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Heavy metals concentration in spotted babylon snail, *Babylonia areolata* from Kemasin Coast, Kelantan

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Abstract. Bioaccumulation of toxic metals in marine gastropods, including spotted babylon snail may cause a serious treats to human via food chain. Therefore, the present study investigates the metal contents in the spotted babylon snail, *Babylonia areolata* based on two sample groups, which are the whole sample and sample without the digestive system using Inductively Coupled Plasma with Atomic Emission Spectrometry (ICP-MS). The concentration pattern of the metals in sample with digestive system was As > Fe > Cu > Zn > Cd > Hg > Sn > Cr and As > Fe > Zn > Cu > Cd > Cr > Hg for sample without digestive system. Overall, the concentrations of these heavy metals were lower in samples without digestive system. Meanwhile, lead (Pb) and antimony (Sb) were not detected in both sample groups. Maximum permissible limits of toxic metals in food were compared and indicated that the heavy metals in *B. areolata* were within the safety levels except for As (sample with and without digestive system) and Cd (sample with digestive system). The exposure risk of heavy metals in *B. areolata* at the Kemasin coast, Kelantan is at a permissible level for the consumer provided that the digestive system is removed from the flesh.

1. Introduction

Heavy metals in the aquatic system have become a significant problem in the environment. These trace metal occurs in all compartments of the environment and tends to accumulate in organisms from different trophic levels. The persistence of metallic compounds with high levels of toxicity in the environment cannot be neglected. It can be bio-accumulated in organisms, magnified in the food chain and affects human health [1]. Using marine species as bio-indicators to track metal emissions has become standard practice around the world [2]. Arsenic (As), cadmium (Cd), copper (Cu), chromium (Cr), iron (Fe), mercury (Hg), lead (Pb), antimony (Sb), tin (Sn) and zinc (Zn) are all classified as environmental risks, with maximum residue amounts in seafood [3][4][5].

Metals discharged can bio-accumulate in the mollusk tissues. Mollusks are the most reliable method for detecting sources of bioavailable pollution because they have spatial sensitivity. [6][7][8]. A mollusk,



on the other hand, is a more reliable bio-indicator of heavy metal contaminations [9]. Spotted babylon snail, *Babylonia areolata* (*B. areolata*) is an invertebrate that belongs to the phylum Mollusca, class Gastropoda and family Buccinidae. According to [10], *B. areolata* is a mollusk that can be found in a number of habitats, including deep-sea vents, coral reefs, estuaries, rocky and sandy shorelines, freshwater lakes, rivers and almost anywhere on land from deserts to rainforests. It has a typical fresh sea or seaweed odour, bright, glossy orange colour, moist in appearance and firm, tight structure [11]. Matured sizes of spotted *B. areolata* are normally around 4 cm to 8 cm [12]. Spotted *B. areolata* is going to be a target species of commercial fisheries in Malaysia. In fact, Chaitanawisuti *et al.* [13] reported that spotted babylon snails are a good candidate for commercial mariculture. However, the existence of *B. areolata* in Malaysia is currently abandoned and the study of the heavy metal contents is still lacking.

Therefore, the study aims to determine the heavy metal content and its concentrations in *B. areolata* at the Kemasin coast, Kelantan. The present study is pertinent since this seafood also served as a delicacy.

2. Methodology

2.1 Samples collection and preparation

A sampling of *B. areolata* was carried out at Kemasin during the non-monsoon season between March to May 2017 using the custom-made trap (Figure 1). The collected samples were washed with distilled water and transferred into a clean incubator outdoor food preservation box. The samples were kept at -10°C in the lab before further study. Heavy metal analysis was carried out at the Fisheries Bio-security Centre, Department of Fisheries, Kuantan, Pahang. The sample of *B. areolata* was prepared into two groups, namely the whole individual sample and sample without digestive system. Only market-sized samples (4.0 to 5.0 cm) were used in this research. Among 1.0 kilogram of sample collected, only a total of 1.6 gram was used for digestion. All samples were determined in duplicate reading.



Figure 1. L1 represent the sample collection site, Kemasin coast Bachok, Kelantan ($6^{\circ}07'48.2''\text{N}$ $102^{\circ}22'34.0''\text{E}$, $6^{\circ}07'46.7''\text{N}$ $102^{\circ}22'32.1''\text{E}$ and $6^{\circ}07'41.4''\text{N}$ $102^{\circ}22'34.8''\text{E}$).

2.2 Metal analysis

The analytical procedure was conducted following Shazili *et al.*[14] with a slight modification. The samples were homogenized and subsequently mixed with nitric acid, HNO₃ and hydrogen peroxide, H₂O₂ Suprapur. Samples were then digested in Multiwave 3000 for 45 minutes to 1 hour and 10.0 – 12.0 ml filtrate aqueous phase were transferred into 16.0 ml test tube using the 0.45 μ membrane filter. The trace of heavy metals such as As, Cd, Cu, Cr, Fe, Hg, Pb, Sb, Sn and Zn were measured using Inductively Coupled Plasma with Atomic Emission Spectrometry (ICP-MS) and the mean concentration (part per million) were recorded. Statistical calculation was performed using SPSS version 23 software. The significant difference between heavy metal concentrations was analysed using one-way analysis of variance (ANOVA) (p<0.05). Bonferroni post-hoc comparison was carried out to examine the statistical difference between the metals. Comparison of means of two groups (the whole individual sample and sample without digestive system) was conducted by using t-test.

3. Results and Discussion

The metal concentrations of As, Cd, Cr, Cu, Fe, Hg, Sn and Zn in *B. areolata* are shown in Table 1. The highest metal concentration in sample group with digestive system was As (190.036 ± 5.267 ppm) while the lowest was Cr (0.065 ± 0.014 ppm). For sample group without digestive system, the highest metal concentration was As (90.033 ± 1.623 ppm) while the lowest was Hg (0.032 ± 0.006 ppm). Metal Pb and Sb were not detected in both sample groups. The lowest concentration of Hg was expected as this metal was not needed in metabolism. Organisms accumulate this non-essential metal in proportion to the degree of pollution in the environment. Meanwhile, Sn was detected only in sample group with digestive system. The results showed a significant difference (p<0.05) between heavy metals in this study. Meanwhile, the Bonferroni post-hoc comparison test reveals a significant difference between metals: As and Cd (p<0.05), As and Cu (p<0.05), As and Cr (p<0.05), As and Fe (p<0.05), As and Hg (p<0.05), As and Sn (p<0.05), As and Zn (p<0.05). However, there is no significant difference (p>0.05) of metals between the two sample groups (with and without digestive system).

Table 1. Heavy metals concentration in *B. areolata*.

Heavy metals	Mean ± SD concentrations (ppm)		Permitted level (ppm)
	With digestive system	Without digestive system	MFAR
As	190.036 ± 5.267*	90.033 ± 1.623*	1
Cd	2.184 ± 0.309*	0.106 ± 0.017	2
Cu	26.664 ± 3.427	4.050 ± 1.509	30
Cr	0.065 ± 0.014	0.068 ± 0.033	NL
Fe	98.274 ± 2.916	63.64 ± 16.82	NL
Hg	0.094 ± 0.004	0.032 ± 0.006	0.5
Pb	nd	nd	2
Sb	nd	nd	1
Sn	0.069 ± 0.0678	nd	50
Zn	12.420 ± 1.186	6.751 ± 1.100	100

nd = not detected

SD = Standard Deviation

NL = No Limit

MFAR = Malaysian Food Act 1983 and Food Regulations 1985

*Values that exceed the permissible limits set by MFAR

Hierarchically, the concentration of the metals occurrence for sample group with digestive system was $As > Fe > Cu > Zn > Cd > Hg > Sn > Cr$ and $As > Fe > Zn > Cu > Cd > Cr > Hg$ for sample group without digestive system. In general, the concentrations of each heavy metal in *B. areolata* with the digestive system were higher than the sample group without digestive system. This finding shows that most of the metals accumulated in the digestive system of *B. areolata*. By comparing with the Malaysia Food Act (1983) and Food Regulations (1985) [15], the Cr, Cu, Fe, Hg, Sn and Zn concentrations in *B. areolata* in both samples were much lower than the permitted concentration (Table 1). It shows that all metal concentrations detected in the studied samples were below the permissible limits except for As (sample with and without digestive system) and Cd (sample with digestive system). The accumulation of metals in the *B. areolata* can be related to the carrier assistance of Fe in the marine environment since the Fe concentration was high. Previous study showed that other metals in the marine environment can be carried by the oxyhydroxide phase of Fe since this metal is a geochemically abundant element in the earth's crust [16]. The spotted *B. areolata* has a proclivity for collecting a high concentration of Fe from the surroundings. Fe is thought to play an important role as an integral component of all living systems [17]. These occurrences also contribute to mollusks' natural ability to monitor and assemble higher Fe concentration [18]. Anthropogenic behaviours or activities near the habitat, on the other hand, are one of the factors that influence bioaccumulation in *B. areolata* [19].

In this study, As was detected in both samples and shown a higher level among all metals that exceed the permitted level, especially the whole sample individual. Heavy metals occur naturally and also derive from anthropogenic sources in ecosystems with substantial variations of concentrations. Metal contents are regularly present in a complex form with insoluble inorganic and organic ligands. Inorganic As is the most toxic and considered carcinogenic to humans as it affects lungs, kidneys and skin disorders [20]. Generally, the concentration of As found at the Kemasin coast might be resulting from shipping activities and oil spillage, as the sampling station is located near the estuary. This estuary served as a gateway for the fishing boats to enter the fishing landing jetty (Figure 1). The metal pollution also may derive from the fishing landing jetty itself with heavy boat traffic. This finding is in agreement with [21], whereby the accumulation of high-level concentration of As in the coastal environment has been caused by heavily boating and fishing activities. Besides, it could be due to the different environmental conditions such as water and weather changes [22]. In this study, Cd is slightly higher than the permitted level in samples with the digestive systems. Metal Cd concentration in shellfish was higher than in fishes due to the bioaccumulation process [23]. Due to intense industrial discharge, port activity, and mining activity in the related rivers, Cd concentrations in coastal and estuarine areas increased in marine environments [24].

Table 2 showed tolerable daily intake of heavy metals by humans as prescribed by The Joint Food and Agriculture Organization / World Health Organization Expert Committee on Food Additives (JECFA). Metal Fe was detected in both samples and the levels were considered very high. Indeed, it can contaminate mollusks due to its tendency to bind with organic matter in the marine environment [25]. Accumulation of high concentration of Fe is also due to the heavy activity of boating and fishing [21]. It is worthy of note that the high accumulation of metals in the digestive system of *B. areolata* has been reported by a previous study [26]. Hence, further monitoring of metals accumulation in this species at the sampling site is indeed needed.

Table 2. Tolerable Daily Intake of Heavy Metals by Human as Prescribed by JECFA (1982,1983,1993 and 2000).

Heavy metals	Provisional Maximum Tolerable Daily Intake (PMTDI)
	ppm
As	0.015
Cd	0.007
Cu	0.05-0.5
Cr	0.1-1.2
Fe	0.8
Hg	0.0016
Pb	0.025
Sb	-
Sn	-
Zn	0.4-10

The comparison of heavy metals concentration (ppm) in *B. areolata* with various gastropods from similar and different regions and countries were depicted in Table 3. Cadmium concentration in *T. clavigera* showed almost similar reading with *B. areolata*. The concentration of Cd in *Thais. sp.*, *Adelomelon ancilla*, *Buccinanops globulosus* and *Trophon geversianus* was higher compared to *B. areolata*. Meanwhile, the concentration of Cu in *Adelomelon ancilla*, *Buccinanops globulosus*, *Trophon geversianus*, *T. clavigera* and *Thais. sp.* showed higher reading compared to *B. areolata*. Iron (Fe) concentration in *B. areolata* was lower than *Adelomelon ancilla*, *Buccinanops globulosus* and *Trophon geversianus*, while the Hg concentration in this babylon snail was slightly lower than *T. clavigera*. Concentration of Zn in *B. areolata* was much lower than the other gastropods from both regions. On the other hand, As, Cr and Sn were not reported in gastropods by the previous studies.

The present results are not homogeneous with the previous studies, as has been reported in Table 3 [2][30]. It could be due to the difference in environmental conditions and anthropogenic activities at the sampling sites [27]. Besides, the understanding of the shellfish habitat is crucial to assess the bioaccumulation of heavy metals [28]. It is worthy of note that the accumulation rate could be affected by many factors, such as feeding habits (omnivorous or carnivorous), anatomical differences, physiological differences and metabolism reactions [29][30]. Thus, it shows that *B. areolata* can be used for monitoring heavy metals in aquatic environments and included in the national monitoring program of seawater ecosystems.

Table 3. The comparison of heavy metals concentration (ppm) in various gastropods from previous studies.

Species	Region	As	Cd	Cu	Cr	Fe	Hg	Pb	S	Sn	Zn	Referen ce
<i>Thais.sp.</i>	Bari Kecil, Terengganu	-	28.6	155	-	-	-	0.95	-	-	187	[2]
	Teluk Cempedak, Pahang	-	14.3	269	-	-	-	3.81	-	-	808	[2]

	Mersing, Johor	-	28.6	287	-	-	-	12.9	-	-	529	[2]
	Sedili Kecil, Johor	-	27.1	256	-	-	-	0.95	-	-	253	[2]
<i>T. clavigera</i>	Coastal waters of the East Coast of Peninsular Malaysia	-	0.21-2.14	70.8-215.87	-	-	0.1-0.4	0.21-2.14	-	-	80.76-868.78	[2]
<i>Adelomelon ancilla</i>	Nuevo gulf, Argentina SH & LPBH	-	8.02-53.12	59.7-586.31	-	526.7-723.63	-	1.81-2.61	-	-	781.6-1091.15	[31]
<i>Buccinanops globulosus</i>	Nuevo gulf, Argentina PC, CA & LPBH	-	38.8-190.24	45.2-68.93	-	351.5-621.69	-	1.95-10.37	-	-	583.5-959.42	[31]
<i>Trophon geversianus</i>	Nuevo gulf, Argentina PC & CA	-	64.7-104.24	37.3-75.41	-	298.0-400.80	-	1.94-3.51	-	-	296.0-504.52	[31]
<i>B. areolata</i>	Kemasin coast, Kelantan	90.0-190.34	0.10-2.184	4.05-26.66	0.0-0.07	63.64-98.27	0.0-0.03	-	-	0.0-7	6.75-12.42	Present study

Abbreviations: Cerro Avanzado beach, LPBH: Luis Piedra Buena harbour, PC: Punta Cuevas beach, SH: Storni harbour

4. Conclusion

The result showed that *B. areolata* contained As, Cd, Cr, Cu, Fe, Hg, Sn and Zn. The concentration patterns of metals for sample group with digestive system was As > Fe > Cu > Zn > Cd > Hg > Sn > Cr and As > Fe > Zn > Cu > Cd > Cr > Hg for sample group without digestive system. Most of the studied metals accumulated in the digestive system of *B. areolata*. The total concentration of heavy metals in *B. areolata* is within the permissible levels except for As (sample with and without digestive system) and Cd (sample with digestive system). The elimination of *B. areolata* digestive system is pertinent before consumption to reduce the exposure of metals. Further investigation should be carried out to determine the level of organic and inorganic for each element Long-term monitoring of metals in *B. areolata* is pertinent to evaluate its potential health risks, considering the level of Cd and As are higher than the safety limits.

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