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# METAL CONCENTRATIONS IN *ANADARA GRANOSA* COLLECTED FROM INTERTIDAL MUDFLATS ON THE WEST COAST OF PENINSULAR MALAYSIA

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**Abstract:** The concentrations of Cd, Cu, Fe, Ni and Zn were analysed in different parts (shells, gill, mantle, muscle and remaining soft tissues) of the red blood cockle *Anadara granosa* collected from six geographical populations in the west coast of Peninsular Malaysia. The metal concentrations (mg/g dry weight) in the total soft tissues of *A. granosa* were 0.07-1.74 (mean: 1.18) for Cd, 2.67-20.10 (mean: 9.34) for Cu, 452-1202 (mean: 768) for Fe, 0.74-1.35 (mean: 0.94) for Ni and 63-190 (mean: 114) for Zn. Evidence of higher concentrations of Cu, Fe and Zn were found in some tissues of cockles collected from Kuala Juru while populations of Hutan Melintang and Bayan Lepas also showed some elevation of metals in certain tissues. This may indicate metal contamination and higher metal bioavailability of the above mentioned sampling sites. All the different tissues were useful in the assessment of trace metal bioavailabilities and contamination. Therefore, further attention on these sites is needed. All metal concentrations in the tissues of cockles were lower than the maximum permissible limits established by standard guidelines for food safety.

KEYWORDS: *Anadara granosa*, biomonitoring, different tissues, trace metals

## Introduction

Cockles are chosen for the biomonitoring studies based on their characteristics as being wide geographical distribution in the intertidal mudflats in Peninsular Malaysia (Yap *et al*., 2008), sedentary lifestyle, reasonable abundance and yearly available, easily identified and sampled, bioaccumulative and correlative properties with the average pollutants of the environment, tolerance to natural environmental fluctuations and pollution, and ecologically and economically important (Phillips and Rainbow, 1993; Dean, 1999; Yap *et al*., 2008). Cockles also occupy an important position in the food chain, as a primary consumer. This will create a food web for the survivals of other trophic level organisms such as fish and crabs (Vadas, 1989). The cockles could act as filter feeders that remove some of the suspended particulate materials from coastal waters and also act as a nutrient retention mechanism over a coastal area (Kuenzler, 1961).

Provision of trace metal data based on total soft tissue is sometimes rather difficult to understand since the data could be misleading or confusing that do not conform with the site description or when there is no other supporting information on the high metal level found in the total soft tissue of bivalves. The difficulty in the data interpretation, of course, can still be solved by explanation such as seasonal factor, physiological conditions, size or weights of bivalves and other intrinsic and extrinsic factors. Nevertheless, in biomonitoring studies, these factors are sometimes difficult to be normalized for all the sampling sites due to limited budget and time constraint. Still, many such or related biomonitoring studies using total soft tissue of mollusks are reported in the literature. In this study, metal concentrations were determined in different tissues of *A. granosa*. The metal levels in the different tissues of all cockle populations will be interpreted based on the description of the sampling site. That which is in agreement with the anthropogenic activities of the sampling sites will be proposed for a better biomonitoring organ in future studies.

Table 1: Global Positioning System (GPS), date of sampling (DS) and description of all sampling sites.

| No. | Sites | DS | L-N | Sites description |
|---|---|---|---|---|
| 1. | Sg. Bahru, Perlis | 11 May 08 | N 6°19'53.40"; E 100°09'24.82" | Aquacultural and agricultural areas |
| 2. | Kuala Juru, Penang | 10 May 08 | N 5°19'41.40"; E 100°23'1.44" | Potentially receiving industrial effluents |
| 3. | Bayan Lepas, Penang | 10 May 08 | N 5°16'60.00"; E 100°16'0.00" | A shipping lane |
| 4. | Batu Melintang, Perak | 11 May 08 | N 5°37'21.58"; E 100°24'06.18" | Industrial and agricultural areas |
| 5. | Kuala Sg. Ayam, Johore | 2 May 08 | N 01°45.316'; E 102°55.842' | Urban area |
| 6. | Sg. Minyak Beku, Johore | 2 May 08 | N 01°, 45.315'; E102°, 55.480' | Industrial and agricultural areas |

L-E=Longitude-East; L-N=Latitude-North

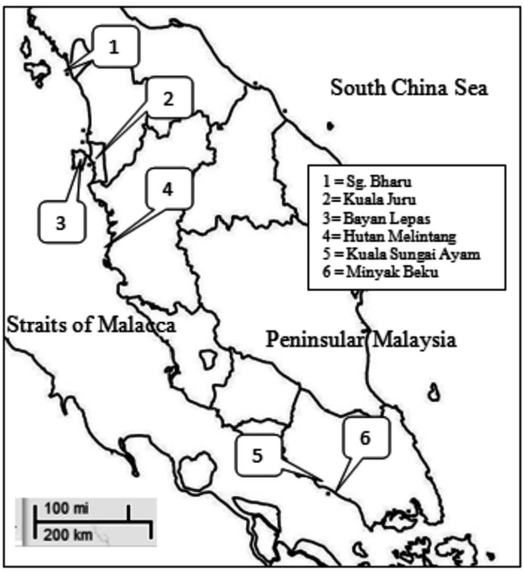


Figure 1: The sampling sites for *Anadara granosa* in the west coast of Peninsular Malaysia.

The ability of bivalves to accumulate metals, hydrocarbons and chlorinated compounds has long been recognized and bivalves are now used in global marine monitoring programs, including the well known 'Mussel Watch' program (Goldberg, 1975). This is the reason why there is a lot of literature documenting contaminant concentrations within a variety of bivalve species which span virtually all classes of anthropogenic contaminants and all regions of the world from the tropics to the poles (Negri *et al*., 2006; Abbas Alkarkhi *et al*., 2008).

Biological samples analysis is more advantageous when compared to the water and sediment for the trace metal concentration analysis because the accumulation of trace metals in their tissues of bivalves is the reflection of the trace metal contamination in the habitat environment (Bu-Olayan and Subrahmanyam, 1997) and it can provide the measurement of the time-integrated of trace metal accumulation and bioavailability (Phillips and Rainbow, 1993; Rainbow, 1995). The objective of this study was to determine the concentrations of Cd, Cu, Fe, Ni and Zn in the different parts of the edible tissues and the total shells of *A. granosa* collected from intertidal mudflats from the west coast of Peninsular Malaysia.

## Materials and Methods

Sampling was conducted between 2 and 11 May 2008. The sampling locations and site descriptions are shown in Figure 1 and Table 1, respectively. The surface water parameters recorded during sampling time included temperature, conductivity, total suspended solids (TDS) and salinity and they were measured by using an YSI meter Model 57.

About 50 individuals of cockles were collected from each sampling site. The collected samples were placed in polyethylene bags and stored in cold ice boxes and were taken back to the laboratory. In the laboratory, the samples were stored at -10ºC.

The measurements of length-width-height of the cockles were recorded by using a vernier calliper to an accuracy of 0.01 cm. The soft tissue dry weight of the cockles was recorded with an electronic balance to an accuracy of 0.001g. All the above allometric data and water contents are given in Table 2.

Table 1: Global Positioning System (GPS), date of sampling (DS) and description of all sampling sites.

| No. | Sampling site | Shell length (cm) | Shell width (cm) | Shell height (cm) | STDW (g) | WC (%) |
|---|---|---|---|---|---|---|
| 1. | Sg. Bahru | 3.29 ± 0.10 ((2.95-4.05) | 2.27 ± 0.08 (1.83-2.82) | 2.30 ± 0.06 (2.05-2.75) | 0.36 ± 0.05 (0.13-0.60) | 83.3 ± 1.15 (77.3-89.4) |
| 2. | Kuala Juru | 2.01 ± 0.08 (1.50-2.39) | 2.04 ± 0.09 (1.50-2.47) | 2.81 ± 0.14 (2.03-3.32) | NA | NA |
| 3. | Bayan Lepas | 2.96 ± 0.10 (2.60-3.62) | 2.07 ± 0.09 (1.68-2.56) | 2.16 ± 0.07 (1.93-2.54) | 0.32 ± 0.02 (0.21-0.42) | 83.4 ± 1.53 (72.8-88.5) |
| 4. | Batu Melintang | 2.36±0.05 (2.15-2.74) | 3.48 ± 0.08 (3.10-3.98) | 2.37 ± 0.07 (2.18-2.95) | 0.39 ± 0.04 (0.10-0.54) | 83.1 ± 1.59 (76.3-94.1) |
| 5. | Sg. Ayam | 3.08 ± 0.07 (2.80-3.54) | 3.08 ± 0.07 (2.80-3.54) | 2.21 ± 0.04 (2.07-2.49) | 0.50 ± 0.03 (0.35-0.64) | 77.5 ± 2.08 (67.0-86.7) |
| 6. | Sg. Minyak Beku | 3.14 ± 0.05 (3.00-3.54) | 2.20 ± 0.03 (2.03-2.34) | 2.15 ± 0.05 (1.98-2.57) | 0.37 ± 0.02 (0.31-0.53) | 84.2 ± 0.48 (82.3-86.3) |

Note: NA= Data not available. STDW= total soft tissue dry weight; WC= water content of the total soft tissue.

For metal analysis, the frozen samples were thawed at room temperature on a clean tissue paper. After cleaning, the samples were dissected and pooled into different parts including gill, mantle, muscle and remainder for the soft tissues (later referred to as remainder) while whole shells were also analysed. They were dried in 60°C for 72 hours in an oven until constant dry weights. Dried samples were weighed for 0.5g and triplicates were analysed for each pooled samples. They were digested with 10 ml concentrated $HNO_3$ (Analar grade. BDH 69%) in a hot-block digester first at low temperature (40°C) for 1 hour and were completely digested at a high temperature (140°C) for 3 hours (Yap *et al.*, 2002a). The digested samples were diluted up to 40 ml with double distilled water and filtered with Whatman filtered paper No. 1 into acid-washed polyethylene bottles.

The concentrations of Cd, Cu, Fe, Ni and Zn were determined by an air-acetylene flame Atomic Absorption Spectrophotometer (AAS) Perkin-Elmer Model AAnalyst 800. The data were presented in μg/g dry weight. Multiple-level calibration standards were analysed to generate calibration curves against which sample concentrations were calculated. Standard solutions for each metal were prepared from 1000 mg/L per stock solution of each metal (MERCK Titrisol).

Table 3: Comparison of metal concentrations (mean µg/g dry weight) between certified values of the certified reference material (DOLT-3 Dogfish liver) and the analytical measured values for each metal.

| Metals | Certified reference material (CRM) | Measured value | Percentage of recovery (%) |
|---|---|---|---|
| Cd | 19.72 ± 0.13 | 19.40 ± 0.00 | 101.66 ± 0.65 |
| Cu | 32.33 ± 0.23 | 31.20 ± 0.00 | 103.61 ± 0.72 |
| Fe | 1322.29 ± 6.59 | 1484.00 ± 0.00 | 89.10 ± 0.44 |
| Ni | 3.95 ± 0.19 | 2.72 ± 0.00 | 145.38 ± 7.06 |
| Zn | 86.74 ± 0.04 | 86.60 | 100.16 ± 0.05 |

For quality control, all glasswares and equipments used were acid-washed with 10% nitric acid solution. Quality control samples made from standard solution for Cd, Cu, Fe, Ni and Zn were analysed after five to ten samples for accuracy check. The percentages of recoveries were acceptable at 80-110% for each of the trace metal analyses. The analytical procedures for the samples were checked with the Certified Reference Material (CRM) for dogfish liver (DOLT-3, National Research Council Canada). All recoveries for the metals were satisfactory (Table 3).

For statistical analysis, one-way independent ANOVA (Analysis of Variance) was used to determine the difference between the means of trace metal concentrations in the different parts of cockles by using statistical software SPSS (version 15.0).

## Results

The cockle sizes for all populations were almost similar (Table 2). Their shell lengths ranged from 2.01-3.29 cm, shell widths ranged from 2.15 to 2.81 cm and shell heights ranged from 2.04 to 3.48 cm. The water contents found in all cockles populations (77.5-83.4%) were comparable to *A. granosa* as reported by Yap *et al*. (2008).

The values of temperatures, conductivity, TDS and salinity are presented in Table 4. They ranged from 28.16-31.07°C for temperatures, 26359-49911 µs/cm for conductivity, 15.79-29.52 mg/L for TDS and 14.66-27.13 ppt for salinity. Although these water parameters were difficult to explain the metals accumulated in the different tissues of *A. granosa*, these parameters indicated that the cockle habitats had certain ranges of the water temperature, conductivity, TDS and salinity.

The metal concentrations in the different parts of *A. granosa* for all sampling sites are presented in Table 5. Based on gills, the highest levels of Fe and Zn are found in Kuala Juru population while the highest levels of Cu and Ni are found in Bayan Lepas population. The highest Cd in gill is found in Minyak Beku population. Based on mantle, the highest levels of Cu and Zn are found in Kuala Juru population while the highest levels of Cd, Fe and Ni are found in Sg. Bharu, Batu Melintang and Bayan Lepas, respectively. However, there is no consistent pattern found based on cockle shells and the results are difficult to interpret. Based on muscle, the highest levels of Fe and Zn are again found in Kuala Juru population. However, highest levels of Cu and Ni are found in Batu Melintang population. The highest Cd level is found in Minyak Beku population.

Based on 'remainder', the highest Zn level is again consistently found in Kuala Juru population, Ni in Bayan Lepas, Cu and Fe in Batu Melintang, and Cd in Sg. Bharu. Based on total soft tissue, the highest levels of all metals are found in Batu Melintang, except for Cd in Minyak Beku. However, this comparison is based on all populations except for Kuala Juru.

The overall concentrations of metals for all sampling sites with their ratios of maximum to minimum values are given in Table 6. It is found that the highest Ni ratios are found in the gills, mantle and muscle, which indicated these three tissues had the bioaccumulative capacity for Ni when compared to other tissues of *A. granosa*. Interestingly, these gills, mantle and muscle had a similar order of increment: Fe< Cd< Zn< Cu< Ni. Comparatively higher Cd and Ni ratios are also found in the 'remainder' with an order of increment: Zn< Fe< Cu< Ni< Cd. However, this trend seems to reverse in the shell in the following order of increment: Cu= Ni< Zn< Cd< Fe. For total soft tissues, the order: Cd< Ni< Fe< Zn< Cu, which indicated the nonessential Cd had the lowest level compared to other metals.

Table 4: Some physico-chemical parameters (mean ± SE) of the surface waters recorded during the sampling time at the sampling sites of cockles. N= 3.

| No. | Sampling site | Temp (°C) | Cond. (µs/cm) | TDS (mg/L) | Salinity (ppt) |
|---|---|---|---|---|---|
| 1. | Sg. Bahru, Perlis | 28.16 ± 0.05$^{A}$ | 35336 ± 6.77$^{B}$ | 21.73 ± 0.00$^{B}$ | 17.40 ± 0.00$^{A}$ |
| 2. | Kuala Juru, Penang | 30.09 ± 0.05$^{A}$ | 44721 ± 3.56$^{C}$ | 26.47 ± 0.00$^{C}$ | 25.90 ± 0.00$^{B}$ |
| 3. | Bayan Lepas, Penang | 29.95 ± 0.03$^{A}$ | 46385 ± 17.1$^{C}$ | 27.58 ± 0.00$^{C}$ | 27.13 ± 0.00$^{B}$ |
| 4. | Batu Melintang, Perak | 29.09 ± 0.05$^{A}$ | 26359 ± 11.0$^{A}$ | 15.79 ± 0.00$^{A}$ | 14.66 ± 0.00$^{A}$ |
| 5. | Sg. Ayam, Johore | 31.07 ± 0.00$^{A}$ | 46773 ± 11.2$^{C}$ | 27.27 ± 0.00$^{C}$ | 26.73 ± 0.00$^{B}$ |
| 6. | Sg. Minyak Beku, Johore | 30.18 ± 0.00$^{A}$ | 49911 ± 5.12$^{D}$ | 29.52 ± 0.00$^{D}$ | 24.00 ± 0.00$^{B}$ |

Note: Temp = Temperature; Cond = Conductivity; TDS = Total dissolved solid.
Values sharing a common letter in the post hoc column are not significantly different (P>0.05).

Table 5: Metal concentration (mean µg/g dry weight ± SE) in the different parts of *Anadara granosa* collected from the intertidal areas of Peninsular Malaysia and their comparisons with the maximum permissible limits of metals (µg/g dw) in seafood of different guidelines.

| Site | Parts | Cd | Cu | Fe | Ni | Zn |
|---|---|---|---|---|---|---|
| Sg. Bahru | Gills | 2.53 ± 1.12$^{A}$ | 5.96 ± 0.53$^{C}$ | 1253 ± 9.95$^{C}$ | 1.68 ± 0.01$^{C}$ | 174 ± 2.49$^{C}$ |
| | Mantle | 1.31 ± 0.71$^{A}$ | 3.78 ± 0.10$^{B}$ | 561 ± 10.2$^{AB}$ | 0.08 ± 0.00$^{A}$ | 108 ± 3.84$^{B}$ |
| | Shell | 3.99 ± 0.12$^{A}$ | 7.41 ± 0.02$^{C}$ | 789 ± 34.9$^{BC}$ | 25.8 ± 0.10$^{D}$ | 12.6 ± 0.18$^{A}$ |
| | Muscle | 1.69 ± 0.31$^{A}$ | 2.46 ± 0.19$^{A}$ | 305 ± 0.6$^{4A}$ | 0.07 ± 0.00$^{A}$ | 84.4 ± 0.92$^{B}$ |
| | Remainder | 1.97 ± 0.26$^{A}$ | 6.12 ± 0.16$^{C}$ | 698 ± 13.3$^{BC}$ | 0.50 ± 0.07$^{AB}$ | 85.0 ± 1.35$^{B}$ |
| | TST | 1.59 ± 0.37$^{A}$ | 6.60 ± 0.15$^{C}$ | 750 ± 34.1$^{BC}$ | 0.74 ± 0.02$^{B}$ | 93.9 ± 1.92$^{B}$ |
| Kuala Juru | Gills | 0.90 ± 0.00$^{C}$ | 11.4 ± 0.00$^{B}$ | 1892 ± 0.00$^{B}$ | 7.67 ± 0.00$^{A}$ | 472 ± 0.00$^{A}$ |
| | Mantle | 0.57 ± 0.12$^{B}$ | 26.4 ± 2.12$^{A}$ | 583 ± 49.7$^{A}$ | 0.60 ± 0.00$^{B}$ | 293 ± 0.58$^{B}$ |
| | Shell | 3.67 ± 0.05$^{D}$ | 4.90 ± 0.23$^{A}$ | 268 ± 8.88$^{C}$ | 16.0 ± 0.77$^{C}$ | 6.68 ± 0.24$^{A}$ |
| | Muscle | 0.57 ± 0.08$^{B}$ | 8.46 ± 1.28$^{A}$ | 808 ± 55.2$^{A}$ | 1.80 ± 0.44$^{B}$ | 334 ± 5.33$^{A}$ |
| | Remainder | 0.07 ± 0.02$^{A}$ | 13.6 ± 6.66$^{A}$ | 452 ± 27.1$^{A}$ | 0.94 ± 0.08$^{A}$ | 107 ± 3.55$^{A}$ |

| | | | | | | |
|---|---|---|---|---|---|---|
| Bayan Lepas | Gills | 1.77 ± 0.00C | 52.4 ± 0.00D | 1206 ± 0.00D | 17.9 ± 0.00B | 98.1 ± 0.00E |
| | Mantle | 0.98 ± 0.21B | 4.10 ± 0.42A | 442 ± 16.4AB | 9.74 ± 0.11C | 145 ± 0.28D |
| | Shell | 2.82 ± 0.18B | 5.72 ± 0.16B | 60.8 ± 2.73E | 23.6 ± 0.76E | 12.8 ± 0.30A |
| | Muscle | 1.62 ± 0.06B | 3.95 ± 0.42A | 387 ± 67.4A | 6.67 ± 0.12B | 139 ± 3.19D |
| | Remainder | 1.29 ± 0.12A | 6.72 ± 0.11C | 496 ± 14.9B | 11.8 ± 1.05D | 67.3 ± 1.23B |
| | TST | 1.26 ± 0.08B | 6.66 ± 0.09C | 623 ± 18.3C | 1.00 ± 0.08A | 131 ± 8.92C |
| Batu Melintang | Gills | 1.17 ± 0.06B | 10.8 ± 0.75B | 1560 ± 55.3D | 8.66 ± 0.97D | 366 ± 7.03E |
| | Mantle | 0.60 ± 0.05A | 13.6 ± 1.07C | 672 ± 35.5B | 5.51 ± 0.53B | 178 ± 9.53C |
| | Shell | 4.42 ± 0.12C | 7.14 ± 0.05A | 417 ± 7.13A | 23.5 ± 0.51E | 15.4 ± 0.69A |
| | Muscle | 1.00 ± 0.06B | 8.54 ± 0.18A | 717 ± 76.8B | 7.10 ± 0.70C | 212 ± 3.36D |
| | Remainder | 0.75 ± 0.03A | 16.5 ± 0.42D | 765 ± 34.5B | 1.14 ± 0.13A | 86.3 ± 5.19B |
| | TST | 1.17 ± 0.08B | 20.1 ± 0.79E | 1202 ± 47.3C | 1.35 ± 0.18A | 190 ± 6.94C |
| Sg. Ayam | Shell | 4.20 ± 0.06A | 7.87 ± 0.13A | 307 ± 4.55A | 25.3 ± 1.74A | 8.62 ± 0.55A |
| | TST | 1.22 ± 0.10B | 6.43 ± 0.18B | 1018 ± 8.52A | 0.76 ± 0.05B | 98.7 ± 2.58B |
| Minyak Beku | Gills | 3.15 ± 0.07D | 2.99 ± 0.18BC | 680 ± 18.6E | 0.40 ± 0.34AB | 101 ± 0.69E |
| | Mantle | 1.01 ± 0.08C | 3.21 ± 0.08C | 318 ± 13.2B | 0.08 ± 0.00A | 80.1 ± 5.47CD |
| | Shell | 7.03 ± 0.04F | 5.09 ± 0.09D | 272 ± 5.70A | 16.7 ± 0.32D | 6.53 ± 0.56A |
| | Muscle | 2.16 ± 0.37E | 0.83 ± 0.07A | 388 ± 19.1C | 1.07 ± 0.03C | 86.6 ± 4.97D |
| | Remainder | 0.99 ± 0.07A | 2.75 ± 0.06B | 386 ± 13.8C | 0.97 ± 0.06BC | 72.4 ± 1.35C |
| | TST | 1.74 ± 0.15B | 2.67 ± 0.18B | 562 ± 12.8D | 0.83 ± 0.06BC | 63.0 ± 2.56B |
| 1. Maximum permissible levels established by the Brazilian Ministry of Health (ABIA 1991) | | 5.00 | 150 | NA | NA | 250 |

| | | | | | |
|---|---|---|---|---|---|
| 2. Permissible limits set by the Ministry of Public Health, Thailand (MPHT 1986) | NA | 133 | NA | NA | 667 |
| 3. Food and Drug Administration of the USA (USFDA 1990) | 25.0 | NA | NA | NA | NA |
| 4. Permissible limits set by the Hong Kong Government (1989) | 2.0 | NA | NA | NA | NA |
| 5. Australian Legal Requirements (NHMRC 1987) | 10.0 | 350 | NA | NA | 750 |

Note: Metal concentration of different parts sharing a common letter in the post hoc column are not significantly different (P>0.05). TST= Total soft tissue. NA= not available.

Table 6: Mean, minimum and maximum concentrations (µg/g dry weight) and ratios of maximum to minimum values of trace metals in the different tissues of *Anadara granosa* investigated in this study.

| | Gills (5) | | | | Mantle (5) | | | | Shell (6) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | mean | min | max | ratio | mean | Min | max | ratio | mean | min | max | ratio |
| Cd | 1.90 | 0.90 | 3.15 | 3.5 | 0.89 | 0.57 | 1.31 | 2.3 | 3.82 | 2.82 | 7.03 | 2.5 |
| Cu | 16.7 | 2.99 | 52.4 | 17.5 | 10.2 | 3.21 | 26.4 | 8.2 | 6.35 | 4.90 | 7.87 | 1.6 |
| Fe | 1318 | 680 | 1892 | 2.8 | 515 | 318 | 672 | 2.1 | 252 | 60.8 | 417 | 6.9 |
| Ni | 7.26 | 0.40 | 17.9 | 44.8 | 3.20 | 0.08 | 9.74 | 121.8 | 21.8 | 16.0 | 25.8 | 1.6 |
| Zn | 242 | 98.1 | 472 | 4.8 | 160.8 | 80.1 | 293 | 3.7 | 10.4 | 6.53 | 15.4 | 2.4 |
| | Muscle (5) | | | | Remainder (5) | | | | Total soft tissue (5) | | | |
| | mean | min | max | ratio | mean | Min | max | ratio | mean | min | max | ratio |
| Cd | 1.41 | 0.57 | 2.16 | 3.8 | 1.01 | 0.07 | 1.97 | 28.1 | 1.40 | 1.17 | 1.74 | 1.5 |
| Cu | 4.85 | 0.83 | 8.54 | 10.3 | 9.14 | 2.75 | 16.5 | 6.0 | 8.49 | 2.67 | 20.1 | 7.5 |
| Fe | 521 | 305 | 808 | 2.6 | 559 | 386 | 765 | 2.0 | 831 | 562 | 1202 | 2.1 |
| Ni | 3.34 | 0.07 | 7.10 | 101.4 | 3.07 | 0.50 | 11.8 | 23.6 | 0.94 | 0.74 | 1.35 | 1.8 |
| Zn | 171 | 84.4 | 334 | 4.0 | 83.6 | 67.3 | 107 | 1.6 | 115 | 63 | 190 | 3.0 |

Note: values in brackets are numbers of sites/replicates.

## Discussion

Table 4 shows the means of temperature, conductivity, TDS and salinity of the habitat surface waters. These water parameters could possibly help us to understand the metal accumulation in cockles. For example, a wide range of salinity (14-27 ppt) shows that the cockles are tolerant of the salinity fluctuations in the intertidal area.

From the study of Selin and Vekhova (2003), the age of molluscs from the different habitats, the shell lengths were also not similar. Therefore, the variability of shell sizes found in the different geographical cockle populations is ecologically common in nature. When bivalves exposed to high pollutants, they are able to accumulate metals to relatively high metal concentrations by their specialised organs without any obvious effect (Hamza-Chaffai *et al*., 1999).

The cockle shells always showed higher concentrations of Cd and Ni. Yap *et al*. (2003a) found the metals could be distributed in the different soft tissues before biodeposition into the shell. At last, these metals substituted the calcium ions in the crystalline phase of the shell. The shell could act as a safe storage for toxic contaminants and detoxification mechanism (Foster and Chacko, 1995; Walsh *et al*., 1995; Edward *et al*., 2009; Yap and Edward, 2009).

The metal accumulations of the marine bivalves can be affected directly in proportion to the temperature (Phillips and Rainbow, 1993) as temperature could affect the metabolic activity of the organisms (Ray, 1986). The salinity of the sea water is inverted proportionally to the accumulation of the metals that increase in salinity will generally decrease the accumulation of metals (Gibb *et al*., 1996). However, the background data of temperature and salinity from the sampling sites from the present study could not explain clearly on the metal accumulation in the different tissues of *A. granosa* collected from different sampling sites.

Essential trace elements, Cu, Fe, Ni, and Zn are part of some enzymes, hormones, and vitamins. These essential metals are known to be regulated from the bivalves (Phillips and Rainbow, 1993) and this was evidenced in gill, mantle, muscle and 'remainder' with the significantly low ratios of maximum to minimum values of Fe and Zn (when compared to other metals) but not clearly shown for Cu and Ni. Low ratio of maximum to minimum values has been used to indicate partial regulative mechanism found in the green-lipped mussels (Yap *et al*., 2002a). Lobel *et al.* (1982) found some individual bivalves were very good regulators of their Zn levels and do not accumulate appreciable amounts of Zn even in highly polluted estuary or the particular bivalves have developed an "impermeability" to Zn. Therefore, the narrowest range of Zn levels were found in 'remainder' (Table 6) of *A. granosa* from the present study, which was found in gill, mantle, muscle and 'remainder', was the indicative of Zn being too partially regulated. The ratios of maximum to minimum in the different tissues of *A. granosa*, whether it can be potentially used to explain the behaviours of metal uptake in the different metals, whether essential or non essential, and whether partially regulated or less regulated, should merit further investigations.

Cockle gills were found to have higher metal concentrations when compared to other tissues. It is because of the direct contact of gills to the surrounding environment (Yap *et al.*, 2006), and reflect short term exposure metal (Roméo *et al*., 2005). The function of the large surface of the gills was to increase metals uptake through facilitated diffusion (Phillip and Rainbow, 1993; Yap *et al*., 2006; Edward *et al*., 2009). In the gills, Fe concentration is also high because the function of gills is for respiration. It has high haemoglobin in blood and in haemoglobin has high content of Fe (Tortora and Grabowski, 2003) in *A. granosa*. The gills and mantles which are in contact with the external environment are considered responsible for the transfer of metals to the organisms. Therefore, differences in the contact surface of the different soft tissues also can influence the accumulation of metals by the different tissues of *A. granosa* (Yap *et al.*, 2008). Furthermore, the soft tissues of cockles, where the specific number of metalloenzymes binds, metabolize, and accumulate excess metal concentrations, acts as a long term metal exposure storage organ (Roméo *et al*., 2005).

Contaminants typically accumulate more efficiently within soft tissues than shells (Brown and Depledge, 1998). Particularly, the essential trace metals are catalytic cofactor for biological process. The essentiality and toxicity of metals requires the presence of precise mechanisms for acquisition that are intimately linked to controlled distribution at the plasma membrane before delivery to subcellular targets (Puig and Thiele, 2002). For example, bivalves need Cu as an essential constituent for their respiratory pigment hemocyanin (Gundacker, 2000). Sometimes, Cu and Zn concentrations in soft tissue were higher than the standard value. It is because the metals are probably taken up actively possibly reflecting their use in metabolism as reported by Genest and Hatch (1981) or storage as a form of detoxification (Lobel *et al.*, 1982).

The present findings on the metal levels in the cockle's shell seem to be difficult to differentiate the known metal polluted site at Kuala Juru (Yap *et al.*, 2002b, 2003b). Hence, more studies are needed before the shells can be confidently used as biomonitoring materials of trace metal pollution. The cockle's shell should merit further studies since bivalve shells have been used to monitor metal contamination (Yap *et al.*, 2003c) and shells accumulate contaminants throughout the entire life of a bivalve and persist after the bivalve has died. The shells can potentially provide a persistent pathway of contaminant exposure to epifaunal organisms (Cravo *et al.*, 2002).

From the comparison of the edible soft tissue in dry weight to the food criteria set or known as maximum permissible limits set from a few countries like Brazil, Thailand, USA, Hong Kong, and Australia (Table 5), it is generally found that the concentrations of Cd, Cu and Zn in the different edible soft tissues are lower than the maximum permissible limits.

## Conclusion

Generally, all the different tissues were useful in the assessment of trace metal bioavailabilities and contamination. The concentrations of Cd, Cu and Zn in the different edible soft tissues are still lower than the maximum permissible limits set for food safety. Although the consumption of cockles from the six populations currently should pose no toxicological risks for Cd, Cu and Zn, the monitoring of metals in the edible cockles should be done regularly since a direct check on the metal contamination of the popular seafood available to the consumers can be easily conducted.

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