EnvironmentAsia
The international journal published by the Thai Society of Higher Education Institutes on Environment

Available online at www.tshe.org/EA
EnvironmentAsia 3(2) (2010) 38-48

Distribution of Heavy Metals in the Different Parts of *Cerithidea Obtusa* and the Relationships between Metal Distribution and Allometric Parameters of the Snail

Chee Kong YAP and Franklin Berandah EDWARD

Department of Biology, Faculty of Science, Universiti Putra Malaysia, UPM, 43400 Serdang, Selangor, Malaysia

Abstract

The intertidal gastropod, *Cerithidea obtusa* were obtained from Bako and Sematan (Sarawak) and Deralik (Perak). Besides the shell, the snails were dissected into five different soft tissues. The soft tissues and the shell were then analysed for heavy metals. It was found that the highest concentrations of Cu (112 - 178 µg/g dw) and Zn (117 - 161 µg/g dw) were found in the tentacle; the highest concentrations of Cd (4.41 - 5.37 µg/g dw), Pb (53.2 - 63.8 µg/g dw) and Ni (26.1 - 27.9 µg/g dw) were found in the shell. On the other hand, the highest Fe concentrations (910 - 2921 µg/g dw) were found in the operculum. The Spearman's correlation coefficient and multiple stepwise linear regression also revealed that the allometric parameters can influence the distribution of heavy metals in some of the different parts. From the present findings indicated that the heavy metals accumulated by the *C. obtusa* from the environment might affect its physical growth, which was shown by the negative correlations found between the metals in the different parts with the allometric parameters.

Keywords: Cerithidea obtuse; heavy metals; allometric parameters; different parts; cluster analysis; multivariate analysis

1. Introduction

Inputs resulted from human activities often deteriorate the health of ecosystems, although the effects of these input to the environment and their interactions on the individual ecosystem components remain unknown (O'Connor, 1996; Connell *et al*., 1999). The stress–effect relationships are difficult to be determined in dynamic environments were natural and/or anthropogenic pressures are known to vary in space and time (Wilson, 2003). For example, estuarine systems, exhibit an extreme level of spatio-temporal variability in their physico-chemical characteristics. As a result, estuaries are regarded as highly complex and dynamic environments that are governed by a variety of natural and anthropogenic stress related gradients (O'Connor, 1996; Telesh, 2004; Elsdon and Gillanders, 2006). Molluscs that inhabit such an extreme environment must be capable of maintaining normal metabolic function despite the constant changes in their external environment (Verslycke *et al*., 2004). Generally, sublethal stress will induce compensatory changes in the organism's energy metabolism (Widdows and Donkin, 1991; De Coen and Janssen, 2003; Smolders *et al*., 2004b). Owing to the majority of the organism's energy budget is used for growth, reproduction and basal metabolism, an increase in energy consumption in the basal metabolism caused by stress will affect the animals' condition, reducing its growth, reproduction potential and susceptibility to other stressors (De Coen and Janssen, 2003).

The study of heavy metal in the different parts of molluscs and relationships with their growth is therefore important, in order to know the accumulation of metal(s) by which tissue(s) that would influence the growth of the molluscs. It is already known that heavy metals can accumulate in tissues during molluscs growth (bioaccumulation) and would disturb the normal metabolisms of the organism (Migliarini *et al*., 2005). For example, toxic effect of Cd can result in the injured cells dying by apoptosis or necrosis (Migliarini *et al*, 2005). Cells normally undergo apoptosis in response to mildly adverse conditions, while exposure to severe conditions (higher doses of the toxin) will result in necrosis (Migliarini *et al*., 2005). These two processes of cells dying definitely will adversely influence the growth of the molluscs.

Present study aims at determining the relationships between heavy metals in the different parts of *C. obtusa* and their allometric parameters. In other words, it is to determine the accumulation of metal by which part(s) that would influence the allometric parameters of the snail.

2. Materials and Methods

A sampling was conducted in February 2006 at Deralik (Perak) to collect the samples. As for the samples from Bako and Sematan (Sarawak), the snails were obtained on December 2006 from the local fishermen which they collected from the mangrove area nearby.

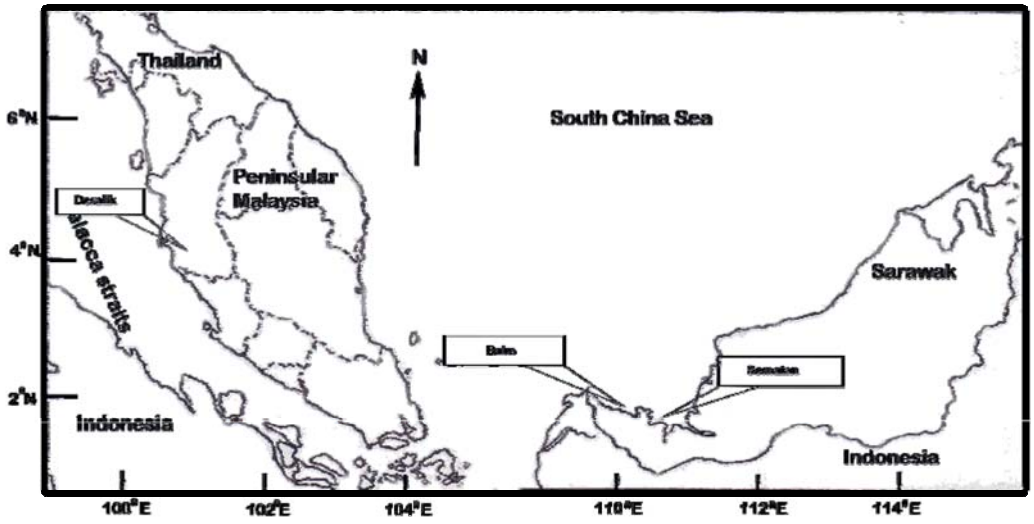

Figure 1. The sampling locations of *Cerithidea obtusa* from Bako and Sematan (Sarawak) and Deralik (Perak)

The sampling map and description of the sampling sites are shown in Fig. 1 and Table 1, respectively. The identification of the species were followed the descriptions by Lim *et al*. (2001). For the analysis, 30-40 individual of snails with almost a similar-size were randomly taken from the main sample and thawed at room temperature (26-29ºC) on a clean tissue paper. The soft tissues were then separated from the shell by crunching (using a clean pestle) the shell carefully. Due to the fragile characteristic of the shell, a mild force was sufficient to break the shell (strong force might destroy the internal organs of the snail). The soft tissues were then dissected and pooled into six different categories namely ceacum, foot, muscle, operculum, remainder, and tentacle besides the shell. The soft tissues and the shell were dried for 72 hours at 60ºC in an oven to constant dry weights (Yap *et al*., 2003a; 2003b). The methodology for measuring the shell width and shell height of the gastropod was based on those described by Ghesquiere (2005).

About 0.5 of the *C. obtusa* tissues were digested in 10 ml of concentrated nitric acid (AnalaR Grade; 69%). They were placed in a hot block digester first at low temperature (40ºC) for one hour and were then fully digested at high temperature (140ºC) for at least three hours. The digested samples were then diluted to a volume of 40ml with double distilled water (DDW). The sample was then filtered through Whatman No.1 filter paper (Dia: 110mm; Schleicher & Schuell, Whatman International Ltd Maidstone England), then they were determined for Cd, Cu, Fe, Ni, Pb and Zn by using an air-acetylene flame Atomic Absorption Spectrophotometer (AAS) Perkin Elmer Model AAnalyst 800. The samples were analyzed in three replicates. The data were presented in µg/g dry weight (dw) basis. Multi-level calibration standards were analysed to generate calibration curves against which sample concentrations were calculated. Standard solutions were prepared from 1000 mg/L stock solutions of each metal (Merck Titrisol).

All the glassware and plastic materials used were acid-washed in 10% concentrations of acid in order to minimize external contamination. Quality control samples made from standard solutions of Cu, Cd, Zn, Pb, Ni and Fe were analyzed once in every ten samples

Table 1. The descriptions of the sampling locations and information on the *Cerithidea obtusa* collected.

No	Location	Sampling date	GPS reading	Site description
1.	Deralik, Perak	25th February2006	N 04º 14’ 53.8” E 100º 42’09.1”	1. Residental area 2. Mangrove area 3. No direct pollution found
2.	Bako, Sarawak	28th December2006	N 01º40’23.36” E 110º 25’54.46”	1. Mangrove areas 2. Fishing village 3. Tourism spot
3.	Sematan, Sarawak	28th December2006	N 01º 48’12.61” E 109º 46’45.65”	1. Intertidal area 2. Fishing village 3. Jetty

Table 2. Analytical results for the Certified Reference Material (CRM) and its certified values for each metal (μg/g dry weight).

Metal	Sample	CRM values	Measured values	Percentage of recovery
Cd	DOLT-3 Dogfish-liver	19.4 ± 0.600	20.5 ± 0.439	106 ± 2.26
Cu	DOLT-3 Dogfish-liver	31.2 ± 1.00	26.5 ± 2.58	85.0 ± 8.28
Fe	DOLT-3 Dogfish-liver	1484 ± 57.0	1070	72.1
Ni	DOLT-3 Dogfish-liver	2.72 ± 0.350	2.77 ± 0.741	102 ± 27.2
Zn	DOLT-3 Dogfish-liver	86.6 ± 2.40	80.9 ± 1.94	93.4 ± 2.24

Note: Pb is not available.

to check for the metal recoveries. The analytical procedures for the snail samples were checked with the Certified Reference Material (CRM) and the recoveries of all metal were satisfactory (Table 2).

For the statistical analysis, the distributions of heavy metals in the different parts were determined by using cluster analysis. The relationships between the heavy metals in the different parts and allometric parameters were analyzed using Spearman's correlation coefficient. Multiple stepwise linear regression was used to determine the influence of heavy metal in the different parts towards the allometric parameters. All data were log10 (X + 1) transformed prior to the statistical analysis. SPSS 12.0 was used to conduct the correlation and multiple stepwise linear regression analyses while STATISTICA 99 edition was used to conduct the cluster analysis.

3. Results

3.1. Distribution of heavy metal in the different parts

Heavy metal concentrations in the different parts of the *C. obtusa* collected from the three sampling sites are shown in Table 4. In general, it was found that the tentacle of the gastropods were highly accumulative of Cu and Zn as shown by the gastropods from all the sites, where they ranged from 112 - 178 μg/g dw and 117 - 161 μg/g dw, respectively. Meanwhile, the operculums were mostly accumulative of Fe, as shown by the gastropods from Bako and Sematan, where they ranged from 638 - 2921 μg/g dw. On the other hand, the shell was highly accumulative of Cd (4.41 - 5.37 μg/g dw), Pb (53.2 - 63.8 μg/g dw) and Ni (24.4 - 27.9 μg/g dw). The ranges of the allometric parameters of the *C. obtusa* are shown in Table 3.

3.2. Relationships between the heavy metals and allometric parameters

The correlations between the heavy metal concentrations between the different parts of the *C. obtusa* and their allometric parameters are shown by the Spearman's correlation coefficients in Table 5. It is found that the accumulation of some metals by the different parts were positively and also negatively correlated with the allometric parameters studied. However, the relationships which were focused upon in the present study were the negative relationships between the metal concentrations in the different parts with the allometric parameters. The outcome of negative correlation coefficients formed was due to the fact that any increment in

Table 3. Ranges of allometric parameters [shell height, shell width, shell volume, soft tissue dry weight (STDW) and Condition index (CI)] of the *Cerithidea obtusa*.

	Site	Shell height, mm	Shell width, mm	Shell volume, cm^3	STDW, g	CI, g/cm^3
1.	Bako, Sarawak	28.5 – 43.8	13.4 – 20.8	1.46 – 4.96	0.117 - 0.571	57.4 - 141
2.	Sematan, Sarawak	23.0 – 43.7	12.8 – 23.0	1.36 – 5.92	0.081 – 0.596	46.0 – 125
3.	Deralik, Sitiawan	33.3 – 52.0	14.0 – 27.0	1.75 – 9.93	0.160 – 1.21	69.1 - 143

the metal concentrations in the different parts will cause decrement in the values of the allometric parameters. In other words, high metal concentrations accumulated by the gastropod may retard/alter it physical growths. Negative correlations are found between the Cu in the shell with the shell width (*P<0.05; R=-0.720*) and shell volume (*P<0.05; R=-0.683*). Negative correlations are also found between Cu-caecum versus (vs) soft tissues (ST) dry weight (*P<0.05; R=-0.800*); Cu- tentacle vs ST dry weight (*P<0.05; R=-0.733*). Significant negative correlations are also found between Cd-remainder vs shell height (*P<0.05; R=-0.695*), shell width (*P<0.01; R=-0.803*) and shell volume (*P < 0.01; R=-0.800*). In the mean time, Cd accumulation by the foot are negatively correlated with the shell height (*P<0.05; R=-0.745*); shell width (*P<0.05; R=-0.745*) and shell volume (*P<0.01; R=-0.833*). In the literature, the relationships of heavy metals and growth rate were also being reported in gastropods *Nucella lapillus* of Loch Fyne, where individuals accumulated relatively high heavy metals have lower growth rates (Leung *et al*., 2001), therefore, the correlations above are in agreement with those reported in the gastropod, *N. lapillus*.

Similarly for Ni, significant negative correlations are found between Ni-caecum vs shell width (*P<0.05; R=-0.787*) and shell volume *(P<0.05; R=-0.700*).

Table 4. Heavy metal concentrations (µg/g dw, mean±SE) of (Cu, Cd, Zn, Pb, Ni and Fe) in the different soft tissues of *Cerithidea obtusa* collected from Bako, Sematan and Deralik.

	Site	Cu	Cd	Zn	Pb	Ni	Fe
Caecum	Bako, Sarawak	95.3 ± 15.8	1.79 ± 0.185	113 ± 18.3	10.0 ± 0.193	9.44 ± 0.207	782 ± 86.2
	Sematan, Sarawak	94.7 ± 2.61	1.43 ± 0.086	113 ± 10.6	7.94 ± 0.433	9.26 ± 0.530	543 ± 47.9
	Deralik, Perak	51.1 ± 1.77	3.45 ± 0.169	318 ± 5.67	6.2 ± 1.00	7.54 ± 0.350	661 ± 71.9
Foot	Bako, Sarawak	118 ± 4.21	1.48 ± 0.071	74.4 ± 8.77	5.94 ± 0.234	2.80 ± 0.192	177 ± 15.9
	Sematan, Sarawak	113 ± 11.3	0.984 ± 0.096	97.7 ± 2.67	5.10 ± 0.606	3.58 ± 0.269	121 ± 4.07
	Deralik, Perak	124 ± 9.36	1.22 ± 0.115	113 ± 0.183	14.0 ± 0.553	5.79 ± 0.615	255 ± 3.47
Muscle	Bako, Sarawak	64.8 ± 7.72	1.16 ± 0.111	82.4 ± 6.21	5.88 ± 0.434	0.660 ± 0.086	156 ± 12.1
	Sematan, Sarawak	83.4 ± 3.79	0.901 ± 0.141	92.4 ± 3.30	4.12 ± 0.178	3.33 ± 0.539	124 ± 10.0
	Deralik, Perak	77.3 ± 0.526	1.39 ± 0.075	79.0 ± 1.57	13.7 ± 0.548	5.08 ± 0.137	254 ± 5.11
Operculum	Bako, Sarawak	51.7 ± 0.000	2.78 ± 0.000	43.9 ± 0.000	9.45 ± 0.000	0.222 ± 0.000	2921 ± 0.000
	Sematan, Sarawak	44.7 ± 0.000	2.13 ± 0.000	45.0 ± 0.000	7.50 ± 0.000	3.47 ± 0.000	910 ± 0.000
	Deralik, Perak	59.1 ± 0.000	2.46 ± 0.000	38.9 ± 0.000	43.0 ± 0.000	7.22 ± 0.000	638 ± 0.000
Remainder	Bako, Sarawak	136 ± 13.4	1.54 ±0.202	65.0 ± 5.06	10.7 ± 1.86	6.54 ± 0.848	850 ± 60.8
	Sematan, Sarawak	143 ± 3.25	1.35 ± 0.158	77.5 ± 1.89	7.62 ± 0.550	6.37 ± 0.253	406 ± 29.6
	Deralik, Perak	133 ± 2.51	1.17 ± 0.144	81.1 ± 5.29	17.2 ± 1.71	6.22 ± 0.052	1137 ± 123
Shell	Bako, Sarawak	11.9 ± 0.794	5.12 ± 0.047	5.91 ± 0.255	56.2 ± 1.43	26.1 ± 0.909	240 ± 48.9
	Sematan, Sarawak	11.1 ± 0.750	5.37 ± 0.205	7.04 ± 0.647	53.2 ± 0.483	27.9 ± 0.295	66.3 ± 4.95
	Deralik, Perak	8.65 ± 0.503	4.41 ± 0.118	6.53 ± 1.39	63.8 ± 0.318	24.4 ± 0.143	52.0 ± 2.35
Tentacle	Bako, Sarawak	178 ± 0.000	0.962 ± 0.000	117 ± 0.000	5.70 ± 0.000	20.1 ± 0.000	240 ± 0.000
	Sematan, Sarawak	173 ± 0.000	1.36 ± 0.000	130 ± 0.000	4.54 ± 0.000	16.2 ± 0.000	177 ± 0.000
	Deralik, Perak	112 ± 0.000	2.32 ± 0.000	161 ± 0.000	34.2 ± 0.000	27.9 ± 0.000	283 ± 0.000

Table 5. Spearman's correlation coefficient between Cu concentrations in the different parts of Cerithidea obtusa and their allometric parameter.

Metal		Shell	Caecum	Remainder	Operculum	Tentacle	Muscle	Foot	Shell height	Shell width	Shell volume	STdry	CI
Cu	Shell	1.000											
	Caecum	0.667*	1.000										
	Remainder	0.600	0.767*	1.000									
	Operculum	-0.517	-0.683*	-0.400	1.000								
	Tentacle	0.850**	0.867**	0.717*	-0.700*	1.000							
	Muscle	-0.333	0.167	0.300	-0.400	-0.067	1.000						
	Foot	-0.233	-0.383	-0.517	0.067	-0.433	0.050	1.000					
	Shell height	-0.427	-0.251	0.192	0.092	-0.276	0.720*	-0.084	1.000				
	Shell width	-0.720*	-0.310	0.059	0.393	-0.460	0.561	-0.243	0.697*	1.000			
	Shell volume	-0.683*	-0.250	0.133	0.283	-0.433	0.683*	-0.233	0.778*	0.979**	1.000		
	STdry	-0.467	-0.800**	-0.367	0.483	-0.733*	0.167	0.483	0.435	0.377	0.383	1.000	
	CI	-0.167	-0.667*	-0.333	0.383	-0.500	-0.133	0.633	0.084	0.017	0.000	0.867**	1.000
Cd	Shell	1.000											
	Caecum	-0.667*	1.000										
	Remainder	0.450	-0.383	1.000									
	Operculum	-0.133	0.167	0.117	1.000								
	Tentacle	-0.617	0.533	-0.833**	-0.417	1.000							
	Muscle	-0.550	0.750*	-0.267	0.217	0.267	1.000						
	Foot	0.000	0.300	0.517	0.750*	-0.500	0.250	1.000					
	Shell height	-0.042	0.050	-0.695*	-0.586	0.603	0.209	-0.745*	1.000				
	Shell width	-0.603	0.159	-0.803**	-0.385	0.795*	0.201	-0.745*	0.697*	1.000			
	Shell volume	-0.467	0.083	-0.800**	-0.467	0.750*	0.133	-0.833**	0.778*	0.979**	1.000		
	STdry	-0.483	0.700*	-0.550	-0.117	0.600	0.383	-0.133	0.435	0.377	0.383	1.000	
	CI	-0.350	0.683*	-0.283	0.033	0.433	0.183	0.217	0.084	0.017	0.000	0.867**	1.000

Zn	Shell	1.000													
	Caecum	-0.450	1.000												
	Remainder	0.367	0.500	1.000											
	Operculum	0.333	-0.800**	-0.267	1.000										
	Tentacle	0.050	0.617	0.850**	-0.367	1.000									
	Musscle	0.083	-0.267	-0.200	0.567	-0.267	1.000								
	Foot	0.033	0.583	0.733*	-0.483	0.900**	-0.233	1.000							
	Shell height	0.109	0.326	0.569	-0.159	0.603	-0.209	0.728*	1.000						
	Shell width	0.109	0.318	0.636	-0.259	0.795*	-0.167	0.921**	0.697*	1.000					
	Shell volume	0.133	0.267	0.583	-0.167	0.750*	-0.067	0.900**	0.778*	0.979**	1.000				
	Stdry	-0.550	0.717*	0.300	-0.500	0.600	-0.133	0.633	0.435	0.377	0.383	1.000			
	CI	-0.417	0.550	0.217	-0.350	0.433	-0.100	0.333	0.084	0.017	0.000	0.867**	1.000		
Pb	Shell	1.000													
	Caecum	0.949**	1.000												
	Remainder	0.812**	0.812**	1.000											
	Operculum	0.602	0.602	0.483	1.000										
	Tentacle	0.534	0.534	0.449	0.916**	1.000									
	Muscle	0.402	0.402	0.590	0.788*	0.856**	1.000								
	Foot	0.436	0.436	0.521	0.720*	0.788*	0.932**	1.000							
	Shell height	0.369	0.369	0.411	0.290	0.525	0.360	0.191	1.000						
	Shell width	0.331	0.314	0.449	0.525	0.786*	0.771*	0.670*	0.697*	1.000					
	Shell volume	0.253	0.253	0.388	0.460	0.728*	0.692*	0.557	0.778*	0.979**	1.000				
	STdry	0.549	0.430	0.515	0.619	0.552	0.582	0.549	0.435	0.377	0.383	1.000			
	CI	0.380	0.295	0.262	0.477	0.343	0.397	0.498	0.084	0.017	0.000	0.867**	1.000		

Ni	Shell	1.000											
	Caecum	0.683*	1.000										
	Remainder	-0.117	0.333	1.000									
	Operculum	-0.217	-0.783*	-0.450	1.000								
	Tentacle	-0.667*	-0.733*	-0.400	0.550	1.000							
	Muscle	-0.333	-0.700*	-0.383	0.900**	0.450	1.000						
	Foot	-0.267	-0.617	-0.383	0.883**	0.433	0.967**	1.000					
	Shellheight	0.243	-0.310	-0.151	0.770*	0.092	0.636	0.678*	1.000				
	Shellwidth	-0.159	-0.787*	-0.360	0.895**	0.393	0.695*	0.695*	0.697*	1.000			
	Shellvolume	-0.017	-.700*	-0.317	0.883**	0.283	0.667*	0.650	0.778*	0.979**	1.000		
	STdry	-0.483	-.633	-0.133	0.633	0.483	0.717*	0.583	0.435	0.377	0.383	1.000	
	CI	-0.533	-.433	-0.183	0.333	0.383	0.583	0.433	0.084	0.017	0.000	0.867**	1.000
Fe	Shell	1.000											
	Caecum	0.233	1.000										
	Remainder	-0.517	0.517	1.000									
	Operculum	0.867**	0.300	-0.417	1.000								
	Tentacle	-0.450	0.350	0.867**	-0.417	1.000							
	Muscle	-0.533	0.167	0.800**	-0.583	0.833**	1.000						
	Foot	-0.483	0.350	0.900**	-0.483	0.833**	0.950**	1.000					
	Shell height	-0.828**	-0.310	0.142	-0.603	0.025	0.033	-0.017	1.000				
	Shell width	-0.778*	-0.209	0.343	-0.795*	0.310	0.251	0.176	0.697*	1.000			
	Shell volume	-0.767*	-0.317	0.217	-0.750*	0.217	0.133	0.033	0.778*	0.979**	1.000		
	STdry	-0.567	0.167	0.383	-0.600	0.550	0.450	0.383	0.435	0.377	0.383	1.000	
	CI	-0.250	0.233	0.283	-0.433	0.417	0.433	0.400	0.084	0.017	0.000	0.867**	1.000

* Correlation is significant at the 0.05 level (2-tailed); ** Correlation is significant at the 0.01 level (2-tailed); N=9; ST dry= soft tissue dry weight; CI=condition index

Accumulation of Fe by some of the different parts too, could cause decrement in some of the allometric parameters as shown by Fe-shell vs shell height ($P < 0.01$; $R=-0.828$), shell width ($P<0.05$; $R=-0.778$) and shell volume ($P<0.05$; $R=-0.767$); and Fe-operculum vs shell width ($P<0.05$; $R=-0.795$) and shell volume ($P<0.05$; $R=-0.750$). The negative correlation found could also be due to the reasons which were being discussed in the previous paragraph.

4. Discussion

4.1. Distribution of heavy metal in the different parts

The differential affinities of the metals to the binding sites might be associated with the different metal concentrations found in the different tissues. The high level of a metal found in a particular tissue could be due to the metal being tightly bound to the metallothionein as was reported by Viarengo *et al*. (1985) and Roesijadi (1992) in mussel. The formation of a metal-thiolate complex with the cysteine residues inside the lysosomes caused the slower depuration of the metals found in the different tissues (Yap *et al*., 2003c) which could result in high levels of metals being found in the mentioned tissues. This mechanism would reduce a metal's toxicity by preventing it from disturbing the cell activities (Webb, 1987).

The accumulation of metals in the different tissues mentioned could also be related to the functions of the organs. The tentacle which are in contact with the external medium and are considered responsible for the metal transfer to the organism. This indicated that the differences in the surface of contact of the different soft tissues may affect the accumulation of metals by the gastropods tissues (Yap *et al*., 2003c). Besides, different rates of accumulation and excretion of metals in the different tissues could also result in the different concentrations found in each of the tissues of molluscs analyzed (Yap *et al*., 2003c).

4.2. Relationships between the heavy metals and allometric parameters

It is known that growth performance of marine organisms depends on many environmental factors such as temperature, bottom types (rocks, sands, pebbles, silts, and etc), oxygen availability, salinity, etc (Belcheva *et al*., 2006). The growth performance of the marine organisms in the heavy metals polluted area is limited by some of these environmental factors which put the organisms into a lower metabolic state and therefore, affecting their growth rate thus lowering the values of allometric parameters. In the oyster *Crassostrea gigas* which has been introduced to two areas with different levels of pollution indicated the reverse relationship between Cd concentration and body size (Boyden and Phillips, 1981; Belcheva *et al*., 2006), indicated that the Cd accumulation influenced the body size of the oyster.

No significant correlations ($P>0.05$) were observed between the accumulation of Zn and Pb by the different parts with the allometric parameters. This may indicate that accumulation of the two metals by the different parts would not cause decrement in the allometric parameters which was in contrast with those reported by Gifford *et al*. (2006) where they reported significant reductions in total oyster growth when they were exposed to high concentrations of Zn and Pb. The result could indicate that the *C. obtusa* were tolerant with Zn and Pb contaminants and have regulative mechanisms for these two metals. However, in normal circumstances, the higher allometric parameters could also influence lower accumulation of heavy metals in the snail (Larger/adult individual accumulate lower concentrations of metal than smaller/young individual). Therefore, future laboratory study should focus on the levels of metal that would influence the allometric parameter(s).

On the other hand, the relationships between the allometric parameters with the heavy metal concentrations in the different soft tissues indicated two different phenomena: 1) The higher metal concentrations in the different tissues could cause a decrement in the values of allometric parameters. 2) The larger allometric parameters could cause a lower metal accumulation in the different tissues of the snails. From ecotoxicological points of view, the first phenomena seems to be of much concern. This is due to higher metal accumulated in the tissues could cause a lower growth rate and subsequently a lower values of the shell length, shell width and ST dry weight of the gastropods. However, the second phenomena is explainable when the snails are able to grow up to the adult size provided that the heavy metal pollution is not a major factor in influencing the growth rate of the snails. Therefore, the snails can grow to a maximum size range. The second phenomena can also be explained by the lower growth rate in the larger sized group and lower total surface area per volume in the larger sized group. These two factors could potentially cause a lower metal accumulation in the larger sized group.

The relationship between the distribution of heavy metals in the different tissues and the allometric parameters could also be explained by the multiple stepwise linear regressions shown in Table 6. The allometric parameters with significant relationships ($P < 0.05$) with the metal concentrations in the different parts are included in Table 6. From the regression equations,

Table 6. Stepwise linear regression between the allometric parameters and heavy metal distributions of the *Cerithidea obtusa*

Metal	Multiple Linear regression
Cu	ST dry = 0.977 + 0.115 (shell) – 0.486 (caecum) ; $P < 0.05$; $R = 0.831$; $R^2 = 0.690$ Condition index = 3.006 + 1.424 (shell) – 1.334 (caecum); $P < 0.05$; $R = 0.863$ $R^2 = 0.744$ Shell height = no significant independent variables have been selected Shell width = no significant independent variables have been selected Shell volume = no significant independent variables have been selected
Cd	ST dry = 9.110 – 3.872 (shell) – 0.183 (caecum) – 6.957 (remainder) – 8.581 (operculum) – 2.579 (tentacle) + 2.296 (muscle) + 4.536 (foot); $P < 0.05$; $R = 1.000$; $R^2 = 0.999$ Condition index = no significant independent variables have been selected Shell height = 1.519 + 0.433 (shell) + 0.112 (caecum) – 0.737 (remainder); $P < 0.05$; $R = 0.778$; $R^2 = 0.606$. Shell width = no significant independent variables have been selected Shell volume = no significant independent variables have been selected
Zn	ST dry = 0.006 – 0.353 (shell) + 0.218 (caecum); $P < 0.05$; $R = 0.802$; $R^2 = 0.644$ Condition index = no significant independent variables have been selected Shell height = no significant independent variables have been selected Shell width = no significant independent variables have been selected Shell volume = no significant independent variables have been selected
Pb	ST dry = -2.220 + 1.364 (shell); $P < 0.05$; $R = 0.690$; $R^2 = 0.476$. Condition index = no significant independent variables have been selected Shell height = no significant independent variables have been selected Shell width = -5.951 + 4.836 (shell) – 1.165 (caecum); $P < 0.05$; $R = 0.890$; $R^2 = 0.791$. Shell volume = - 16.766 + 11.614 (shell) – 2.776 (caecum); $P < 0.05$; $R = 0.859$; $R^2 = 0.737$
Ni	ST dry = no significant independent variables have been selected Condition Index = 4. 381 – 2.133 (shell) – 1.634 (caecum) + 2.010 (remainder) – 1.885 (operculum) – 0.334 (tentacle) + 2.388 (muscle); $P < 0.05$; $R = 0.997$; $R^2 = 0.995$. Shell height = 1.382 + 0.698 (shell) – 0.755 (caecum); $P < 0.05$; $R = 0.760$; $R^2 = 0.577$ Shell width = 1.349 + 0.633 (shell) – 0.928 (caecum); $P < 0.05$; $R = 0.876$; $R^2 = 0.767$ Shell volume = 0.598 + 1.711 (shell) – 2.312 (caecum); $P < 0.05$; $R = 0.848$; $R^2 = 0.720$
Fe	ST dry = no significant independent variables have been selected Condition index = no significant independent variables have been selected Shell height = 1.801 -0.083 (shell) Shell width = -3.210 -0.964 (shell) + 0.198 (caecum) -0.447 (remainder) + 0.721 (operculum) + 3.902 (tentacle) + 0.128 (muscle) – 2.027 (foot); $P < 0.05$; $R = 1.000$; $R^2 = 1.000$ Shell volume = no significant independent variables have been selected

Note: ST dry = Soft tissue dry weight

generally, it was found that the accumulation of metals (Cu, Zn, Pb and Ni) by the shells and caecums significantly influenced most of the allometric parameters such as ST dry weight and condition index for Cu; ST dry weight for Zn; shell width and shell volume for Pb; and shell height, shell width and shell volume for Ni. On the other hand, the accumulations of Cd and Fe by the shell, caecum, remainder, operculum, tentacle, muscle and foot significantly influenced the ST dry weight and shell width, respectively. In the mean time, the accumulation of Ni by the shell, caecum, remainder, operculum, tentacle and muscle significantly ($P<0.05$) influenced the condition index of gastropods. These relationships indicated that different allometric parameters of *C. obtusa* are significantly influenced by different metals in the different tissues of the snails.

5. Conclusions

Present study found that the tentacle of *C. obtus*a was highly accumulative of Cu and Zn; while the operculum was highly accumulative of Fe. The shell meanwhile was accumulative of metals such as Cd, Pb and Ni. From the negative Spearman's correlation coefficient and stepwise linear regression, it was found that accumulation of heavy metals by the different parts of *C. obtusa* were significantly influenced most of the allometric parameters. This preliminary study revealed that the metal concentrations in the different parts are important factors that could influence the allometric parameters of the *C. obtusa*.

Acknowledgement

The authors wish to acknowledge the financial support provided through the Research University Grant Scheme (RUGS), [Vote no.: 91229], by Universiti Putra Malaysia and e-Science Fund [Vote no.: 5450338], by the Ministry of Science, Technology and Innovation, Malaysia.

References

Belcheva NN, Zakhartsev M, Alla V, Silina AV, Slinko EN, Chelomin VP. Relationship between shell weight and cadmium content in whole digestive gland of the Japanese scallop *Patinopecten yessoensis* (Jay). Marine Environmental Research 2006; 61: 396–409.

Boyden CR, Phillips DJH. Seasonal variation and inherent variability of trace elements in oysters and their implications for indicator studies. Marine Ecology Progress Series 1981; 5: 29–40.

Connell D, Lam P, Richardson B, Wu R. Introduction to ecotoxicology. Oxford: Blackwell Science; 1999. 170.

De Coen WM, Janssen CR. The missing biomarker link: relationships between effects on the cellular energy allocation biomarker of toxicant-stressed Daphnia magna and corresponding population characteristics. Environmental Toxicology and Chemistry 2003; 22: 1632–41.

Elsdon TS, Gillanders BM. Temporal variability in strontium, calcium, barium, and manganese in estuaries: implications for reconstructing environmental histories of fish from chemicals in calcified structures. Estuarine, Coastal and Shelf Science 2006; 66: 147–56.

Gifford SP, Macfarlane GR, O'Connor WA, Dunstan RH. Effect of the pollutants lead, zinc, hexadecane and octocosane on total growth and shell growth in the Akoya Pearl Oyster, *Pinctada imbricata*. 2006; 25(1): 159-65.

Leung KMY, Morgan IJ, Rudolf S SW, Lau TC, Svavarsson J, Furness RW. Growth rate as a factor confounding the use of the dogwhelk *Nucella lapillus* as biomonitor of heavy metal contamination. Marine Ecology Progress Series 2001; 221: 145–59.

Lim KKP, Murphy DH, Morgany T, Sivasothi N, Ng PKL, Soong BC, Tan HTW, Tan KS, Tan TK. Volume 1: The Ecosystem and Plant Diversity and Volume 2: Animal Diversity. In *A Guide to angroves of Singapore*, (*Eds*. P.K.L. Ng, N. Sivasothi). BP Guide to Nature Series published by the Singapore Science Centre 2001, sponsored by British Petroleum. Singapore: Raffles Museum of Biodiversity Research, The National University of Singapore & The Singapore Science Centre.

Migliarini B, Campisi AM, Maradonna F, Truzzi C, Annibaldi A, Scarponi G, Carnevali O. Effects of cadmium exposure on testis apoptosis in the marine teleost *Gobius niger*. General and Comparative Endocrinology 2005; 142: 241-47

O'Connor RJ. Toward the incorporation of spatiotemporal dynamics into ecotoxicology. In: Rhodes OE, Chesser RK, Smith MH, editors. Population dynamics in ecological space and time. Chicago: University of Chicago Press 1996; 281–317.

Roesijadi G. Metallothionein in metal regulation and toxicity in aquatic animals, Aquatic Toxicology 1992; 22: 81–113.

Smolders R, Bervoets L, De Coen W, Blust R. Cellular energy allocation in zebra mussels exposed along a pollution gradient: linking cellular effects to higher levels of biological organization. Environmental Pollution 2004; 129: 99–112.

Ghesquiere SAI. Applesnails. www.applesnail.net. Retrieved February 2007.

Telesh IV. Plankton of the Baltic estuarine ecosystems with emphasis on Neva Estuary: a review of present knowledge and research perspectives. Marine Pollution Bulletin 2004; 49: 206–19.

Verslycke T, Ghekiere A, Janssen CR. Seasonal and spatial patterns in cellular energy allocation in the estuarine mysid Neomysis integer (Crustacea: Mysidacea) of the Scheldt estuary (The Netherlands). Journal of Experimental Marine Biology and Ecology 2004; 306: 245–67.

Vierengo A, Palmero S, Zanicchi G, Capelli R, Vaissiere R, Orunesu M. Role of metallothioneins in Cu and Cd accumulation and elimination in the gill and digestive gland cells of *Mytilus galloprovincialis* (Lam.), Marine Environmental Research 1985 ; 16: 23-36.

Webb M. Toxicological significance of metallothionein. Experientia Supplement 1987; 52: 109–34.

Widdows J, Donkin P. Role of physiological energetics in ecotoxicology. Comparative Biochemistry and Physiology - Part C: Toxicology & Pharmacology 1991; 100: 69–75.

Wilson JG. Evaluation of estuarine quality status at system level with the Biological Quality Index and the Pollution Load Index. Biology and Environment 2003; 103B: 49–57.

Yap CK, Ismail A, Omar H, Tan SG. Accumulation, depuration and distribution of cadmium and Zinc in the green-lipped mussel *Perna viridis* (L) under Laboratory conditions. Hydrobiologia 2003c; 498: 151-60.

Yap CK, Ismail A, Tan SG. Different soft tissues of the green-lipped mussel *Perna viridis* (L.). as biomonitoring agent of copper: Field and laboratory studies. Malaysia's Application in Biology 2003a; 32(2): 9-18.

Yap CK, Ismail A, Tan SG. Background concentrations of Cd, Cu, Pb and Zn in the green-lipped mussel *Perna viridis* (Linnaeus) from Peninsular Malaysia. Marine Pollution Bulletin 2003b; 46: 1035-48.

Received 2 June 2010
Accepted 30 June 2010

Correspondence to
Chee Kong YAP
Department of Biology,
Faculty of Science,
Universiti Putra Malaysia,
UPM, 43400 Serdang,
Selangor,
Malaysia.
Tel: 603-89466616
Fax: 603-86567454
Email: yapckong@hotmail.com