim Ahmad[2] and Masni Mohd Ali[2]

[1]Waste Technology and Environment Division, Malaysian Nuclear Agency, Bangi, 43000, Kajang, Selangor, Malaysia
[2]Faculty of Science and Technology, Universiti Kebangsaan Malaysia (UKM), 43000 UKM Bangi, Selangor, Malaysia
*Corresponding author email: munirahzali@nm.gov.my

ABSTRACT

Arsenic and mercury levels in marine organisms are critical environmental and public health concerns. These toxic elements can accumulate in bivalves and pose significant consumer risks. Monitoring of these elements is essential to ensure the safety of seafood consumption. In this study cockles, clams and mussels were collected from Melaka and Pahang states for the determination of total arsenic and total mercury levels. The bivalve tissues were digested using microwave digester and analyzed using inductively coupled plasma-mass spectrometer (ICP-MS). The total arsenic in the bivalve samples ranged between 0.85 ± 0.04 mg kg^{-1} and 1.60 ± 0.27 mg kg^{-1}. Meanwhile, the total mercury in the bivalve samples was from 0.004 ± 0.002 to 0.007 ± 0.002 mg kg^{-1}. The result of the elements was compared with the local and global guidelines. This research provides the latest information for food safety and risk assessment studies in the regional and national levels.

Keywords: bivalvia, arsenic, mercury, inductively coupled plasma-mass spectrometer.

ABSTRAK

Paras arsenik dan merkuri dalam kerang-kerangan adalah antara perkara kritikal yang dibimbangkan pada alam sekitar dan kesihatan awaml. Unsur-unsur toksik ini boleh berkumpul dalam organisma marin dan boleh memberi risiko yang ketara pada pengguna. Pemantauan unsur-unsur ini adalah penting untuk memastikan keselamatan pengambilan makanan laut. Di dalam kajian ini kerang, lala dan kupang telah diambil dari negeri Melaka dan Pahang untuk menentukan jumlah arsenik dan jumlah merkuri. Tisu kerang-kerangan ini telah dicerna menggunakan pencerna gelombang mikro dan dianalisis menggunakan spektrometer jisim plasma (ICP-MS) yang digabungkan secara induktif. Jumlah arsenik dalam sampel kerang berjulat antara 0.85 ± 0.04 mg kg-1 dan 1.60 ± 0.27 mg kg-1. Manakala, jumlah merkuri dalam sampel kerang daripada tidak dapat dikesan kepada 0.01 ± 0.002 mg kg-1. Hasil daripada elemen tersebut dibandingkan dengan garis panduan tempatan dan global. Penyelidikan ini menyediakan maklumat terkini untuk kajian keselamatan makanan dan penilaian risiko di peringkat serantau dan nasional.

Kata kunci: *kerang-kerangan, arsenik, merkuri, spektrometer jisim plasma yang digabungkan secara induktif*

INTRODUCTION

Bivalve, as filter feeders, play a significant role in the marine ecosystem by filtering water and accumulating various substances, including contaminants. Among these contaminants, arsenic and mercury are of particular concern due to their toxicity and potential health impacts on both marine life and humans. Arsenic (As) is a naturally occurring element found in the Earth's crust, and its presence in marine environments can result from natural processes such as volcanic activity and the weathering of rocks, as well as from anthropogenic sources like industrial discharges and agricultural runoff (Luvonga et al., 2020). Similarly, mercury (Hg), primarily released into the environment through industrial activities such as coal combustion and mining, poses severe ecological and health risks due to its ability to bioaccumulate in marine organisms and biomagnified through the food web (Fui et al., 2022; Haris et al., 2020).

The study of As and Hg in bivalve samples is crucial for understanding the extent of marine pollution and its implications for food safety. Bivalve are considered good bioindicators for monitoring the presence of heavy metals in marine environments due to their sedentary nature and ability to accumulate contaminants in their tissues over time (Alina et al., 2012; Lucero Rincón et al., 2023). This makes them an excellent model for assessing the levels of these toxic elements and the potential risks they pose to human health when contaminated mussels are consumed. Monitoring these levels helps in the implementation of regulations and policies aimed at reducing pollution and protecting marine and human health.

Recent studies have shown varying levels of As and Hg in bivalvia samples, reflecting the diversity of contamination sources and environmental conditions (Al-Sulaiti et al., 2022; Luvonga et al., 2020). For instance, recent research in Klang River, Malaysia revealed significant accumulation of Hg in gastropod samples, highlighting the impact of local industrial activities and urbanization towards marine environment (Haris et al., 2020). Similarly, investigations in other coastal regions have demonstrated that bivalvia populations in proximity to urbanized and industrialized areas tend to exhibit higher concentrations of these metals compared to those from less impacted areas (Alina et al., 2012).

The quantification of the total As and Hg in biological samples such as bivalve often employs the use of microwave digestion followed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) for precise quantification. The microwave digestion technique is utilized to efficiently break down the organic matrix of the samples, ensuring complete extraction of the metals. ICP-MS is then used for its high sensitivity and ability to detect trace levels of As and Hg. To ensure method accuracy and reliability, analysis of Standard Reference Materials (SRMs) and/or certified reference material (CRM) is essential to validate the performance of the digestion and quantification procedures. This practice ensures that the results obtained are accurate and precise.

In Malaysia, the guidelines of As and Hg levels in bivalves are established based on organic As and methyl-Hg. According to the Malaysian Food Regulation 1985, the Malaysian Food Regulations 1985, the maximum allowable limit for organic As is 1.0 mg kg^{-1}, meanwhile for methyl-Hg is set at 0.5 mg kg^{-1}. Due to the high cost and tedious analytical methods for speciation analysis, the routine bivalve samples analysis for organic As and methyl-Hg were screened for total As and Hg levels prior to speciation analysis in the majority of laboratories. Therefore, the precise and accurate determination of total As and Hg was vital to identify the level of these metals and understand their distribution and behavior in the environment. This study aims to determine the level of total As and Hg in selected bivalve samples from Melaka and Pahang states and compare with the local and global guidelines.

METHODOLOGY

Sample collection and preparation

A total of 20 to 40 samples of three different bivalvia species (cockle, clam and mussel) were purchased from the local fisherman at Kuala Sungai Baru, Melaka and Kuantan, Pahang (Figure 1). The locations were selected based on the abundance of bivalvia from the two states. The three bivalvia samples were cockle, clam and mussel (Figure 2). The lengths of the bivalvia samples were 2-3 cm, 3-4 cm and 6-7 cm respectively. The samples were purchased from local fishermen between July and December 2022. The bivalvia samples were stored in the plastic zip lock bag and kept chilled at 4°C during transportation. In the laboratory the samples were stored frozen below 0°C prior to extraction and analysis.

Figure 1. Sampling locations

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Figure 2. The bivalvia samples in this study. Cockle (i), clam (ii), and mussel (iii)

Digestion of samples using microwave digester

Frozen bivalvia samples were thawed at room temperature and dissected using plastic forceps. The soft edible tissues were pooled in between 10 and 20 samples for each bivalvia species. The pool bivalvia samples were ground using stainless steel grinder and stored in propylene containers. The bivalvia tissue samples were digested according to the Association of Analytical Collaboration (AOAC) Official Method 2015.01 Heavy Metals in Food. In brief, 1.0 g of wet-weight soft bivalvia tissues (n=3) were weighted using analytical balance and transferred to microwave digester vessels. The tissues were added with 10ml concentrated nitric acid and allowed to stand for 10 minutes.

The tissue samples were digested using microwave digester (MARS 6, USA). The digestion program was set at 200°C for 15 minutes ramping followed by 15 minutes holding times. The pressure was set at 800 psi and the power was between 900-1050 W during the entire digestion. The digested tissue samples were allowed to cool and mark up to 25 ml using deionized water. The samples were stored in polypropylene tube. In every batch of digestion procedure, two method blanks and standard reference materials (SRMs) were digested for method performance analysis. The SRM used in this study are trace elements and methylmercury in mussel tissue (National Institute of Standard and Technology (NIST) SRM 2976), Dogfish liver certified reference material (CRM) for trace metals and other constituents (National Research Council Canada (NRCC) DOLT-5) and oyster tissue (NIST SRM 1566b). The limit of detection (LOD) was determined through the analysis of 23 method blanks and calculated as 3 times the standard deviation (SD) of the method blanks results.

Quantification of arsenic and mercury in the bivalvia samples

The determination of As and Hg in the bivalvia tissue samples was performed using ICP-MS. As was quantified using kinetic energy discrimination (KED) mode using helium gas with 3.5 ml min^{-1} flow and Hg was determined using standard mode. To compensate for the percentage of acidity in samples and the carbon buffer content in the samples, the internal and calibration standards were prepared in 20% acid and 15% methanol. The calibration standards were prepared using As and Hg single-element standards (CPA Chem, Bulgaria). The internal standard rhodium (Rh) and Iridium (Ir) mixture was prepared at 0.020 mg L^{-1} and 0.040 mg L^{-1} respectively for instrument internal standardization. Three calibration points consisted of 0.010 mg L^{-1}, 0.025 mg L^{-1}, and 0.050 mg L^{-1} were established with correlation coefficient (R^2) of more than 0.995. Calibration verification (quality control standards) was analyzed for every ten samples and the values ranged between 90 and 110%. The concentration of samples was reported in mg kg^{-1} (wet weight) units by following formula:

$$mg\ kg^{-1} = mg\ L^{-1}\ x\ fv\ (L)\ x\ \frac{1}{ws\ (kg)}$$

Where *fv* is the final volume mark up after digestion and *ws* is the weight of sample. The LOD of As and Hg for this method was 0.004 and 0.002 mg kg^{-1} for As and Hg respectively. The spatial difference of As and Hg in both locations were plot using ArcGIS software and statistical analysis (t-test and *F*-test) was performed using Excel software.

RESULTS AND DISCUSSION

Method performance using certified reference material (CRM)

Table 1 shows the concentration obtained from this research analytical procedures, the certified values of the SRM/CRM and the percentage recovery. The percentage recovery (%) for As was between 85.17 ± 5.35 and 113.83 ± 3.24%. The percentage recovery (%) for Hg in SRM/CRM was between 76.5 ± 9.17 and 91.56 ± 1.59. Based on the percentage of recovery results, the microwave digestion and the quantification procedures were in good performance, produce satisfactory precision and reliable results.

Table 1. SRM and CRM recovery results

SRM/CRM	Arsenic (As)			Mercury (Hg)		
	Obtain result (mg kg^{-1})	Certified Value (mg kg^{-1})	Recovery (%)	Obtain result (mg kg^{-1})	Certified Value (mg kg^{-1})	Recovery (%)
Mussel Tissue NIST SRM 2976 (n=2)	15.14 ± 0.43	13.3 ± 1.8	113.83 ± 3.24	0.056 ± 0.001	0.061 ± 0.04	91.56 ± 1.59
Dogfish Liver NRCC CRM DOLT-5 (n=2)	29.32 ± 0.99	34.6 ± 2.4	87.4 ± 2.86	0.36 ± 0.02	0.44 ± 0.18	80.64 ± 5.2
Oyster Tissue NIST SRM 1566b (n=2)	6.52 ± 0.40	7.65 ± 0.65	85.17 ± 5.35	0.028 ± 0.003	0.037 ± 0.0013	76.56 ± 9.17

Level of arsenic (As) and mercury (Hg) in the bivalvia samples

Figure 3 displays the level of As in the bivalvia samples from Kuala Sungai Baru (i) and Kuantan (ii). In Kuala Sungai Baru, As concentrations in cockle, clam and mussel samples were 1.48 ± 0.10, 1.23 ± 0.09 and 0.94 ± 0.02 mg kg $^{-1}$ respectively. Meanwhile, in Kuantan, the level of As in cockle, clam and mussel samples were 1.04 ± 0.10, 1.60 ± 0.09, and 0.85 ± 0.02 mg kg^{-1} respectively. The As in the bivalvia samples may derived from natural input and also anthropogenic activities such as mining, industrial discharge and agricultural input runoff

(Luvonga et al., 2020). Based on the study by Alina et al. (2012), the As level in their cockle samples (0.88 ± 0.02 mg kg $^{-1}$) from the Strait of Malacca was lower than the As level in the cockle samples from Kuala Sg Baru Melaka. The increase of As in bivalvia samples from the same region of sampling locations and after more than ten years of study may indicate the increase of this element may derive from the urbanization and industrialization nearby the sampling locations (Salam et al., 2021)

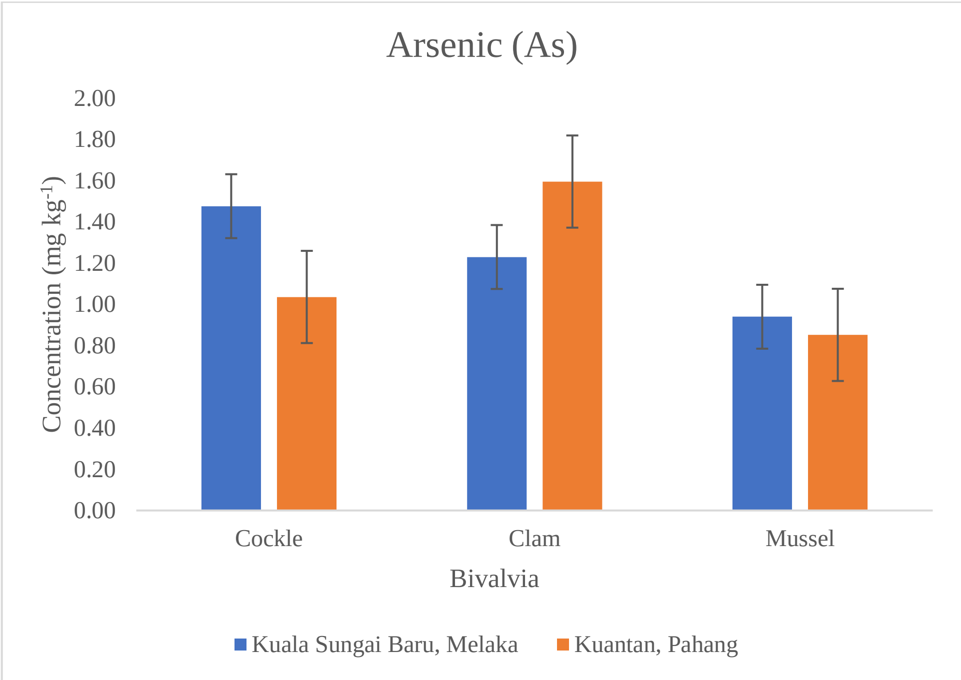

Figure 3. As level in the bivalvia from Kuala Sungai Baru, Melaka and Kuantan, Pahang

Figure 4 shows the level of Hg in bivalvia samples from Kuala Sungai Baru (i) and Kuantan (ii). In Kuala Sungai Baru, the concentration of Hg in cockle, clam and mussel samples were 0.006 ± 0.06, 0.007 ± 0.07, and 0.007 ± 0.003 mg kg^{-1} respectively. Meanwhile in Kuantan, the level of Hg in cockle, clam and mussel samples were 0.006 ± 0.001, 0.005 ± 0.001, and 0.004 ± 0.0003 mg kg^{-1} respectively. The Hg concentration in bivalvia samples in both locations was similar with the exception to the mussel sample in Kuantan region. Similar to As, the bioavailability of Hg in bivalvia samples may derived from natural and anthropogenic sources (Al-Sulaiti et al., 2022). The Hg natural sources originated from earthquake activities, volcanic eruptions, and geothermal springs meanwhile mining, smelting, coal burning, and oil refinery were due to Hg anthropogenic activities. Based on the previous study by Fui et al. (2022), the Hg in their bivalve samples (0.23 mg kg-^{1}) at Setiu, Terengganu was higher than the Hg in the bivalve of Kuantan. The difference of Hg concentration in the bivalve samples may due to the Hg availability in the water ecosystem, the hydrodynamic of the environment and also detoxifying capabilities of the bivalves (Fui et al., 2022; Haris et al., 2020).

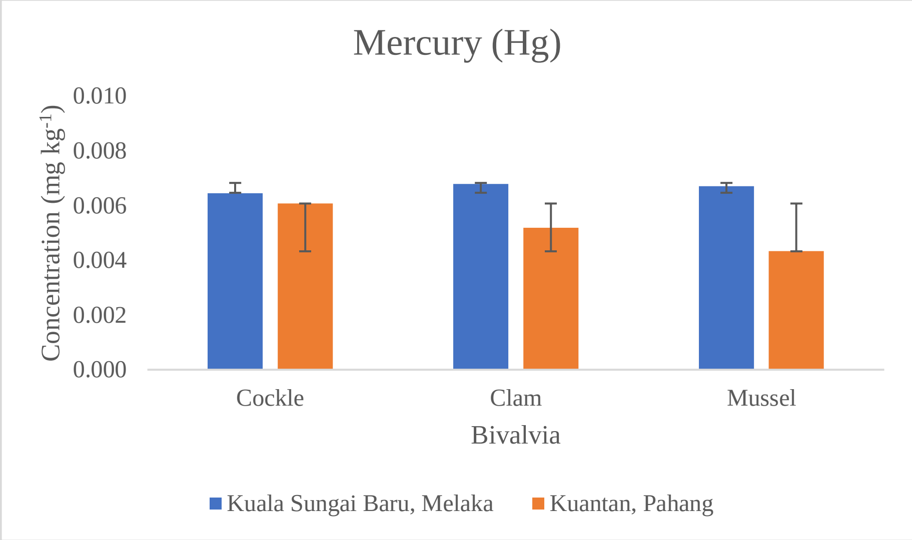

Figure 4. Hg level in the bivalvia from Kuala Sungai Baru, Melaka and Kuantan, Pahang

Spatial difference of arsenic (As) and mercury (Hg) between Melaka and Pahang states

Based on the *t*-test (Table 2), the level of As and Hg in all bivalvia samples showed insignificant differences between the two sampling locations (p>0.05). The similar distribution of As and Hg in all bivalve samples from both sampling stations may due to the similar sampling location (muddy coastal ecosystem) with moderate urbanization areas. Kuala Sungai Baru, Melaka and Kuantan, Pahang areas were occupied by medium industrialization with shipping port, petroleum and tourism industries.

Table 2. Spatial difference of As and Hg in all three bivalvia species based on *t*-test

Bivalvia	As		Hg	
	Melaka	Pahang	Melaka	Pahang
Cockle	$p > 0.05$		$p > 0.05$	
Clam	$p > 0.05$		$p > 0.05$	
Mussel	$p > 0.05$		$p > 0.05$	

Based on *F*- test, the level of As in both locations was significantly difference between bivalvia samples (p<0.05) (Table 3). The difference in As accumulation between bivalvia samples was due to the biological parameters such as body sizes, growth, reproductive status and genotype (Harsono et al., 2017). In contrast, based on the *F*-test, the concentration of Hg in both locations was similar between bivalvia samples (p>0.05) (Table 3).

Table 3. F-test result for As and Hg difference in all three bivalvia species in Melaka and Pahang

Location	As			Hg		
	Cockle	Clam	Mussel	Cockle	Clam	Mussel
Melaka	p<0.05			p>0.05		
Pahang	p<0.05			p>0.05		

Comparison of arsenic (As) and mercury (Hg) levels with guideline

The Malaysian Food Act 1985 guidelines specify the maximum permissible level of organic As at 1.0 mg kg^{-1}. Based on the analysis of total As in bivalve samples from both locations, the clam and cockle samples exceeded the total of 1.0 mg kg^{-1} level. Therefore, it is compulsory and recommended for further As speciation analysis for clam and cockle samples from both locations to investigate the species of As in the samples as the amount of As detected may derived from the total As. However, according to the Food and Agricultural Organization (FAO) of the United Nations, guidelines for total As in bivalvia samples have not been established. Meanwhile, for Hg, all bivalve samples in both locations were below the methyl-Hg guideline from the Malaysian Food Act 1985 and lower than the international guideline for total Hg in bivalvia sample based on FAO (0.5 mg kg^{-1}).

CONCLUSIONS

As for conclusion, the distribution of total As and Hg levels of cockle were examined for the clam, cockle and mussel samples from Kuala Sungai Baru, Melaka and Kuantan, Pahang. The As and Hg distribution patterns for all three bivalve samples were similar due to the similar geological and urbanization regions. However, the different levels of these elements between bivalve samples may due to biological factors. It is recommended to perform further As speciation analysis for clam and cockle samples to understand the safety risk of bivalve consumption in the local areas. The further analysis of Hg in the food chain also was important to investigate the potential of Hg biomagnification across the food chain. This study newest data on As and Hg food safety and risk assessment in the bivalve samples from local areas of Melaka and Pahang states. Furthermore, it is recommended to conduct the food contaminants monitoring program more frequently in the study area to ensure food safety compliance and address human risk potential.

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