The use of bivalves as bio-indicators in the assessment of marine pollution along a coastal area

A. M. Yusof,¹* N. F. Yanta,¹ A. K. H. Wood²

¹ Department of Chemistry, Universiti Teknologi Malaysia, 81310 UTM Skudai, Malaysia ² Malaysia Institute of Nuclear Technology Research, 43000 Bangi, Malaysia

(Received July 21, 2003)

The assessment of environmental pollution of the coastal areas of the Malaysian Peninsula was done by analyzing the contents of the heavy and trace elements in the bivalves blood clams (*Anadara granosa*) and green mussels (*Perna viridis*) and sediments at twenty-two sampling stations to look for prevailing trends. Heavy and trace elements analyzed in this study were As, Cd, Cr, Cu, Pb, Se and Zn. Two techniques, namely the neutron activation analysis (NAA) and atomic absorption spectrophotometry (AAS) were used in the quantitative determination of the heavy metals while Marine Sediment Reference Material (BCSS) and Lobster Hepatopancreas (TORT-1) provided the certified reference materials in the quality assurance control. The potential use of these bivalves as suitable bio-indicators was evaluated from correlation tests based on the concentrations of heavy and trace elements in the sediment-metals system to those in the bivalves.

Introduction

Trace elements from seawater and marine sediments are known to be accumulated by many species of marine invertebrates such as oysters, mussels, clams and shells. Their usefulness as bio-indicator organisms provide, ideally, an estimate of trace elements availabilities to the biomass of different areas and localities. They should be able to accumulate the pollutant in a sedentary manner without being killed.¹ There are three possible routes by which metals can be derived namely, (1) from solution, (2) from the ingestion of food and (3) from the ingestion of particulate matter containing metals.² One of the major requirements is that all species to be used as bioindicators should exhibit the same correlation in their elemental contents with those in the surrounding marine environment namely water, at all locations in the study area under all conditions. The body burdens of trace metals in most bivalves have been used to identify and map areas with exceedingly high levels of trace metals and organic pollutants, hence they can be used as biomonitors for aquatic environment.³⁻⁵ Concentrations of the various trace metals can differ in the type of tissue analyzed and so it is with variations in body size or growth rates which can influence the quantity of metals in the tissues.⁶

This study attempts to look at the distribution and dispersion of some heavy and trace elements namely As, Cd, Cr, Cu, Pb, Se and Zn along the coastal area of varied pollution levels using the bivalves blood clam (*Anadara granosa*) and green mussel (*Perna viridis*) as the bio-indicators since they accumulate heavy and trace elements from water, sediments and/or from food. Bivalves (such as oysters) and mussels especially (such as green mussels) have been used widely as sentinel

organisms for monitoring the concentration of marine pollutants.⁷ Heavy metals, organochlorine compounds and petroleum hydrocarbons have long been recognized as the most deleterious pollutants to biota in the world's marine, coastal and estuarine waters.⁸ However, some alarm has been expressed concerning possible health related problems associated with seafood consumption due to the presence of certain heavy metals in quantities exceeding those of the maximum permissible limit (MPL) allowed under the standards,⁹ for example the presence of copper residues in ovsters.¹⁰ The level of contamination in these organisms provides a timeintegrated measure of elements bioavailability,¹¹ responding essentially to that fraction of the total environmental load which is relevant directly to the ecotoxicological nature of the contaminants.¹²

Experimental

Study area

The coastal area of the Peninsula of Malaysia was chosen as the study area since it was thought that varying levels of marine pollution were taking place due to different non-point source pollutants originating from various types of land use activities namely land development, both chemical and petrochemical industries and wastewater from domestic sources which eventually find their way into the coastal and estuarine environment. A total of 22 sampling sites were earmarked based on the availability of both localized species of blood clams and green mussels. Sampling was done twice over a period of one year and the average readings taken for analysis.

Samples

The two marine species, blood clams and green mussels were collected and hand-picked to ensure that for each site a total of 30 animals of size between 30–35 mm for green mussels and between 8–10 mm for blood clams were used as worthy samples. Sediment samples were collected about 500 m from the shoreline at the same site where the animal samples were gathered, using a grab as well as a hand sediment sampler model US DH-48. The sediment samples were placed into polyethylene bags, sealed and taken to the laboratory for further analysis.¹³

Chemicals

All reagents were of analytical grade (Analar) and were prepared by diluting Analar acids (HNO₃) obtained form Fluka with quartz-distilled water (Q-H₂O) to the appropriate molarity or percentage. Certified reference materials *Lobster Hepatopancreas* (TORT-1) and Marine Sediment (BCSS-1) supplied by the National Research Council of Canada (NRCC) were used in this study for quality assurance controls. All containers were washed by soaking them for at least 24 hours in a 10% HNO₃ solution after washing them with Triton-X100 detergent solution. This was followed by thorough rinsing with DDW before storing them in fume cupboards providing a Class-100 working environment.

Sample preparation

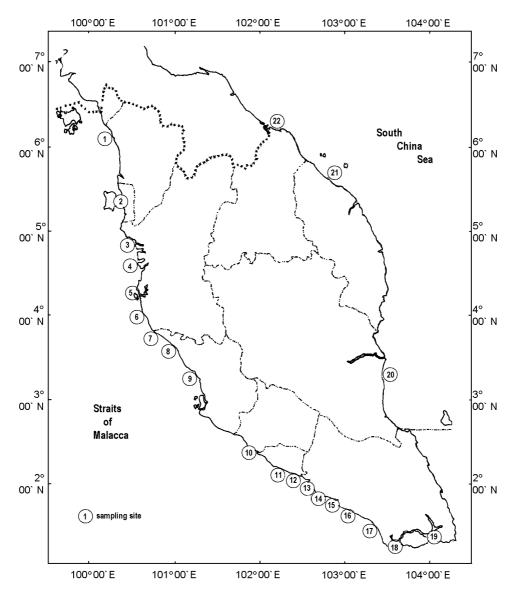
The outer shells of the animals were carefully cleaned, the shells opened with a stainless steel knife and the cavity fluid of the animals drained. The remaining shell contents were freeze-dried using an Edward Mudalyo freeze-dryer and later ground to <200 mesh with a Herzog HSM-100 grinder. Sediment samples were freeze-dried in the original polyethylene containers after subjecting to dialysis with a semipermeable membrane to get rid of the excess seawater and ground to about <200 mesh with a Herzog HSM-100 grinder. About 1 g of samples were carefully weighed and transferred to a Teflon[®] PFA decomposition vessel of a CEM Model MDS-81D microwave digester followed by the addition of between 5-10 ml of 70% HNO3. About 2-2.5 minutes were sufficient for most samples to be completely digested. Details of the heating conditions required for different sample types are described elsewhere.^{13–15} The elements Cd, Cu, Pb and Zn were analyzed spectrophotometrically with a GBC Avanta System 3000 (GF 3000) with a PAL 3000 Autosampler.

Irradiations and counting

The sediments were oven-dried for at least 2 weeks at 60 °C or until a constant dry weight was observed followed by grinding using a grinder mill (Herzog) to get the homogenous powdered form of approximately <200 mesh size (±75 µm). The elemental concentrations of As, Se and Cr were determined by instrumental neutron activation analysis (INAA). Sample weight each of about 0.1 g was placed in small polyethylene vials (i.d. 1 cm×3 cm) and heat-sealed for the irradiation process. Analytical accuracy was obtained by using certified reference materials Lobster Hepatopancreas (TORT-1) and Marine Sediment (BCSS-1) supplied by the National Research Council of Canada (NRCC). They were irradiated together with the samples in the 1 MW Triga Mark II reactor at the Malaysian Institute for Nuclear Technology Research (MINT) in a thermal flux of $5 \cdot 10^{12} \text{ n} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$ with irradiation of up to 6 hours followed by varying cooling times of between 1 to 2 days for ⁷⁶As, and 3 weeks for ⁷⁵Se and ⁵¹Cr followed by a counting time of 3600 seconds to ensure good statistics. The induced activity was measured by gammaray spectrometry using a large volume, coaxial hyperpure germanium detector (HPGe) from Ortec Model GC-1520 (Canberra, Australia) with a resolution of 2.0 keV FWHM for the 1332 keV gamma of ⁶⁰Co with an efficiency of 10% relative to NaI.

Results and discussion

The study area as shown in Fig. 1, covers the entire coastal line of the Malaysian Peninsula and the 22 sites chosen represented areas of varying anthropogenic activities where either one or both species can be found. Collection of samples was done within the pH range of 6–8. Animals of almost similar sizes were chosen to represent species of similar age and exposure to the environment. Certified and obtained values in the *Lobster Hepatopancreas* TORT-1 and Marine Sediment BCSS-1 SRMs used in this study are shown in Table 1. Most of the values obtained for elemental recovery using the same procedures used in the sample preparations exceeded 85% and some showed recovery of more than 95%. This is considered quite substantial, thus enabling this procedure to be used throughout the study.





	Lobster Hep	patopancreas	Marine	Sediment
Element	Element certified, ^a $\mu g \cdot g^{-1}$	Element found, ^b µg ⁻¹	Element certified, ^a $\mu g \cdot g^{-1}$	Element found, ^b µg·g ⁻¹
As	24.6 ± 2.2	23.7 ± 1.9	11.1 ± 0.06	$10.5~\pm~0.06$
Cd	26.3 ± 2.1	25.8 ± 2.1	0.25 ± 0.04	0.22 ± 0.04
Cr	2.4 ± 0.6	2.1 ± 0.5	123 ± 14	119 ± 13
Co	0.42 ± 0.05	$0.40~\pm~0.04$	11.4 ± 2.1	10.8 ± 2.5
Cu	439 ± 22	$428~\pm~19$	18.5 ± 2.7	18.2 ± 2.5
Pb	10.4 ± 2.0	9.8 ± 1.7	$22.7~\pm~3.4$	$22.0~\pm~3.6$
Zn	177 ± 10	168 ± 11	119 ± 12	111 ± 11

Table 1. Elemental determinations in Lobster Hepatopancreas TORT-1 and Marine Sediment BCSS-1 SRMs

^a Mean and 95% tolerance limits.

^b Mean and standard deviation for three determinations.

The dissolution of marine and sediment samples in this study was done by microwave heating with programmed heating and this procedure was proven to be suitable and useful in reducing materials loss during heating.^{14–16} The reproducibility of this technique in some environmental studies was reported by YUSOF et al.^{13,14} Reactions during the digestion step between oxidizing acids and the samples will also generate heat especially when exothermic reactions were involved and this contributes to the loss of some of the volatiles such as metalloids. Most samples exhibited percentage loss of volatiles between 2.9–5.5%, depending on the sample matrix and duration of digestion.

The elemental concentrations in the two species, blood clams and green mussels used in this study from various sites of the Malaysian Peninsula (Fig. 1) are reported in Tables 2 and 3, respectively, while corresponding sediment data pertaining to the 22 sites chosen are given in Table 4. The reported values are averaged for triplicate subsamples of blood clams, green mussels and sediment and the precision was found to be more than 90%.

Table 2 shows the data for heavy and trace elements concentrations determined in blood clams. The results indicated that the elemental distribution in this animal were both element and site specific with some indications of preference in the uptake. The relative uptake for As is within 4–7 $\mu g \cdot g^{-1}$ for most samples with the exception of sites 14 (14.8 \pm 1.3 µg·g⁻¹) and 16 $(9.0\pm0.8 \ \mu g \cdot g^{-1})$ for the higher end and as low as that found at site 21 ($2\pm 0.2 \ \mu g \cdot g^{-1}$). Most of the samples taken were within the vicinity of agricultural areas mostly large oil palm plantations heavy in pesticides and herbicides use. Because of the high concentration of industrial areas on the west coast of the Malaysian Peninsula compared to the east coast, the Cd determined is relatively higher in samples taken at sites on the west coast. This phenomenon is similar for Cr, and Pb. There is no direct trend for the distribution of Se since the average concentrations determined were in the range of $1.6\pm0.2 \ \mu g \cdot g^{-1}$ to $3.7\pm0.3 \ \mu g \cdot g^{-1}$. Blood clams and green mussels have always indicated a higher tolerance for Zn compared to other heavy metals and this is reflected in the data given in Table 2. Relatively higher Zn concentrations were reported in blood clams collected at site 2, 7, 8, 9, 12 and 20 and they are site specific in the sense that incidentally these sites are all in the vicinity of ports busy with navigational activities and cargo handling particularly petroleum and petroleum products.

For all the samples analyzed for As, as shown in Table 3, the green mussels in general have shown a selectivity in the uptake of this element compared to blood clams. The uptake of As by green mussels in most cases exceeded those recorded for blood clams. The same relatively high distribution pattern is also indicated in Cu.

Concentrations distribution of various heavy and trace elements in sediments, as shown in Table 4, taken along the coastal line, showed varying patterns in accordance with site specific relationship. The relatively high As contents in sediment samples is attributed to the agricultural runoff from large plantations using pesticides and herbicides in their operation and this is evident at sites 20, 21, and 22. Sites exhibiting high As concentrations on the west coast could be the result of either agricultural runoffs or industrial discharge into the waterways which will eventually find its way into the marine ecosystem including sediment. Almost similar concentrations of Cr, Cu, Pb and Zn found in the average shale^{17,18} has been recorded while Se showed a rather low accumulation in the sediment. Incidentally, both blood clams and green mussels accumulate higher Se contents in their tissue compared to the amount determined in the sediment samples due to the fact that Se is an essential element for living organisms including blood clams and green mussels. It is obvious that both these species are element specific in nature with a high preference for Se uptake. Thus these species are not suitable candidates as bio-indicators for Se. It was reported that in fresh water the usual range of inorganic selenium is between $0.02-1.0 \text{ ng} \cdot \text{ml}^{-1}$ and in sea water $0.004 \text{ ng} \cdot \text{ml}^{-1}$ at the surface and $0.06 \text{ ng} \cdot \text{ml}^{-1}$ in the deep ocean, mostly in the selenite form. Groundwater in contact with different geological environments may reach much higher concentrations of inorganic selenium, sometimes as high as 6000 ng·ml⁻¹.¹⁹ However, Cd which is not an essential element in most living organisms, has shown a proportional relationship in its concentration in both the species and in the sediment samples. Almost 80% of the sediment samples analyzed for Cd showed concentrations of more than $0.5 \,\mu g \cdot g^{-1}$. This is also true for blood clams samples (~87%). Unlike blood clams, green mussels do not seem to exhibit the same uptake characteristics, making it an unsuitable bio-indicator for Cd.

Data given in Tables 2 to 4 have been rearranged and the concentrations of individual element in the two localized species were plotted against those found in the sediment where the animals were collected. A positive correlation of relatively acceptable coefficient value between the animal and sediment samples for a specific element can be construed as the animal being a suitable bio-indicator.

Accordingly, in some instances the elemental contents in either blood clams and/or green mussels was found to be relatively high at the locations where the elemental contents in sediment samples was also high.

XXX			Ca	Ċ	Cu	Pb	Se	Zu
ХX	CRG-1	+1	+I	+1	2.01 ± 0.32	+1	2.24 ± 0.23	57.20 ± 2.51
Х	CRG-2	+1	+I	8.51 ± 0.97	3.27 ± 0.52	+I	1.68 ± 0.18	H
	CRG-3	+1	+I	6.24 ± 0.71	+I	+1	+1	+I
K	CRG-4	+1	+I	+1	+I	+1	+1	+I
Х	KRG-5	7.25 ± 0.62	2.14 ± 0.10	8.42 ± 0.84	2.42 ± 0.11	0.79 ± 0.04	2.01 ± 0.27	73.41 ± 0.60
K	CRG-6	+I	+I	+1	H	H	+1	+1
K	CRG-7	+1	+1	6.42 ± 0.73	+I	+1	+1	+I
Х	CRG-8	+1	+I	8.22 ± 0.94	+I	+I	+I	+I
K	CRG-9	+1	+1	+1	+1	+1	+1	+1
K	RG-12	+1	+1	+1	+1	+1	+1	+1
K	RG-14	+1	+I	+I	+I	+I	+I	+I
K	RG-16	+1	+1	+1	+I	+1	+1	+I
K	RG-20	+1	+1	+1	+I	+1	+1	+1
K	KRG-21	+1	+I	2.33 ± 0.26	+I	+I	3.73 ± 0.35	64.50 ± 0.51
Ki	RG-22	+1	0.18 ± 0.04	+1	1.84 ± 0.14	0.99 ± 0.02	2.96 ± 0.26	41.80 ± 3.09
			Table 3. Concentra	tion of heavy metals in g	Concentration of heavy metals in green mussles (<i>Perna viridis</i>) (in $\mu g \cdot g^{-1}$ dry weight)	iis) (in μg·g ⁻¹ dry weight)		
Site	Sample	As	Cd	ç	Cu	Рb	Se	Zn
[KPG-1	+	+	5.60 ± 0.20	3.50 ± 0.30	+	+	72.50 ± 1.70
10	KPG-10	+I	+1	+I	+1	+I	+I	+I
1	KPG-11	27.70 ± 2.50	0.10 ± 0.05	+I	4.78 ± 0.36		3.10 ± 0.31	77.70 ± 0.80
12 1	KPG-12	+I	+I	+I	+1	+I	+I	+I
13 1	KPG-13	H	+1	+1	+1	+1	H	+I
14	KPG-14	+I	+I	7.52 ± 1.90	+I	+I	+I	+I
15 1	KPG-15	+1	+1	+1	+1	+I	+I	68.52 ± 1.10
17 1	KPG-17	+1	+1	2.81 ± 0.19	+1	+1	+1	+1
18	KPG-18	+I	+	+	+	+	+	87.90 + 2.50
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2. Concentration of heavy metals in blood clams (Anadara granosa) (in $\mu g \cdot g^{-1}$ dry w

A. M. YUSOF et al.: THE USE OF BIVALVES AS BIO-INDICATORS IN THE ASSESSMENT OF MARINE POLLUTION

Site	Sample	As	Cd	Cr	Cu	Pb	Se	Zn
_	[-]	15.51 ± 2.51	0.93 ± 0.01	40.31 ± 1.60	14.22 ± 1.21	14.60 ± 1.70	0.69 ± 0.03	157.03 ± 8.01
5	L-2	21.31 ± 2.10	+1	H	26.30 ± 1.29	19.93 ± 3.10	1.20 ± 0.08	167.00 ± 16.0
ŝ	L-3	16.93 ± 2.29	+1	87.82 ± 5.10	22.61 ± 2.40	10.90 ± 0.31	1.84 ± 0.12	113.01 ± 2.30
4	L4	+I	2.56 ± 0.12	70.71 ± 1.18	26.33 ± 1.72	15.81 ± 1.10	0.84 ± 0.01	150.03 ± 6.04
5	L-5	+I	+1	112.00 ± 13.00	23.74 ± 2.88	12.49 ± 2.10	+1	+1
9	L-6	+I	+1	75.10 ± 2.21	12.40 ± 2.01	+	0.80 ± 0.02	+I
7	L-7	+I	0.87 ± 0.03	+I	26.80 ± 1.80	+I	+1	+1
~	L-8	+I	+	57.90 ± 1.60		H	1.80 ± 0.57	+1
6	6-1	+I	- ++ 6	+		+I	0.80 ± 0.06	+1
10	L-10	+I	+ -	+I	8.90 ± 0.31	+I	0.70 ± 0.09	+1
II	L-11	+I	1.98 ± 0.12		12.64 ± 1.30	13.52 ± 0.59	+I	+1
12	L-12	+I	+ 9	98.70 ± 2.20		+I	+I	+1
13	L-13	+I	- +			+I	+I	+1
4	L-14	+I	+ 0	53.60 ± 1.93	11.91 ± 2.59	+I	0.74 ± 0.05	+1
15	L-15	+I	- +	+1	33.48 ± 2.73	+I	+I	+I
16	L-16	+I	++	+1		+I	+I	+I
17	L-17	9.89 ± 1.22	3.03 ± 0.05	2.81 ± 0.22	29.40 ± 1.30	7.34 ± 0.50	0.41 ± 0.05	170.03 ± 6.92
18	L-18	+I	+1 &	5.77 ± 0.23	16.33 ± 1.01	+1	0.48 ± 0.02	+1
61	L-19	+I	+1 &	6.80 ± 0.25	37.40 ± 2.60	11.90 ± 0.74	0.60 ± 0.09	+1
20	L-20	+I	4	64.70 ± 2.60	15.82 ± 1.58	H	1.03 ± 0.16	118.99 ± 1.60
21	L-21	H	+ 0	17.20 ± 1.80	11.60 ± 2.62	7.80 ± 0.62	1.45 ± 0.05	85.03 ± 1.83
22	1-22	16.81 ± 0.86	0.93 + 0.09	62.93 ± 2.79	11.04 ± 0.57	9.04 ± 0.91	1.06 ± 0.10	89.33 ± 1.04

This would indicate a positive correlation between the concentrations of the element in the species and in the sediment (Figs 2 and 3). Positive correlation was observed for As, Cd, Cr, Pb, Se and Zn in blood clams as compared to green mussles and the order of good correlation is Pb>Cd≅Se>Zn>Cr>As. Preferentially, blood clams would be a potential candidate as a good bio-indicator for Pb, Cd and Se accumulation or pollution in coastal sediments though Se is an essential element. Green mussles on the other hand showed

relatively good correlation only for Cr in sediments as compared to blood clams while the rest of the elements in the sediments did not indicate significant correlation with those in green mussels. For some metals, with a poor correlative pattern, inverse behavior between the animals and sediment can be explained on the basis of exchange and readjustment of the metal ion concentrations in the cell membrane-sediment system.²⁰

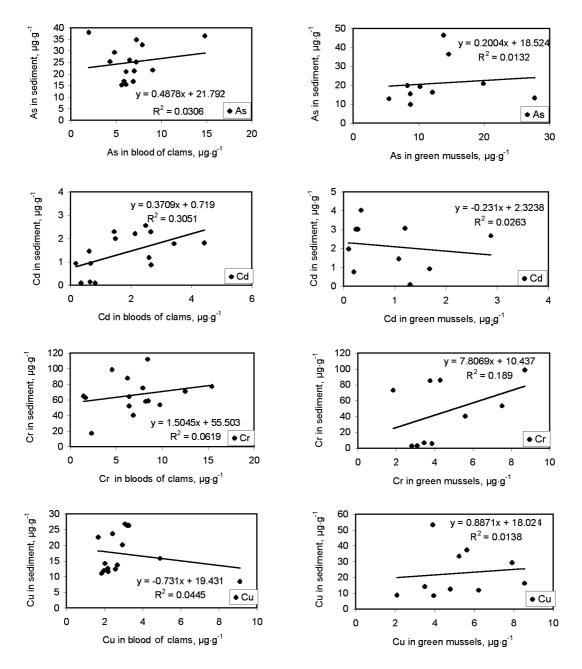


Fig. 2. Correlation plots of As, Cd, Cr and Cu concentrations against those in sediments

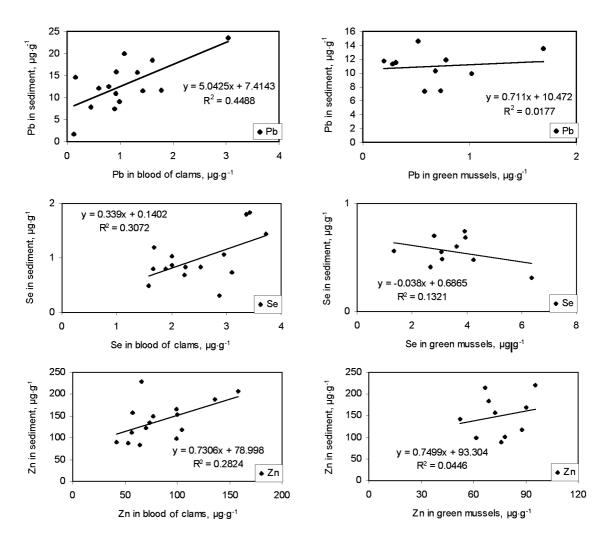


Fig. 3. Correlation plots of Pb, Se and Zn concentrations against those in sediments

Conclusions

Depending upon species-specificity towards the element uptake and specific regulatory mechanisms, the bivalves chosen from the sampling sites can exhibit varying amounts of elemental contents in their tissues. Most of the analysis is shown to be a valuable method for monitoring pollution in coastal sediments due to some heavy and trace elements. The feeding habits of the two species used in this study enabled some heavy and trace elements present in the sediment to be taken up by these animals, thus making them potential bio-indicators for these elements. The use of correlation coefficients for different elements present in sediments and in the animals proved to be useful in determining the suitability of one or both of these species as bio-indicators for specific elemental pollution. Comparatively, blood clams have demonstrated to be a good bio-indicator for Pb, Cd and possibly Se while green mussels may be used for Cr. The usefulness of these bivalves can be further explored by including some other parameters usually employed in controlled conditions such as temperature, pH and also the age of the animals. This would hopefully give a more accurate interpretation of the uptake pattern of the elements by these animals.

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One of us (A.M.Y.) would like to thank the National Science Development and Research Council (MPKSN) and Universiti Teknologi Malaysia for supporting the grant given under Contract No. IRPA 08-02-06-0032, the Malaysian Institute of Nuclear Technology and Research (MINT) for the irradiation facilities and all those involved directly or indirectly during the tenure of this work.

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