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SHORT COMMUNICATION

Human Health Risk Assessment of Heavy Metals in Shellfish from Kudat, Sabah

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ABSTRACT

Introduction: Shellfish is likely to be contaminated with heavy metals brought about by various environmental factors such as climate change, bioaccumulation of environmental contaminants and imbalanced natural ecosystem. **Methods**: Shellfish were selected for heavy metal detection as they are mainly consumed by the locals in Kudat. Arsenic, Cadmium, Chromium, Nickel, and Plumbum (As, Cd, Cr, Ni, and Pb) content in clam (*Meretrix* spp.), scallop (*Amusium pleuronectes*) and conch (*Strombus canabrium*) were determined by the US EPA 200.3 acid digestion method and Inductively Coupled Plasma Mass Spectrophotometer (ICP-MS). Risk assessment was calculated to assess the total exposure of heavy metals among the population of Kudat. **Results:** Among all the heavy metals, studied, As was found to have the highest concentration and this was found in scallop with the concentration level being 18.93±5.30 µg/g compared to clam and conch. Estimated daily intake of the heavy metals by the population ranged from 0.60-6.82 g/ day/ kg for As, 0.02-1.58µg/day/kg for Cd, 0.37-0.94µg/day/kg for Cr, 0.16-0.61 g/day/ kg for Ni and 0.10-0.25µg/day/kg for Pb based on previous calculation to exposure. The hazard quotient of As and Cd in scallop was greater than 1.0. No acceptable exposure level for these shellfish has been previously reported. The rate of consumption of these metals did not exceed the standards prescribed in the Food Act 1983 and Food Regulations 1985. **Conclusion:** Based on this study, it is concluded that the exposure to heavy metals risk from the consumption of these shellfish among the population in Kudat, Sabah is at an acceptable level.

Key words: Heavy metals, Kudat, risk assessment, shellfish

INTRODUCTION

Seafood products such as shellfish are a rich source of protein as well as an important source of income for the fishing community in Malaysia. Shellfish is nutritious given its protein and mineral contents. However, shellfish may contain heavy metals harmful to human health (Koh, 2011). The presence of heavy metals is ubiquitous in the environment, but the naturally occurring heavy metals are generally low in concentration. Rapid industrialisation in Malaysia has led to an alarming level of heavy metal pollution (Liu *et al.*, 2011). As a result, heavy metals may accumulate in shellfish through the food chain, and

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consumption of these contaminated seafood pose risks to human health (Yap *et al.*, 2008). Thus, risk assessment of shellfish should be carried out as a preventive measure to safeguard public health. This study was aimed at investigating human health risk assessment of heavy metals from the consumption of shellfish obtained from the Kudat area in Sabah.

METHODS

Three types of shellfish that are commonly consumed were selected for analysis namely, clam (*Meretrix* spp.), conch(*Strombus canabrium*) and scallop (*Amusium pleuronectes)*. A total amount of 200g of each sample was purchased in the fish market in Kudat. All samples were stored at -3^{o}C before analysis.

All reagents were of analytical reagent grade. Concentrated nitric acid (69%, England), hydrogen peroxide (60%, Germany), hydrochloric acid (37%, Germany) and deionised water were used for all dilutions.

Sample preparation and analysis procedures were according to US EPA method (EPA 2000) with some modifications. Samples were thawed to room temperature. The tissue of the shellfish was removed from the shell using a stainless steel knive and washed with deionised water three times to make sure all dirt was removed. Samples were then oven dried to constant weight at 60-65^{o}C for few days. The dried samples were kept in a dessicator until further analysis.

Approximately 1.0 g of dried sample was processed in a triplicate for acid digestion. All samples were digested in concentrated nitric acid and placed on a hot plate in a 50 ml beaker until boiling point and fully digested. The digested samples were then allowed to cool to room temperature before 2 ml of 60% hydrogen peroxide was added and the sample reheated. This step was repeated until the solution turned clear from dark brown colour, then 10 ml of 60% hydrogen peroxide was added for oxidation. The solution was allowed to cool once again and digested with another 2 ml of 37% hydrochloric acid and reheated for several minutes until only 5 ml of the solution was left. The solution was diluted with deionised water to a total volume of 50 ml and filtered through 0.6 µm Whatman filter paper and transferred to a 50 ml centrifuge tube using a syringe with 0.45 µm syringe head filter. The tubes were stored until further analysis.

The analysis was conducted using Inductively Coupled Plasma Mass Spectrophometer (ICP MS Model Elan 9000 Perkin Elmer). Experiments were conducted in triplicates.

Calculation of health risk

Uptake rates of heavy metals by consuming shellfish or the estimated daily intake (EDI), can be calculated using the following formula:

$$EDI = \frac{C \times IR \times EF \times ED}{BW \times AT}$$

where, *C* (mg/g) is the concentration of heavy metals in the shellfish of edible tissue, obtained from the analysis; *R* (ingestion rate), is the rate of consumption of shellfish a day (160g/day/person)(Agusa *et al.*, 2007); *EF* is the exposure frequency, for example, 365 days for those who eat shellfish seven times a week and 52 days for those who eat shellfish once a week; *ED* is the duration of exposure (ED is usually calculated according to the study objective, and is usually calculated for a period of one year or 365 days); *AT* is the average exposure to non-carcinogens for 365 days/year and multiplied by ED; *BW* is the average body weight. According to Azmi *et al.* (2009) in Malaysia, the average weight for men is 66.56kg and for women, it is 58.44kg while the average weight is 62.65kg.

Hazard quotient (HQ) is the ratio used for the characterisation of risk and also to estimate whether a particular risk has a significant impact. HQ is calculated using the following equation:

$$HQ = \frac{EDI}{RfD}$$

where

EDI = intake of heavy metals through the consumption of clam-shells (As described in previous equation)

RfD = estimated rate of safe shellfish consumption in terms of heavy metal

RfD, reference dose, is an estimated value that is recommended by international agencies such as US EPA, FAO and WHO. *RfD*, as described above, is an estimated safe limit of daily intake for a chemical. *RfD* is usually expressed in units of mg/kg/day or mg/kg/day. According to US EPA, Pb does not come with a *RfD* and this concept does not fit Pb as there are a few potential adverse health effects that could occur even if the concentration level of Pb does exceed the threshold limit.

Statistical analysis

One-Way ANOVA was performed on all experimental data with SPSS Version 21.0. The significance level was set at $p<0.05$.

RESULTS & DISCUSSION

Concentration of metals in shellfish

The highest level of heavy metal observed was arsenic with the highest concentration of 18.93±5.30µg/g being found in scallop. With regard to Cd, scallop presented the highest level at 4.38±1.37µg/g. As reported by Olmedo *et al.* (2013), Cd concentration in shellfish was higher than in fishes due to bioaccumulation process. Meanwhile, there was a significant difference in Cr concentration level between scallop (2.61±0.67) and clam (0.08±0.05) at $p<0.05$ but no significant difference was observed for conch (1.56±0.38). The same situation was observed for Pb concentration, where a significant difference was observed between scallop (0.69±0.16) and clam (0.28±0.10). As for Ni concentration level, all three study samples indicated no significant difference at $p<0.05$ (Table 1).

Knowledge of the habitat is crucial in order to identify the bioaccumulation basis of heavy metal in shellfish (Hajeb & Jinap 2009). At the same time, anthropogenic activities carried out near to the habitat of shellfish is also one of the factors that affect bioaccumulation in shellfish (Zhang *et al.*, 2007). In addition, climate change or weather changes could be contributory factors to heavy metal concentration in shellfish. According to a study conducted by Ruelas Inzunza, Garate-Vierra & Paez-Osuna (2007), Pb concentration in shellfish is higher during the rainy season compared to the drought period. Our study showed that the concentration of all studied heavy metals was at "high" but did not exceed the permissible limits as recommended by Malaysia Food Act 1983 (Act 281), and this could be due to sampling being done in November which is the rainy season. In Malaysia, maximum rainfall commonly occurs from October to November. Different species of shellfish have different trends of bioaccumulation as there are many factors that could affect the bioaccumulation rate such as eating habits (omnivorous or carnivorous), anatomical difference, physiological difference and metabolism reactions (Yilmaz & Sadikoglu, 2011).

Health risk estimation

Exposure to these studied heavy metals through shellfish consumption has potential adverse health effects.

Concentration levels in scallop have a relatively higher potential health risks for people consuming shellfish once a week (6.81 µg/kg/bw/day). The EDI of Cd through scallop consumption was 1.58µg/kg/bw/day which is the highest observed. The EDI and HQ of all studied heavy metals in the three different shellfish species are calculated and shown in Table 2.

From the calculation of the HQ, the results indicate that HQ for As and Cd

Table 1. Heavy metal concentrations in shellfish samples (μg/g)

Parameter	**As*	**Cd*	**Cr*	**Ni*	**Pb*
Clam (*Metretrix* spp.)	0.95-2.89 (1.66±1.07)	0.04-0.13 (0.08±0.05)	0.47-1.69 (1.03±0.61)	0.81-2.15 (1.70±0.78)	0.18-0.37 (0.28±0.10)
Conch *(Strombus canarium)*	1.98-3.07 (2.50±0.55)	0.04-0.05 (0.05±0.01)	1.19-1.94 (1.56±0.38)	0.37-0.55 (0.45±0.09)	0.45-0.64 (0.54±0.10)
Scallop (*Amusium pleuronectes*)	12.81-22.10 (18.93± 5.30)	2.84-5.45 (4.38±1.37)	2.20-3.39 (2.61±0.67)	0.9-2.23 (1.48±0.68)	0.59-0.88 (0.69±0.16)

* Data are expressed as range of concentration and (mean ± SD)

Table 2. Summary of Estimated Dietary Intake (EDI) and Hazard Quotient (HQ)

Heavy metal	*shellfish*	*EDI (μg/kg/day)*	*HQ*
As	Clam (*Metretrix* spp.)	0.60	0.2
	Scallop (*Amusium pleuronectes*)	6.81	2.27
	Conch (Strombus canarium)	0.9	0.3
Cd	Clam (Metretrix spp.)	0.03	0.03
	Scallop (*Amusium pleuronectes*)	1.58	1.58
	Conch (*Strombus canarium)*	0.02	0.02
Cr	Clam (Metretrix spp.)	0.37	0.12
	Scallop (*Amusium pleuronectes*)	0.94	0.31
	Conch (*Strombus canarium*)	0.56	0.19
Ni	Clam (Metretrix spp.)	0.61	0.03
	Scallop (*Amusium pleuronectes*)	0.53	0.03
	Conch (*Strombus canarium*)	0.16	0.01
Pb	Clam (Metretrix spp.)	0.10	-
	Scallop (*Amusium pleuronectes*)	0.25	-
	Conch (*Strombus canarium*)	0.19	-

in scallop exceeds 1.0 (2.27 and 1.58 for As and Cd, respectively). According to Gerba (2000) HQ is a ratio that used in estimating the potential health effects; an HQ ratio that exceeds 1.0 shows a higher potential for health effects. However, HQ is a probability assumption in estimating likelihood for potential health effects to occur and the result could vary as there are many factors that could affect the concentration level of heavy metals in shellfish such as cooking method (Amiard *et al.*, 2008). Furthermore, despite the HQ for As and Cd in scallop exceeding 1.0, the concentration level of As and Cd in scallop did not exceed the maximum permissible limit recommended by the Malaysia Food Act 1983 and Food Regulation 1985(Act 1983).

CONCLUSION

The results indicate significant variation between heavy metals levels of all studied shellfish species (clam, conch and scallop). The levels of all heavy metals in the shellfish were lower than the recommended maximum permissible levels bythe Malaysia Food Act 1983 and Food Regulation 1985.

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