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Heavy Metals Concentration Relationship with *Perna viridis* Physical Properties in Mengkabong Lagoon, Sabah, Malaysia

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Abstract: *Perna viridis* (*P. viridis*) has been identified as a good biological indicator in identifying environmental pollution, especially when there are various types of Heavy Metals Accumulations (HMA) inside its tissue. Based on the potential of *P. viridis* to accumulate heavy metals and the data on its physical properties, this study proffers to determine the relationships between both properties. The similarities of the physical properties are used to mathematical model their relationships, which included the size (length, width, height) and weight (wet and dry) of *P. viridis*, whilst the heavy metals are focused on concentrations of Pb, Cu, Cr, Cd and Zn. The concentrations of metal elements are detected by using Flame Atomic Adsorption Spectrometry. Results show that the mean concentration of Pb, Cu, Cr, Cd, Zn, length, width, height, wet weight and dry weight are: 1.12±1.00, 2.36±1.65, 2.12±2.74, 0.44±0.41 and 16.52±10.64 mg kg^{-1} (dry weight), 105.08±14.35, 41.64±4.64, 28.75±3.92 mm, 14.56±3.30 and 2.37±0.86 g, respectively. It is also found out that the relationships between the Heavy Metals Concentrations (HMA) and the physical properties can be represented using Multiple Linear Regressions (MLR) models, relating that the HMA of Zinc has affected significantly the physical growth properties of *P. viridis*.

Key words: *Perna viridis*, biological indicator, heavy-metals-accumulations (HMA), mathematical model, multiple linear regressions (MLR)

INTRODUCTION

Pollutants as heavy metals can be distributed to aquatic environment through many pathways. These pathways may involve processes, what are also known as bioaccumulation. The accumulation of heavy metals in living tissues, especially mollusc, may cause the increasing of the toxicity levels in living tissues (Dobrowolski and Skowronska, 2001). However, some molluscs, such as *P. viridis*, have a natural technique to reduce the heavy metals concentration from their tissues which in turn, is related to its dual-shell activity; open and closed feeding method. In addition, *P. viridis* had also been known as a biological indicator for heavy metals concentration by many researchers (Sivalingam, 1977; Yap *et al*., 2002; Widmeyer and Bendell-Young, 2007).

According to Widmeyer and Bendell-Young (2007), active feeding behavior of mollusc may increase the concentrations availability of pollutant in its tissue. Mollusc is also exposed to different food suspensions consisting mixtures of sediment, particulate matter and seston. The different concentrations of pollutant in aquatic environment may affect the growth of mollusc in aquatic environment. So, the purpose of this study is to determine the relationships between the heavy metals accumulations in total soft tissue with the physical properties of *P. viridis*.

MATERIALS AND METHODS

Study site: Mengkabong Lagoon is located in the Tuaran District, which is 53 km away from the city of Kota Kinabalu in Sabah. It is dominated by a mangrove ecosystem which is suitable for aquaculture activities (Fig. 1). There are four stations of aquaculture activities being identified, namely B1, B2, B3 and B4, as shown in Fig. 1.

Data samplings of *P. viridis*: Fresh samples (n = 120) of *P. viridis* in Fig. 2 are collected randomly in different length sizes, ranging from 60 mm to 113 mm. Each individual is separated from its shell and the constant weights of tissue samples are taken at 60°C (Silva *et al*., 2006; Blackmore, 2001; Nair *et al*., 1993).

The digestion method of tissue samples is carried out by first adding 10 mL of concentrated nitric acid to it and then heated on a hotplate at 70°C (Silva *et al*., 2006;

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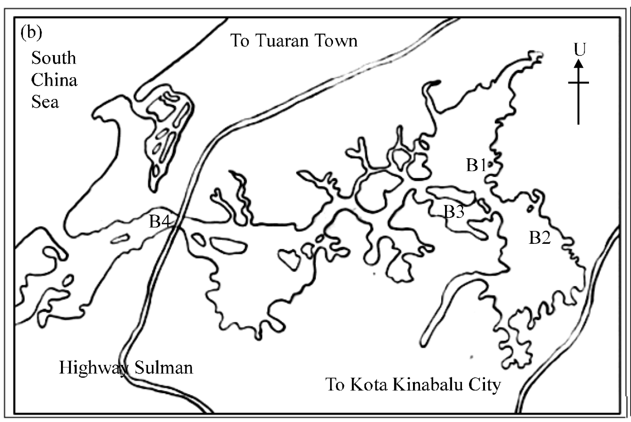

Locations	Coordinate
B1	06° 09.067' N 116° 11.907' E
B2	06° 08.527' N 116° 12.308' E
B3	06° 08.661' N 116° 11.590' E
B4	06° 08.257' N 116° 09.400' E

Fig. 1(a-b): Study sites at Mengkabong Lagoon in Tuaran district, Sabah (Scale 1 cm: 500 m)

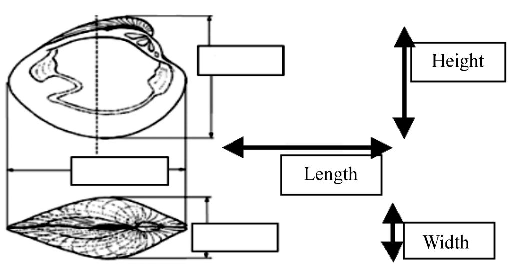

Fig. 2: Schematic size measurement of *P. viridis*

Yap *et al*., 2002; Blackmore, 2001). Distilled water is then added to it up to 50 mL. The heavy metals contents are then measured using FAAS after filtering it with a 45 μm sized Withman membrane. The SI unit for the concentration of heavy metals in the tissues is presented in mg kg^{-1} (dry weight). The accuracy and precision of the procedures are compared to a standard reference material, Lobster Hepatopancreas (TORT-2) which is provided by the National Research Council of Canada. The recovery ranges from 89 to 108%, as shown in Table 1.

Table 1: Concentration of heavy metals in a standard reference material, lobster hepatopancreas (TORT-2)

Value	N	Pb	Cu	Cr	Cd	Zn
Certified	-	0.35	106.00	0.77	26.70	180.00
SE		0.13	10.00	0.15	0.60	6.00
Observed	5	0.33	114.00	0.69	26.30	187.00
SE		0.06	10.10	0.02	2.10	3.80
Recovery (%)		94.60	107.70	89.00	98.30	104.30

All data as Mean±SE in mg kg^{-1} dry weight (n = 5)

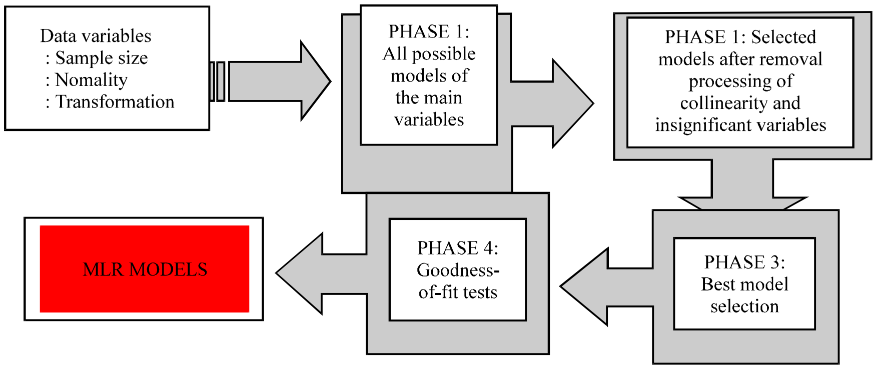

Fig. 3: Procedures in mathematical modeling phases using Multiple Linear Regressions (MLR)

Normality, multicollinearity and model-building: The variables are initially tested for their normality distributions and are based on the Kolmogorov-Smirnov statistics with Lilliefors significance level of more than 0.05, since the sample size, n = 120 is large (>50). For non-normal data, appropriate transformations has to be done first, followed by minimizing collinearity between the variables (if there exist), based on the Pearson Correlation Coefficient matrix. Next, follows the model-building procedures which involve the following phases, as shown in Fig. 3, before finally the selection of the model equations representing the relationships between the heavy metals concentration with the respective physical properties.

RESULTS

Appropriate transformations have been carried out to normalize the data variables. Table 2 shows the definitions of the newly transformed variables and its units used. New variables are assigned to it for model simplicity.

Table 3 depicts the normality tests of the variables before and after transformation, showing that most of the variables have turned to normal after transformation. However, only three of the variables are not normal, although they have undergone their best transformations. Their significant p-values do not exceed 0.05 to indicate normality, but their normality plots exhibit nearly normal distributions.

Table 4 depicts the Pearson Correlation Coefficient matrix of the transformed variables model say M31. Any existence of multicollinearity between the variables has to be remedied first before further analysis can be done. It can be seen that there are no absolute coefficient values of $|r|\geq 0.95$ exist in the model. Therefore, no multicollinearity effects exist in this model function.

The number of the all possible models would be given by:

$$\sum_{j=1}^{q=5} j({}^{q}C_{j})$$

where, 'q' would be the number of single independent variables. Table 5 depicts some selected models from the thirty-one (31) possible models for Lead (Pb) for the five single independent variables. The all possible models for Lead are depicted in the Appendix. Simultaneously, similar models are also applied to the other heavy metals concentrations. Hence, overall there are 155 models to be selected from in relating the HMA as the dependent and the physical growth factors as the independent variables. However, in this work the models with interactions between the independent variables are not carried out.

Table 6 below depicts the Pearson Correlation Coefficient matrix for model M31 of Pb. No multicollinearity exists, so the procedures of removing insignificant variables will then be carried out using backward elimination method of the Coefficient test. The number of eliminated insignificant variables will then follow after the parent model as shown by Abdullah *et al.* (2008).

Table 7 tabulates the results of the best mathematical models which represent the relationships of the heavy metals accumulation versus the physical properties of *P. viridis.* Comparisons of the best models after elimination amongst the heavy metals would show that Pb has a negative relationship with the wet weight, while Cu and Cd have negative relationships with the dry weight properties. In addition, Zn has positive relationships with two of the physical properties, i.e., the width and dry weight, while Cr has a good relationship with three of them, namely, the width, height and dry weight properties. Further comparisons based on the least sum of square

Table 2: Transformations of Defined Variables (heavy metals concentration and physical properties)

Variables	Pb	Cu	Cr	Cd	Zn	Length	Width	Height	Wet Wt.	Dry wt.
Transformed	$\sqrt[3]{Pb}$	$\frac{(Cu^{0.39}-1)}{0.39}$	$\sqrt[3]{Cr}$	Ln(Cd)	$\frac{(Zn^{0.225}-1)}{0.225}$	Length	$\frac{1}{Wide}$	Height	Wet weight	$(\sqrt[3]{DW})^2$
New variables	Y1	Y2	Y3	Y4	Y5	X1	X2	X3	X4	X5
Unit	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mm	mm	mm	g	g

Table 3: Normality tests before and after transformations of heavy metals concentrations and physical properties

Before transformation					After transformation				
Variable	Statistic	df	Sig.		Variable	Statistic	df	Sig.	
Pb	0.133	120	0.000	Not normal	Y1	0.076	120	0.089	Normal
Cu	0.119	120	0.000	Not normal	Y2	0.101	120	0.004	Not normal
Cr	0.220	120	0.000	Not normal	Y3	0.093	120	0.013	Not normal
Cd	0.177	120	0.000	Not normal	Y4	0.064	120	0.200*	Normal
Zn	0.154	120	0.000	Not normal	Y5	0.124	120	0.000	Not normal
Length	0.077	120	0.081	Normal	X1	0.077	120	0.081	Normal
Wide	0.095	120	0.010	Not normal	X2	0.073	120	0.171	Normal
Height	0.074	120	0.099	Normal	X3	0.074	120	0.099	Normal
Wet weight	0.055	120	0.200*	Normal	X4	0.055	120	0.200*	Normal
Dry weight	0.086	120	0.029	Not normal	X5	0.080	120	0.056	Normal

Table 4: Pearson correlation coefficient matrix of transformed variables (heavy metals concentration and physical properties of *P. viridis*)

Variable	Y1	Y2	Y3	Y4	Y5	X1	X2	X3	X4	X5
Y1	1	0.543	0.476	0.280	0.0234	-0.1470	0.229	-0.170	-0.235	-0.220
Y2		1	0.577	0.371	0.2840	-0.3110	0.379	-0.310	-0.343	-0.444
Y3			1	0.373	0.1410	-0.0138	0.238	-0.102	-0.247	-0.291
Y4				1	-0.075	-0.1010	0.171	-0.092	-0.206	-0.257
Y5					1	0.0830	-0.021	0.153	0.187	0.193
X1						1	-0.843	0.794	0.544	0.761
X2							1	-0.737	-0.548	-0.752
X3								1	0.564	0.759
X4									1	0.764
X5										1

Table 5: Some selected possible models for lead (Pb)

Models with single independent variables for pb	
M1	$Y_1 = \beta_0+\beta_1X_1+\mu$
M2	$Y_1 = \beta_0+\beta_2X_2+\mu$
:	: : : :
M6	$Y_1 = \beta_0+\beta_1X_1+\beta_2X_2+\mu$
M7	$Y_1 = \beta_0+\beta_1X_1+\beta_3X_3+\mu$
:	: : : :
M11	$Y_1 = \beta_0+\beta_2X_2+\beta_4X_4+\mu$
M12	$Y_1 = \beta_0+\beta_2X_2+\beta_5X_5+\mu$
:	: : : : :
M16	$Y_1 = \beta_0+\beta_1X_1+\beta_2X_2+\beta_3X_3+\mu$
M17	$Y_1 = \beta_0+\beta_1X_1+\beta_2X_2+\beta_4X_4+\mu$
:	: : : : : :
M29	$Y_1 = \beta_0+\beta_1X_1+\beta_3X_3+\beta_4X_4+\beta_5X_5+\mu$
M30	$Y_1 = \beta_0+\beta_2X_2+\beta_3X_3+\beta_4X_4+\beta_5X_5+\mu$
M31	$Y_1 = \beta_0+\beta_1X_1+\beta_2X_2+\beta_3X_3+\beta_4X_4+\beta_5X_5+\mu$

Table 6: Coefficient matrix of model M31 for Pb

	Y1	X1	X2	X3	X4	X5
Y1	1	-0.170	0.229	-0.235	0.220	0.543
X1		1	-0.737	0.564	0.759	-0.310
X2			1	-0.548	-0.752	0.379
X3				1	0.764	-0.343
X4					1	-0.444
X5						1

Errors (SSE), shows that Zn, is a better indicator amongst all the heavy metals, follow by Pb and Cr. The model equation is transformed back into its defined variables as indicated and is given by:

$$\frac{(Zn^{0.225}-1)}{0.225} = 0.706+\frac{22.783}{\text{Width}}+0.303\ (\text{dry weight})^{2/3}$$

In other words, four HMA dependent variables have shown a good relationship to the dry weight physical independent properties, except for Pb which is negatively related to the wet weight physical properties. It can also be seen from the models that the length of *P. viridis*, has not become one of the determining indicator of the physical properties for HMA identification. Thus, it can be said that the HMA has been affected significantly by the physical growth properties, especially the width, height, wet and dry weight properties of *P. viridis*.

After deriving the mathematical model which can represent the best indicator for HMA, the goodness-of-fit tests (i.e., the normality test and randomness test) are carried out on the standardized residuals so as to ascertain the validity of the derived model. The choice of the best mathematical model is based on the least sum of square error (SSE) where the assumptions of normality and linearity of the model residuals are verified. Figure 4 above indicates that these assumptions of the goodness-of-fit the best mathematical model represented by Zn have been met.

Table 7: Mathematical models equations for Y1-Pb, Y1-Cu. Y3-Cr, Y4-Cd, Y5-Zn

Primary model equation	Best model after elimination	SSE
M31:Y1 =-0.25+0.006 X1+37.851 X2-0.001X3-0.017 X4-0.034 X5	M31.5: Y1 = 1.286-0.025 X4	0.3414
M31:Y2 =-1.537+0.14 X1+93.586 X2+0.016 X3-0.002X4-1.056 X5	M31.5Y2 = 2.622-1.049 X5	0.9306
M31:Y3 =-0.764+0.009X1+37.67X2+0.032X3 = -0.012X4-0.459X5	M31.3: Y3 =-0.205+29.225 X2+0.47 X3-0.470X5	0.5188
M31:Y4 =-3.110+0.015 X1+35.641X+0.040X3-0.005X4-0.885X5	M31.5: Y4 =-0.230-0.555 X5	0.9181
M31: Y5 = 0.583-0.002X1+22.783X2+0.013X3+0.004X4-0.242X5	M31.4: Y5 = 0.706+22.5617 X2+0.303 X5	
	$\frac{(Zn^{0.225}-1)}{0.225} = 0.706 + \frac{22783}{Width} + 0.303\ (dry\ weight)^{2/3}$	0.2586

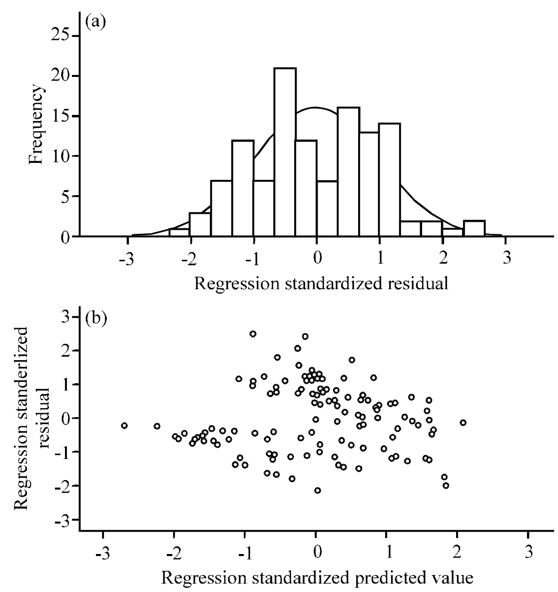

Fig. 4(a-b): Residuals histogram plot and residuals scatter plot for Zn (a) Histogram and (b) Scatter plot, Dependent variable: z-0.22

DISCUSSION

The experimental results show that *P. viridis* has accumulated the Heavy Metals Concentration (HMA) in the following order: Zn>Cu>Cr>Pb>Cd. As a result, high accumulations of Zn and Cu have indicated the high potentiality of these elements being accumulated in the tissue bodies of *P. viridis*. According to Dobrowolski and Skowronska (2001), mollusc was found to be a very effective organism to accumulate heavy metals compared to fish. In addition, Blackmore (2001) had also reported that high accumulation of Zn and Cu were also found in *Saccostrea cucullata*.

The mathematical models of the respective heavy metals (Pb, Cu, Cr, Cd, Zn) in Table 7, show that different heavy metal would affect differently the physical properties. After the procedures of all the phases in model-building have been carried out, the best model equation shows that Zn is the best indicator based on its least SSE value, compared to the other metals. The model also indicates that Zn has a directly positive relationship with the variable, dry weight and inversely proportional to the width of *P. viridis*. The best model with the highest SSE from Table 7, represented by Cu, however, has also shown that dry weight too is a determining factor. These results are also in accordance with Boyden (1974) and Otchere (2003), where dry weight and sizes of each individual mollusc, are important components when measuring the concentration of heavy metals accumulated in its body.

CONCLUSION

P. viridis has accumulated high concentration of Zn and Cu but it is still in the permissible safety levels for human consumption, especially as a seafood resource (100 and 30 mg kg^{-1}). There are significant relationships found between the Heavy Metals Concentrations (HMA) and the physical properties. These relationships can be represented using Multiple Linear Regressions (MLR) models. The models thus relate that the HMA has affected significantly the physical growth properties, especially by the width, height, wet and dry weight properties of *P. viridis*. Further works can hence be done by looking at the interactions between the independent variables. Comparisons of the best model equations based on the least SSE, indicate that Zn is the best indicator and is given by the model equation:

$$\frac{(Zn^{0.225}-1)}{0.225} = 0.706 + \frac{22.783}{Width} + 0.303\ (dry\ weight)^{2/3}$$

ACKNOWLEDGMENT

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Appendix: All possible models with single independent variables

Models with Single Independent Variables for Pb	
M1	$Y_1 = \beta_0+\beta_1X1+\mu$
M2	$Y_1 = \beta_0+\beta_2X2+\mu$
M3	$Y_1 = \beta_0+\beta_3X3+\mu$
M4	$Y_1 = \beta_0+\beta_4X4+\mu$
M5	$Y_1 = \beta_0+\beta_5X5+\mu$
M6	$Y_1 = \beta_0+\beta_1X1+\beta_2X2+\mu$
M7	$Y_1 = \beta_0+\beta_1X1+\beta_3X3+\mu$
M8	$Y_1 = \beta_0+\beta_1X1+\beta_4X4+\mu$
M9	$Y_1 = \beta_0+\beta_1X1+\beta_5X5+\mu$
M10	$Y_1 = \beta_0+\beta_2X2+\beta_3X3+\mu$
M11	$Y_1 = \beta_0+\beta_2X2+\beta_4X4+\mu$
M12	$Y_1 = \beta_0+\beta_2X2+\beta_5X5+\mu$
M13	$Y_1 = \beta_0+\beta_3X3+\beta_4X4+\mu$
M14	$Y_1 = \beta_0+\beta_3X3+\beta_5X5+\mu$
M15	$Y_1 = \beta_0+\beta_4X4+\beta_5X5+\mu$
M16	$Y_1 = \beta_0+\beta_1X1+\beta_2X2+\beta_3X3+\mu$
M17	$Y_1 = \beta_0+\beta_1X1+\beta_2X2+\beta_4X4+\mu$
M18	$Y_1 = \beta_0+\beta_1X1+\beta_2X2+\beta_5X5+\mu$
M19	$Y_1 = \beta_0+\beta_1X1+\beta_3X3+\beta_4X4+\mu$
M20	$Y_1 = \beta_0+\beta_1X1+\beta_3X3+\beta_5X5+\mu$
M21	$Y_1 = \beta_0+\beta_1X1+\beta_4X4+\beta_5X5+\mu$
M22	$Y_1 = \beta_0+\beta_2X2+\beta_3X3+\beta_4X4+\mu$
M23	$Y_1 = \beta_0+\beta_2X2+\beta_3X3+\beta_5X5+\mu$
M24	$Y_1 = \beta_0+\beta_2X2+\beta_4X4+\beta_5X5+\mu$
M25	$Y_1 = \beta_0+\beta_3X3+\beta_4X4+\beta_5X5+\mu$
M26	$Y_1 = \beta_0+\beta_1X1+\beta_2X2+\beta_3X3+\beta_4X4+\mu$
M27	$Y_1 = \beta_0+\beta_1X1+\beta_2X2+\beta_3X3+\beta_5X5+\mu$
M28	$Y_1 = \beta_0+\beta_1X1+\beta_2X2+\beta_4X4+\beta_5X5+\mu$
M29	$Y_1 = \beta_0+\beta_1X1+\beta_3X3+\beta_4X4+\beta_5X5+\mu$
M30	$Y_1 = \beta_0+\beta_2X2+\beta_3X3+\beta_4X4+\beta_5X5+\mu$
M31	$Y_1 = \beta_0+\beta_1X1+\beta_2X2+\beta_3X3+\beta_4X4+\beta_5X5+\mu$

REFERENCES

Abdullah, N., H.J. Zainodin and J.B.N. Jonney, 2008. Multiple regression models of the volumetric stem biomass. WSEAS Trans. Math., 7: 492-502.

Blackmore, G., 2001. Interspecific variation in heavy metal body concentrations in Hong Kong marine invertebrates. Environ. Pollut., 114: 303-311.

Boyden, C.R., 1974. Trace element content and body size in molluscs. Nature, 251: 311-314.

Dobrowolski, R. and M. Skowronska, 2001. Distribution and environmental mobility of selected trace metals in the Zemborzyce reservoir. Pol. J. Environ. Stud., 10: 383-388.

Nair, D., C.L. Angell and R. Hall, 1993. Small-Scale Oyster Culture on the West Coast of Peninsular Malaysia. Nagaraj and Co., Madras, India, Pages: 32.

Otchere, F.A., 2003. Heavy metals concentrations and burden in the bivalves (*Anadara* (*Senilia*) *senilis*, *Crassostrea tulipa* and *Perna perna*) from lagoons in Ghana: Model to describe mechanism of accumulation/excretion. Afr. J. Biotechnol., 2: 280-287.

Silva, C.A.R., B.D. Smith and P.S. Rainbow, 2006. Comparative biomonitors of coastal trace metal contamination in tropical South America (N. Brazil). Mar. Environ. Res., 61: 439-455.

Sivalingam, P.M., 1977. Aquaculture of the green mussel, *Mytilus viridis* Linnaeus, in Malaysia. Aquaculture, 11: 297-312.

Widmeyer, J.R. and L.I. Bendell-Young, 2007. Influence of food quality and salinity on dietary cadmium availability in *Mytilus trossulus*. Aquat. Toxicol., 81: 144-151.

Yap, C.K., A. Ismail, S.G. Tan and H. Omar, 2002. Correlations between speciation of Cd Cu Pb and Zn in sediment and their concentrations in total soft tissue of green lipped mussel *Perna viridis* from the west coast of peninsular Malaysia. Environ. Int., 28: 117-126.