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Comparison of Heavy Metal Concentrations (Cd, Cu, Fe, Ni and Zn) in the Shells and Different Soft Tissues of *Anadara granosa* Collected from Jeram, Kuala Juru and Kuala Kurau, Peninsular Malaysia

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ABSTRACT

The concentrations of Cd, Cu, Fe, Ni and Zn were analysed in different parts (shells, mantle plus gills and foot plus visceral mass) of the red blood cockle, *Anadara granosa* collected from an anthropogenic-receiving site at Kuala Juru and from relatively unpolluted sites at Jeram and Kuala Kurau. The metal concentrations (µg/g dry weight) in the total soft tissues of *A. granosa* ranged from 1.30-9.44 (mean: 4.69) for Cd, 91.9-203.5 (mean: 130.2) for Zn, 0.80-16.15 (mean: 7.67) for Ni, 455.91-1125.5 (mean: 715.3) for Fe and 5.41-7.39 (mean: 6.14) for Cu. The present study revealed that the *A. granosa* was a potential biomonitor for Cu and Zn as observed in the comparison with those metals in the sediment. Although the elevated concentrations of Cu and Zn were found in the soft tissues of Kuala Juru's cockles, these metal concentrations were still below the maximum permissible limits established by the Malaysian Food Regulations 1985 and the WHO standard guidelines but the Cd concentrations of cockles from Jeram were higher than the maximum permissible limits established by both guidelines. As suggested by many reported studies found in the literature, regular biomonitoring of heavy metal concentrations at these three sites is needed since the edible *A. granosa* is a popular commercial bivalve in Malaysia.

Keywords: ***Anadara granosa*, biomonitoring, blood cockle, bivalve, different parts, heavy metals, soft tissues, shells**

INTRODUCTION

Red blood cockle, *Anadara granosa* [Order: Arcoida; Family: Arcidae] is a filter-feeding bivalve that lives in muddy intertidal areas feeding on phytoplanktons and zooplanktons. Their biology and ecology (Broom, 1985) are very interesting from the biomonitoring point of view (Phillips and Rainbow, 1993). Like other bivalves, cockles fulfill most of the requirements for a good biomonitor of heavy metal pollution by having a wide geographical distribution and are easily collected, as well as having a sessile lifestyle (Lowe and Kendall, 1990). The cockles have been shown to be able to accumulate heavy metals to significant levels in their soft tissues (Mat *et al.,* 1994). Besides, the cockles are also an important protein source in the Southeast Asian region including Malaysia.

In the literature, the use of the soft tissues of cockles for biomonitoring studies of heavy metal pollution had been reported in *Cerastoderma edule* from the Moroccan Atlantic coastline (Cheggour *et al.,* 2001) and *A. granosa* from Malaysia (Ibrahim, 1994; Mat, 1994; Mat and Maah, 1994; Alkarkhi *et al.,* 2008). Phillips and Muttarasin (1985) reported concentrations of Cd, Cr, Cu, Fe, Ni, Hg and Zn in the commercial cockle *A. granosa* from Thailand and their findings indicated the potential use of the cockles to estimate the toxicological risks of heavy metals by determining the heavy metal concentrations in the soft tissues of cockles.

Besides, the shell had also been proposed as a biomonitoring material by a few researchers. Yap *et al.* (2003a) suggested that the shell of the bivalve, *Perna viridis* may act as a safe storage

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matrix for heavy metals, which is resistant to soft tissue detoxification mechanisms (Walsh *et al.*, 1995). The metals found in the shell could be explained on the basis that some trace metals are incorporated into the shells of the bivalves through substitution of the calcium ions in the crystalline phase of the shell or are associated with the organic matrix of the shell (Foster and Chacko, 1995; Yap *et al.*, 2003a).

The semi-culture of *A. granosa* is of considerable economic importance in Malaysia. According to Noordin (1988), there were about 4-5000 ha of mudflats along the west coast being utilized for this purpose. Contamination of the highly productive mudflats with heavy metals resulted in the accumulation of these metals in the filter-feeding cockles which often serve as important environmental sinks of heavy metals (Pringle *et al.* 1968).

Based on the aforementioned, *A. granosa* which are easily available in the coastal areas of Peninsular Malaysia (Noordin, 1988); and its ability to accumulate heavy metals (Pringle *et al.* 1968), it fulfils the criteria as a potential biomonitor.

Since the three sampling sites at Kuala Juru, Kuala Kurau and Jeram are located in high anthropogenic activity areas of the west coast of Peninsular Malaysia, the present study is therefore crucial in order to monitor the heavy metal status in the west coast besides the economical importance of *A. granosa*. Based on the sediment samples collected during 1999-2001 (Yap *et al.*, 2002a, 2003b, 2003c, 2005), it was revealed that the heavy metal pollution in the intertidal area of the west coast of Peninsular Malaysia are localized and near to anthropogenic sources (Yap *et al.*, 2008). This study was therefore important.

The objectives of this study were: (i) to determine the concentrations of Cd, Cu, Ni, Fe and Zn in the edible soft tissues of *A. granosa* (shells, mantle plus gills and visceral mass plus foot in addition to total soft tissues) collected from Kuala Juru, Kuala Kurau and Jeram. (ii) to ascertain the potential of *A. granosa* as a biomonitor by comparing metals found in the tissues with those in the sediment.

MATERIALS AND METHODS

The cockles and sediments were collected on 19-20th April 2005 from Kuala Juru, Kuala Kurau and Jeram (*Fig. 1*). Total soft tissues of cockles were dissected from the shells. The soft tissues were separated into two major parts (namely mantle plus gills and foot plus visceral mass).

The shells and the different parts of the soft tissues of cockles were digested in concentrated nitric acid (69%) while the dried and 63 μm sieved sediments were digested in a combination of nitric acid and perchloric acid in the ratio of 4 : 1. They were placed in a hot-block digestion first at low temperature for one hour and then they were fully digested at high temperature (140°C) for at least 3 hours. The digested samples were then diluted to 40 ml with double distilled water. After filtration, the prepared samples were determined for Cd, Cu, Fe, Ni and Zn by using an air-acetylene flame atomic absorption spectrophotometer (AAS) Perkin-Elmer Model AAnalyst 800. The data were presented in μg/g dry weight basis. The analytical procedures for cockles and sediments were checked with the Certified Reference Materials (CRM) for soils and dogfish and the recoveries of all metal were satisfactory (Table 1).

To avoid possible contamination, all the glassware and equipment used were acid-washed and the accuracy of the analysis was checked with the blanks and quality control samples made of standard solutions. The percentage recoveries for the heavy metal analyses were between 90-110%.

RESULTS AND DISCUSSION

The allometric variables which included the water contents, condition indices, shell lengths, shell heights, shell widths, total soft tissue dry weights and total shell dry weights, are presented in Table 2.

Fig. 2 and 3 depict the concentrations of heavy metal in the total soft tissues, mantle plus gills and foot plus visceral mass of the *A. granosa*. Among the three sites, the highest levels of Cd and Ni were found in the shells, total soft tissues and all the different parts of the cockles' soft tissues from Jeram. The highest levels of Zn were found in the shells, total soft tissues and parts of the cockles' soft tissues from Kuala Juru. The highest levels of Cu and Fe were also found in the total soft tissues and all parts of the cockles' soft tissues from Kuala Juru but not in the shells.

From Table 3, the metal concentrations (μg/g dry weight) in the total soft tissues of *A. granosa* ranged from 1.30-9.44 (mean: 4.69) for

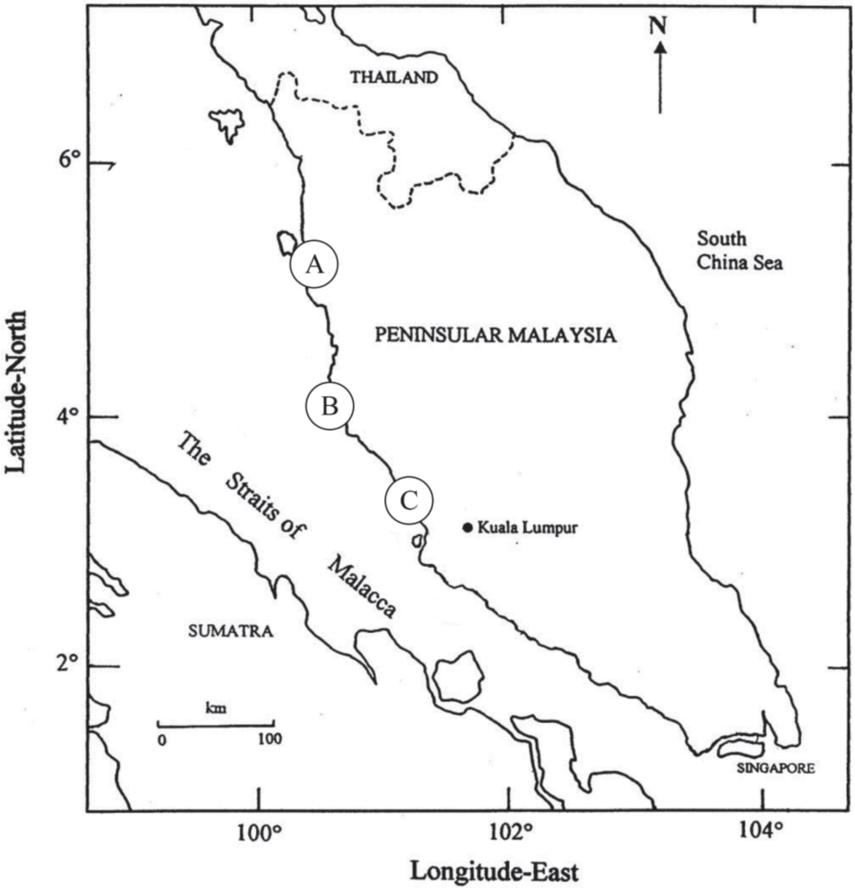

Fig. 1: Sampling sites for the cockle, Anadara granosa *at A) Kuala Juru, B) Kuala Kurau and C) Jeram*

Cd, 91.9-203.5 (mean: 130.2) for Zn, 0.80-16.15 (mean: 7.67) for Ni, 455.91-1125.5 (mean: 715.3) for Fe and 5.41-7.39 (mean: 6.14) for Cu. Based on the various soft tissues and total soft tissues, the metal abundance were Fe> Zn> Ni> Cu> Cd while the metal abundance based on the shells were Fe> Ni> Zn> Cd> Cu. The differences in metal abundance between the soft and hard tissues indicated that the metal accumulation and the metal binding capabilities of the two tissue types vary. Generally, the shells had higher concentrations of Cd and Ni than the soft tissues of the cockles while the soft tissues [including the two different soft parts] contained higher concentrations of Zn, Cu and Fe than in the shells. These results could be related to the differences in the accumulation and depuration of the hard tissues and soft tissues of the cockles. In the soft tissues, the metals were bound to metallothionein, which played an important role in metal detoxification (Roesijadi, 1980) while the metals in the shells were fixed in the crystalline lattices of the carbornate structures of the shells (Watson *et al.,* 1995). However, the questions that arise are 'Why did the soft tissues of cockles have higher levels of Zn, Cu and Fe than the shells?' and 'Why did the cockle shells have higher levels of Cd and Ni than the soft tissues?' Answers to these require future studies. Some authors (Carell *et al.,* 1987; Sturesson, 1976, 1978) suggested that the materials in mollusc's shell could be a permanent record of the environmental changes in heavy metal concentrations. Since the composition of the shell was strongly related to chemical mineralogy which included metals accumulated from the environment, this could be one of the reasons why shell metal concentrations related to the metal concentrations in its environment (Yap *et al.,* 2003a) besides the roles played by the calcium

TABLE 1
Analytical results for the reference material and its certified values for each metal (μg/g dry weight)

Metal	Sample	Certified reference material (CRM)	Measured value	Percentage % of recovery
Cd	DOLT-3 Dogfish-liver (National Research Council Canada)	19.4	20.0	103
	MESS-3 Marine Sediment (Vienna)	NA	NA	NA
Cu	DOLT-3 Dogfish-liver (National Research Council Canada)	31.2	32.0	103
	MESS-3 Marine Sediment (Vienna)	33.9	29.3	86.5
Fe	DOLT-3 Dogfish-liver (National Research Council Canada)	1484	1070	72.1
	MESS-3 Marine Sediment (Vienna)	4340	4013	92.5
Ni	DOLT-3 Dogfish-liver (National Research Council Canada)	NA	NA	NA
	MESS-3 Marine Sediment (Vienna)	46.9	37.6	80.1
Zn	DOLT-3 Dogfish-liver (National Research Council Canada)	86.6	100	116
	MESS-3 Marine Sediment (Vienna)	159	145	91.2

Footnote: NA = Not available

ions in the crystalline phase of the shell as discussed in the introduction.

In supporting the use of cockles as biomonitors of heavy metal pollution in the intertidal mudflats, a comparison of metal concentrations between the sediments and cockles was conducted as shown in Table 4. Based on Table 4, the highest levels of Cu and Zn were found in the surface sediments of Kuala Juru, which was in agreement with the high levels of Cu and Zn found in the soft tissues of cockles from the site. The highest level of Cd was also found in the sediments and cockles from Jeram. Yap *et al.* (2002b) also found a correlation for Cd, Cu and Pb between soft tissues of *Perna viridis* and sediments. However, the highest levels of Ni and Fe found in the cockles from Jeram and Kuala Juru, respectively, were not reflected in the high levels of these two metals found in the surface sediments from both sites. The results of this study indicate that *A. granosa* is a good biomonitor of Cu and Zn but not for Ni and Fe.

Further studies are required to confirm the utility of *A. granosa* as a biomonitor of Ni and Fe. Furthermore, the depletion of Ni and Fe in the surface sediments of Jeram and Kuala Juru could be possibly explained by the speciation chemistry, complexation reactions with cations and bioavailability of these micronutrients in the sedimentary environments. Several studies including those of Martin and Fitzwater (1998), Cullen (1995) and Martin (1990) explained that Fe is needed by phytoplankton to uptake nitrates for their metabolism and/or constituents of enzymes.

Among the three sites, Jeram has the highest bioavailability and contamination of Cd and Ni [as indicated by the hard and soft tissues] while Kuala Juru had the highest bioavailability and

TABLE 2
The allometric variables of *Anadara granosa* from Kuala Juru, Kuala Kurau and Jeram. All sites N= 20

Site	Variable	Mean	SE	Min	Max
Kuala Juru	Water Content [%]	81.01	-	-	-
	Condition index [g/cm^3]	20.05	0.60	14.48	24.21
	Shell length [cm]	2.57	0.05	2.18	2.99
	Shell height [cm]	2.40	0.04	2	2.7
	Shell width [cm]	3.36	0.07	2.84	3.97
	Soft tissue dry weight [g]	0.42	0.02	0.23	0.58
	Shell dry weight [g]	6.68	0.28	3.84	8.66
Kuala Kurau	Water Content [%]	81.14	-	-	-
	Condition index [g/cm^3]	27.71	0.67	22.09	33.24
	Shell length [cm]	2.46	0.04	2.19	2.98
	Shell height [cm]	2.35	0.04	2.06	2.76
	Shell width [cm]	3.29	0.04	2.95	3.63
	Soft tissue dry weight [g]	0.53	0.02	0.33	0.78
	Shell dry weight [g]	6.61	0.30	4.45	9.94
Jeram	Water Content [%]	80.74	-	-	-
	Condition index [g/cm^3]	25.35	0.76	19.55	30.65
	Shell length [cm]	2.62	0.03	2.42	2.92
	Shell height [cm]	2.28	0.02	2.14	2.48
	Shell width [cm]	3.39	0.03	3.18	3.69
	Soft tissue dry weight [g]	0.52	0.02	0.37	0.72
	Shell dry weight [g]	6.39	0.35	0.96	8.32

contamination of Zn [as indicated by the hard and soft tissues] and Cu [as indicated by the different soft tissues]. The highest bioavailability of Cd and Ni found in the cockles collected from Jeram is to our knowledge, inexplicable. Further studies are needed to identify the sources of Cd and Ni in Jeram. Based on the sediment data (Table 4), high levels of Zn and Cu were found in Kuala Juru and therefore supported the use of the shells and soft tissues of *A. granosa* as biomonitors of Cu and Zn. In the literature, Cheggour *et al.* (2001) found that Cu and Zn concentrations in edible cockle, *C. edule,* appeared to be regulated over the concentration ranges that were found in lagoon sediments. Based on our comparative results between the cockles and sediments, the regulative mechanisms for Cu and Zn were not significant since the concentrations of both metals [Cu and Zn] correlated positively between the cockles [whether shells or soft tissues] and the sediments. The highest Cd level found in Jeram was also supported by the high level of Cd in the sediment.

Kumaraguru and Ramamoorthi (1979) reported the Cu concentrations in *A. granosa* inhabiting the Vellar estuary. The Cu levels in the soft body tissues ranged from 7.56 to 11.08 μg/g dry weight in *A. granosa* [comparable to the 5.41-7.39 μg/g dry weight from this study]. Analysis for metals in the different organs (gills, mantle, hepatopancreas, foot and adductor muscles) showed that the gills and mantle contained elevated Cu concentrations, suggesting a binding capacity of the mucous protein to the Cu (Kumaraguru and Ramamoorthi, 1979). In a previous study, Kumaraguru and Ramamoorthi (1978) also reported the toxicity of Cu to *A. granosa* inhabiting the Vellar estuary in Porto Novo (southern India). These studies revealed that the cockle, *A. granosa,* is tolerant to heavy metal pollution in coastal waters. The tolerance exhibited by the cockle also may be due to the formation of metal-thiolate complex with the cysteine residues situated inside the lysosomes, which eventually reduce its toxicity by preventing the metal(s) from interfering with the cellular metabolism (Webb, 1987). Since the cockle is able to survive in the highly polluted waters, the heavy metals accumulated in the hard tissues and soft tissues should be monitored from time to time.

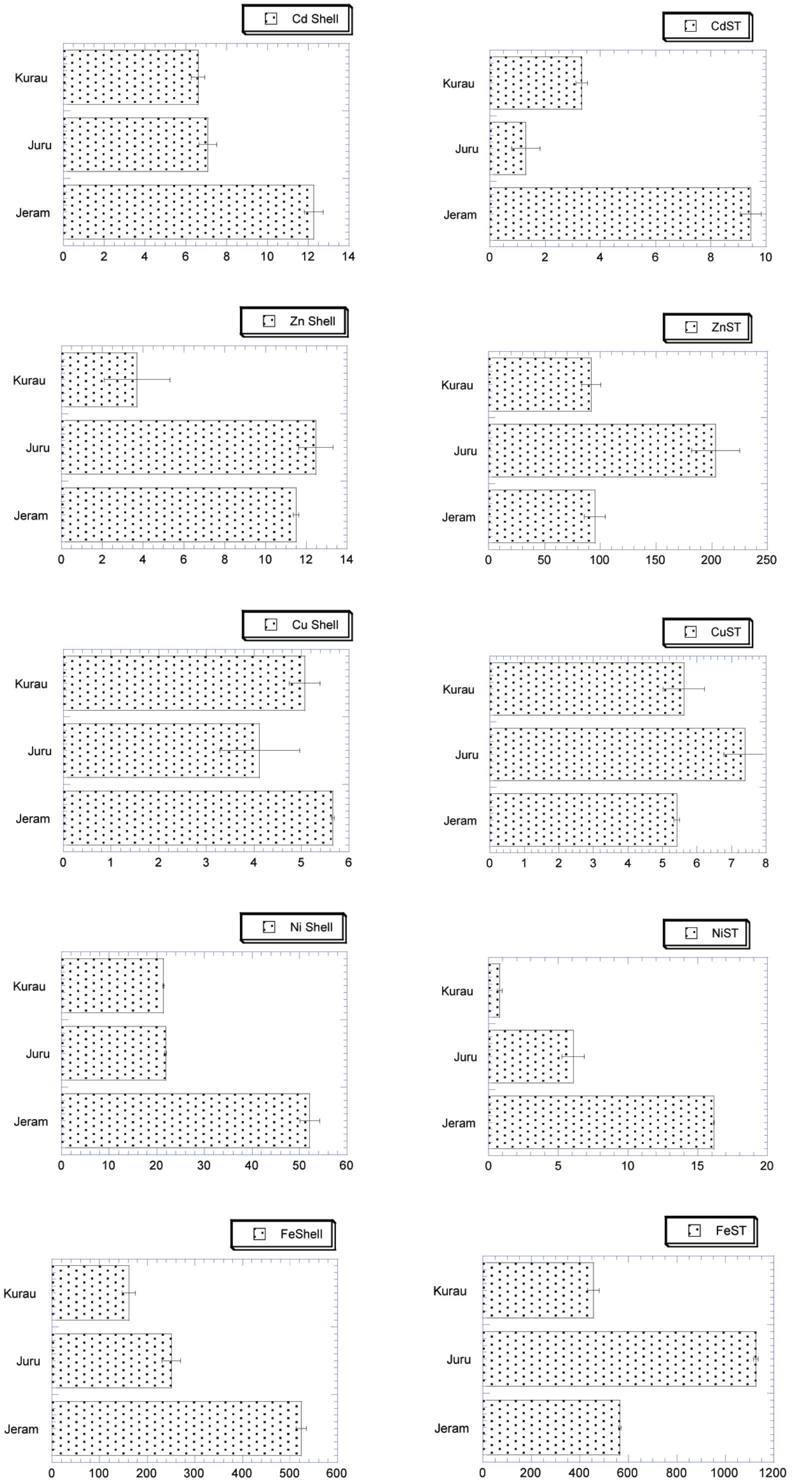

Fig. 2: The concentration (mean (μg/g ± SE dry weight) of heavy metals (Cd, Cu, Ni, Fe and Zn) in the shells and total soft tissues (ST) of the Anadara granosa *from Jeram, Kuala Juru and Kuala Kurau*

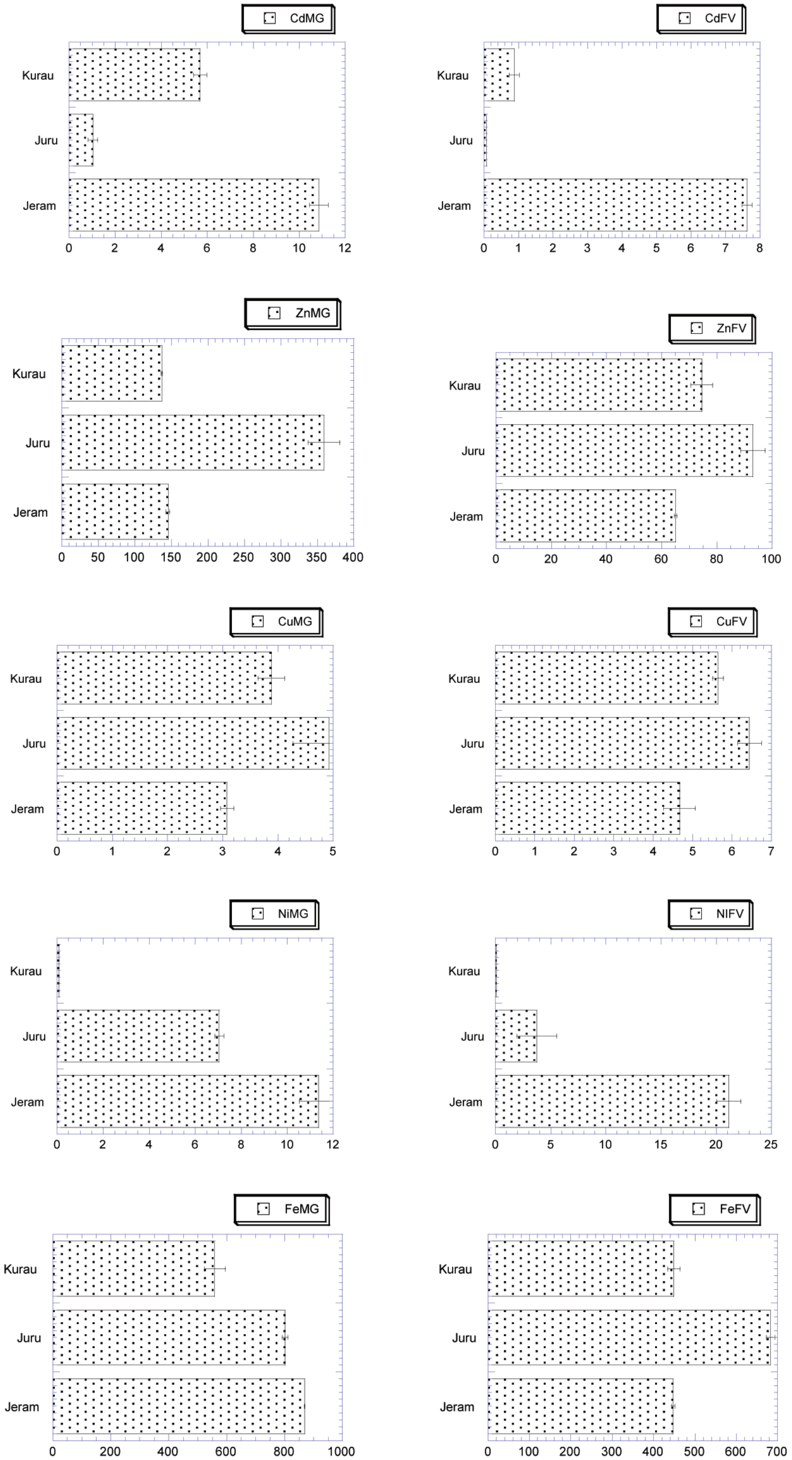

Fig. 3: The concentration (mean (µg/g ± SE dry weight) of heavy metals (Cd, Cu, Fe, Ni and Zn) in the pooled tissues of mantle plus gill (MG) and pooled tissues of foot and visceral mass (FV) of the Anadara granosa *from Jeram, Kuala Juru and Kuala Kurau*

TABLE 3
The concentration [μg/g dry weight] of heavy metals in different parts of the *Anadara granosa*. N= 3

Tissue	Metal	Minimum	Maximum	Mean	Std Error
Total soft tissue	Cd	1.30	9.44	4.69	2.45
	Zn	91.89	203.47	130.20	36.65
	Cu	5.41	7.39	6.14	0.63
	Ni	0.80	16.15	7.67	4.50
	Fe	455.91	1125.50	715.31	207.48
Mantle plus gills	Cd	1.04	10.85	5.86	2.83
	Zn	136.67	358.90	213.58	72.70
	Cu	3.08	4.92	3.96	0.53
	Ni	0.08	11.36	6.16	3.29
	Fe	559.48	868.82	743.19	93.90
Visceral mass plus foot	Cd	0.08	7.62	2.86	2.39
	Zn	65.07	93.05	77.54	8.22
	Cu	4.67	6.44	5.58	0.51
	Ni	0.08	21.13	7.78	6.70
	Fe	447.59	682.77	526.45	78.16
Shell	Cd	6.60	12.27	8.65	1.82
	Zn	3.70	12.47	9.22	2.78
	Cu	4.12	5.66	4.95	0.45
	Ni	21.43	52.15	31.80	10.17
	Fe	161.75	523.38	311.98	108.78

TABLE 4
The mean concentration (μg/g dry weight ± standard error) of heavy metals in the surface sediments collected from Kuala Kurau, Kuala Juru and Kuala Jeram. N= 3

	Kurau	Juru	Jeram
Cd	1.70 ± 0.10	1.24 ± 0.10	2.32 ± 0.08
Zn	74.64 ± 1.05	317.4 ± 2.48	98.97 ± 2.34
Cu	12.91 ± 0.25	32.91 ± 0.41	17.81 ± 0.64
Ni	114.1 ± 3.52	510.93 ± 45.86	24.01 ± 1.15
Fe	35365 ± 403	27595 ± 1795	47242 ± 2281

The heavy metal concentrations in the cockles found in this study were compared with data reported in the literature (Table 5). In general, the metal concentrations from the present study are well within those reported from Thailand (Huschenbeth and Karms, 1975; Phillips and Muttasasin, 1985; Hungspreud *et al.*, 1994), India (Patel *et al.*, 1985), and some previous studies from Malaysia (Jothy *et al.*, 1983; Devi, 1986; Mat *et al.*, 1994; Mat and Maah, 1994; Mat, 1994). Comparing the metal concentrations in the cockles with the permissible limits set by the USFDA, the results indicated no metal toxicolgical threat to consumers. Although high concentrations of Cu and Zn were found in the Kuala Juru cockles, these metal concentrations were still below the maximum permissible limits established by the Malaysian Food Regulations 1985 and the WHO standard guidelines. However, Cd concentrations of Jeram's cockles exceeded the maximum permissible limit as established by the Malaysian Food Regulations 1985 and should be avoided. The high Cd level found from this study [0.25-1.79 µg/g dry weight] was in agreement with those reported by Mat (1994). The Cd concentrations were comparatively higher than those reported by Huschenbeth and Harms (1975), Phillips *et al.* (1982) and Phillips and Mutarasin (1985) for *A. granosa* and *Anadara* sp.

TABLE 5
The comparison of the concentrations (μg/g dry weight) of Cd, Cu, Ni, Fe and Zn in the soft tissues of *Anadara granosa* from the present study with those other reported studies

Samples/locations	WB	Ni	Fe	Cd	Cu	Zn	Authors
Thailand	Wet	NA	NA	0.28	5.60	16.2	Huschenbeth and Karms (1975)
Penang and Perak coastal waters	Wet	NA	NA	1.91	0.51	19.2	Jothy *et al.* (1983)
Northern part of Peninsular Malaysia	Wet	NA	NA	0.20-0.79	0.80-1.60	14.6 -23.1	Devi (1986)
Lekir, Perak	Dry	NA	NA	6.10	6.30	64.0	Mat *et al.* (1994)
Batu Kawan and K. Selangor	Dry	NA	NA	2.10-6.90	3.40-4.50	56.0-64.0	Mat and Maah (1994)
Bombay Harbour (1976-1980)	Dry	3.90-10.8	1400-2000	2.80-4.50	8.10-11.1	70.0-132	Patel *et al.* (1985)
Thailand coastal waters	Dry	1.30-2.00	442.1-1055.6	0.25-0.80	4.55-7.37	42.0-57.9	Phillips and Muttasasin (1985)
Retail outlets in Kuala Lumpur (4 sites)	Wet	0.29-0.54	NA	1.23-1.42	0.64-0.80	12.85-14.73	Mat (1994)
Thailand coastal waters	Dry	NA	NA	1.88-3.71	1.36-2.44	77.75-119.81	Hungspreug *et al.* (1994)
Jeram, Kuala Juru and Kuala Kurau	Dry	0.80-16.15 (7.67)	455.91-1125.5 (715.3)	1.30-9.44 (4.69)	5.41-7.39 (6.14)	91.9-203.5 (130.2)	This study
Jeram, Kuala Juru and Kuala Kurau	Wet*	0.15-3.07 (1.46)	86.6-213.9 (135.9)	0.25-1.79 (0.89)	1.03-1.40 (1.17)	17.5-38.7 (24.7)	This study
Permissible levels of metals on food	Wet	NA	NA	1.00	30.0	100	Malaysian Food Regulations 1985

*Wet weight basis was converted to dry weight basis based on a conversion factor of 0.19.
Note: NA= Not available

(Phillips *et al.*, 1982). However, it was of similar levels as those reported by Jothy *et al.* (1983). Although the degree of coastal water pollution is not serious in Malaysia, the presence of 'hot-spots' cannot be ruled out (Broom, 1985). Therefore, it is important to monitor the level of contamination of Cd in the muddy coastal environment. This is due to the extensive culture of cockles in this area. Contamination in seafoods, caused by industrialization and other anthropogenic activities in the vicinity of coastal areas is always positively associated with elevated levels of Cu, Zn, Ni and Cd in cockles (Phillips, 1978; Phillips *et al.* 1982). In the *A. granosa,* the concentrations of Ni varied from 0.80-16.15 μg/g dry weight. Ni could be found in various forms of alloy in heavy machineries such as turbine blades as well as its variety of uses as industrial catalysts. Therefore, the monitoring of Ni in the environment is also important.

CONCLUSIONS

In conclusion, *A. granosa* can be used as a biomonitor for Cu and Zn. Further studies, however, are necessary to explain the ability of *A. granosa* as a biomonitor of Ni and Fe. On the other hand, the depletion of Ni and Fe may be due to the chemistry speciation, complexation reactions with cations and bioavailability of those micronutrients in the sedimentary environments in Jeram and Kuala Juru. The Cd levels of the cockles from Jeram which exceeded the Malaysian Food Regulation 1985, suggested that the cockles should be avoided for human consumption.

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