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Rare Earth Elements Behaviour at West Coast of Peninsular Malaysia Rocky Shore Ecosystem Using *Saccostrea cucullata* as Bioindicator

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This study focuses on the assessment of rare earth elements (REEs) in rocky shore ecosystem along west coast region of Peninsular Malaysia coastal waters, describing their partitioning in selected body part of *Saccostrea cucullata* and deliberating on interspatial variability. Samples were digested using Teflon Bomb technique and concentrations of 14 naturally occurring REEs were measured using ICP-MS technique, along with selected trace metals for additional data. The REEs fractionation patterns normalized to chondrite were remarkably similar, indicating a common source of the REEs. Consistent REEs abundance patterns were found in all samples, with enrichment of LREE over HREE, which implies that REEs are transported as a coherent group through west coast of Peninsular Malaysia aquatic ecosystems. Quantification of anomalies calculated using values normalized to chondrite data presented ratios higher than unity for Ce and ratios lower than unity for Eu in all sites. Filter feeder *S. cucullata* is considered to be highly potential as a bioindicator for REEs, undoubtedly due to its feeding behaviour that is much related to suspended particles as REEs sources. Comparative results showed that the values are considerably below the safety limit, with the exception of Ce and Nd in the soft tissue of *S. cucullata*.

Keywords: Rare earth elements, Soft tissue, Rocky shore, Chondrite, *Saccostrea cucullata*.

INTRODUCTION

Rare earth elements (REEs) are used in enormous magnitudes in the mechanized of industrial goods including lasers, computers, pigments for glass and plastics and additives, where they influence electric, magnetic, or optical features [1]. The demanding use of REEs in the technological industry results in noticeably higher amounts disposed into the surroundings [2]. Rare earth elements incline to be accumulated by biota and have contaminated consequences similar to those of Cd, Zn and Ni [1]. All aquatic invertebrates gather trace elements in their tissues, whether or not these elements are required for metabolism [3]. With respect to REEs, no established biological functions have been described and thus these elements are considered as non-essential in biota. Various element-accumulating bivalve species show a high abundance and play an important role as bioindicators for pollution in global monitoring programs throughout the world [4]. The objectives of this study are to explore the bioavailability and extent of exposure of REEs in soft tissue of *S. cucullata* from different rocky shore sites along coastal waters of the west coast of Peninsular Malaysia and to provide the baseline data for the west coast region.

EXPERIMENTAL

In general, Peninsular Malaysia can be divided into two parts; east coast area (from Kelantan state to East Johor state) and west coast area (from Perlis state to West Johor state). The west coast area is much more developed compared with the east coast area as it is adjacent to the busiest strait in the world, Malacca strait [5,6]. Six study sites have been selected in west coast area, on the basis of the occurrence of the *S. cucullata* and different site characteristics.

Initially, before sampling, site survey has been done to ensure the accessibility to the study sites, as to minimize the sampling time during next sampling session and the availability of the selected bioindicator. Table-1 displayed a list of sampling sites and their locations determined by coordinate along the west coast of Peninsular Malaysia coastal waters. These recorded locations were also useful for further exploration. All samples were taken during the same period, but from different sites to characterize the spatial variations in the samples. About 20-40 individuals of relatively same size (shell length of 4-5 cm) were hand collected during low tide period and placed in polyethylene bags with native water. They were endorsed to empty their guts for 24 h, following the method by Bustamante and Miramand [7], with little modification. All samples were assigned in plastic loads, sealed, categorized and saved at 4-6 °C during transportation to the laboratory where the samples were cleaned with running Mili-Q water (18.2 Ω) to eliminate sediment and salt particles prior to store frozen. After parameter measurement and tissue extraction, obtained soft tissues were oven dried at 60 °C for 3 days. Oven-dried samples were then minced to a powder and kept at room temperature until analysis.

TABLE-1
LIST OF SAMPLING SITES AND THEIR LOCATIONS

No.	Site	Latitude	Longitude
1	Pulau Sayak, Kedah	5.6548° N	100.3340° E
2	Batu Feringghi, Penang	5.4799° N	100.2616° E
3	Teluk Senangin, Perak	4.3111° N	100.5701° E
4	Teluk Batik, Perak	4.1853° N	100.6093° E
5	Tanjung Bidara, Malacca	2.2956° N	102.0787° E
6	Batu Pahat, Johor	1.7937° N	102.8876° E

Glassware used was treated with 10 % nitric acid solution in advance for contamination prevention. The digestion and analytical procedures were executed using the Teflon Bomb method by Kamaruzzaman *et al.* [8], with little modification. Analysis of REEs was carried out using ICP-MS Perkin Elmer Elan 9000 system using its standard configuration, following method by Kamaruzzaman *et al.* [9] with modification to enhance the accuracy. The HNO_3 (Merck, Suprapur) used for measurement was less than 2 % as to minimize the damage to the interference and to minimize isobaric molecular interferences. Aliquots were typically diluted 10 times for analysis with 2 % HNO_3, made using a combination of pure HNO_3 (Merck, Suprapur) and Milli-Q water. Results were quantified *via* an external calibration curve generated from the response obtained from serial dilutions of a multi-element calibration standard. The isotopes measured were ^{139}La, ^{140}Ce, ^{141}Pr, ^{142}Nd, ^{152}Sm, ^{153}Eu, ^{158}Gd, ^{159}Tb, ^{164}Dy, ^{165}Ho, ^{166}Er, ^{169}Tm, ^{174}Yb and ^{175}Lu. Results achieved were blank corrected and expressed as μg g^{-1} dry weight. Recovery procedure was executed using Standard reference material BCR 668 Mussel Tissue purchased from Institute for Reference Materials and Measurements (IRMM), Belgium.

One-way analysis of variance (ANOVA) was engaged for assessment of dissimilarity and noteworthy differences observed between REEs in different sites. All comparisons were made at least at the 95 % ($p < 0.05$) and 99 % ($p < 0.01$) level of significance. The analytical results and field data were compiled to form a multi-elemental database using Excel 2013 software (Microsoft, Washington, USA) and SPSS software (SPSS Inc., Chicago, USA). To omit the overwhelming Oddo-Harkins' effect in the REEs concentration pattern of samples, concentration data were divided by the average chondrite taken from Mcdonough and Sun [10] and shale concentrations taken from Taylor and McLennan [11]. The anomalies were defined as REE_n/REE_n^* where REE_n is the shale-normalized or chondrite-normalized REE concentration while REE is the calculated normalized concentration. Following the previous studies [12-14], the magnitude of the REEs anomaly were calculated based on the equation:

$$REE_n/REE_n^* = 2REE_n/([REE]_{n-1} + [REE]_{n-1}) \quad (1)$$

where n = 1, 2, 3… in the order of lanthanide series. Adopted from (1), the anomalies of Ce and Eu are calculated as:

$$Ce/Ce^* = 3[Ce]/([La] + 2[Nd]) \quad (2a)$$

$$Ce/Ce^* = 3(Ce_{sample}/Ce_N)/[(La_{sample}/La_N) + 2(Nd_{sample}/Nd_N)] \quad (2b)$$

$$Eu/Eu^* = [Eu]/([Sm] \times [Gd])^{1/2} \quad (3a)$$

$$Eu/Eu^* = (Eu_{sample}/Eu_N)/((Sm_{sample}/Sm_N) \times (Gd_{sample}/Gd_N))^{1/2} \quad (3b)$$

where Ce, La, Nd, Eu, Sm and Gd are a concentration in the sample while the subscript N refers to the chondrite concentration. Therefore, the positive anomaly is defined as the REE/REE* value greater than unity while negative anomaly is defined as the REE/REE* value less than unity. For the calculation of $(La/Yb)_N$, the equation was expressed as:

$$(La/Yb)_N = (La_{sample}/La_N)/(Yb_{sample}/Yb_N) \quad (4)$$

where N is the concentration of La and Yb in Post-Archean Australian Sedimentary rocks (PAAS). Post-Archean Australian Sedimentary rocks values was obtained from Taylor and McLennan [11]. For the calculation of total REEs, LREE and HREE, the calculation method were as follow:

$$\Sigma LREE = La + Ce + Pr + Sm \quad (5)$$

$$\Sigma HREE = Eu + Gd + Tb + Dy + Ho + Er + Tm + Yb + Lu \quad (6)$$

$$\Sigma REE = \Sigma LREE + \Sigma HREE \quad (7)$$

RESULTS AND DISCUSSION

Rare earth elements distribution in *S. cucullata*: The mean rare earth elements (REEs) concentrations with standard deviation in the soft tissue of *S. cucullata* are illustrated in Table-2. Result by using ANOVA indicated that there are significant differences between concentrations of all REEs among sampling sites ($p < 0.05$). By calculating the average of REEs for all sites, Ce was the most abundant element while Lu was the least abundant element.

Table-3 presented the pattern of REEs of the soft tissue of studied *S. cucullata* in decreasing pattern, dividing it into LREE and HREE distribution. The pattern was quite consistent through all sites. In general, the abundance of REEs concentrations in the soft tissue of *S. cucullata* follows the order of Ce > Nd > La > Sm > Pr for the LREE and Gd > Dy > Er > Yb > Ho > Tb > Eu > Tm > Lu for the HREE. The REEs accumulation patterns in this oyster species indicate enrichment of LREE compared to HREE.

Rare earth elements behaviour in *S. cucullata*: The total concentration of REEs (ΣREE), light REEs (ΣLREE), heavy REEs (ΣHREE) and its indicator ratios of ΣLREE/ΣHREE, La/Yb, La/Sm, Ce/La, Eu/Sm and Yb/Sm of the soft tissue of *S. cucullata* are presented in Table-4. LREE enrichment is over HREE as indicated by the higher mean values of ΣLREE compared to ΣHREE. The mean ratio of ΣLREE/ΣHREE between sites did not show significant changes from 3.20 to 5.85. Based

TABLE-2
MEAN CONCENTRATION (µg g^{-1} DRY WEIGHT) OF ΣREE IN SOFT TISSUE OF *S. cucullata*

	P. Sayak	Bt. Feringghi	Tlk. Senangin	Tlk. Batik	Tg. Bidara	Bt. Pahat
La	0.71 ± 0.39	0.30 ± 0.17	1.44 ± 0.61	0.79 ± 0.82	2.50 ± 0.55	5.40 ± 3.03
Ce	1.78 ± 0.82	0.69 ± 0.42	3.51 ± 1.31	2.52 ± 2.74	8.45 ± 2.81	13.24 ± 6.62
Pr	0.21 ± 0.11	0.08 ± 0.06	0.45 ± 0.16	0.31 ± 0.35	4.97 ± 2.26	1.56 ± 0.74
Nd	0.97 ± 0.46	0.42 ± 0.27	1.90 ± 0.64	1.39 ± 1.54	4.93 ± 1.93	6.22 ± 2.72
Sm	0.28 ± 0.15	0.12 ± 0.10	0.49 ± 0.17	0.43 ± 0.49	1.61 ± 0.63	1.54 ± 0.47
Eu	0.03 ± 0.03	0.01 ± 0.01	0.05 ± 0.03	0.06 ± 0.07	1.61 ± 0.71	0.27 ± 0.07
Gd	0.33 ± 0.16	0.14 ± 0.12	0.56 ± 0.19	0.49 ± 0.56	1.76 ± 0.62	1.81 ± 0.51
Tb	0.03 ± 0.02	0.01 ± 0.02	0.07 ± 0.03	0.08 ± 0.09	1.28 ± 0.51	0.23 ± 0.05
Dy	0.28 ± 0.13	0.14 ± 0.12	0.52 ± 0.19	0.49 ± 0.56	0.45 ± 0.08	1.33 ± 0.17
Ho	0.03 ± 0.02	0.01 ± 0.02	0.07 ± 0.03	0.08 ± 0.09	0.52 ± 0.20	0.20 ± 0.03
Er	0.12 ± 0.05	0.05 ± 0.05	0.23 ± 0.08	0.22 ± 0.24	0.63 ± 0.19	0.55 ± 0.09
Tm	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.02 ± 0.02	0.43 ± 0.17	0.04 ± 0.01
Yb	0.08 ± 0.03	0.03 ± 0.03	0.14 ± 0.04	0.12 ± 0.12	0.18 ± 0.01	0.35 ± 0.10
Lu	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.20 ± 0.08	0.00 ± 0.00

TABLE-3
DECREASING PATTERN OF RARE EARTH ELEMENTS OCCURRENCES (µg g^{-1} DRY WEIGHT) IN SOFT TISSUE OF *S. cucullata*

Site	Element pattern	
	Low rare earth elements	High rare earth elements
P. Sayak	Ce > Nd > La > Sm > Pr	Gd > Dy > Er > Yb > Ho > Eu > Tb > Tm > Lu
Blk. Pulau	Ce > Nd > La > Sm > Pr	Gd > Dy > Er > Yb > Ho > Tb > Eu > Tm > Lu
Bt. Feringghi	Ce > Nd > La > Sm > Pr	Gd > Dy > Er > Yb > Ho > Tb > Eu > Tm > Lu
Tlk. Senangin	Ce > Nd > La > Sm > Pr	Gd > Dy > Er > Yb > Ho > Tb > Eu > Tm > Lu
Tlk. Batik	Ce > Nd > La > Sm > Pr	Dy > Gd > Er > Yb > Ho > Tb > Eu > Tm > Lu
Bt. Pahat	Ce > Nd > La > Pr > Sm	Gd > Dy > Er > Yb > Eu > Tb > Ho > Tm > Lu

TABLE-4
MEAN CONCENTRATION OF ΣREE, ΣLREE, ΣHREE, LREE/HREE, Ce/La IN SOFT TISSUE OF *S. cucullata*

Site	ΣREE (µg g^{-1})	ΣLREE (µg g^{-1})	ΣHREE (µg g^{-1})	LREE/ HREE	Ce/La
P. Sayak	6.62	3.95	0.89	4.42	2.51
Blk. Pulau	2.96	1.61	0.39	4.16	2.32
Bt. Feringghi	12.32	7.35	1.68	4.37	2.69
Tlk. Senangin	9.86	5.43	1.57	3.47	3.17
Tlk. Batik	30.53	21.39	6.69	3.20	3.33
Bt. Pahat	38.60	27.96	4.78	5.85	2.45

on this ratio also displayed that LREE to be enriched compared with HREE in the samples. The correlation between LREE in the samples was measured by the determination of Ce/La, which indicated that both ratios were consistent in most of the studied sites.

Rare earth elements anomalies in *S. cucullata*: Chondrite has been used for normalization of REEs since the bulk composition of the globe is assumed to be close to chondrite meteorites that represent the primordial earth [15,16]. Referring to Fig. 1, Ce and Eu deviations in the chondrite-normalized REEs pattern are expected as these REEs are expected to behave differently from the other REEs due to changes in their oxidation states in a specific environment. They behave differently, in which Ce^{3+} under oxidizing conditions becomes insoluble Ce^{4+} while under reducing condition Eu^{3+} becomes Eu^{2+} [16-19].

Fig. 1 displays the chondrite-normalized pattern for REEs concentrations in soft tissue of *S. cucullata*. Results of the analysis presented patterns of LREE enrichment, gradual downward pattern and depletion through HREE concen-

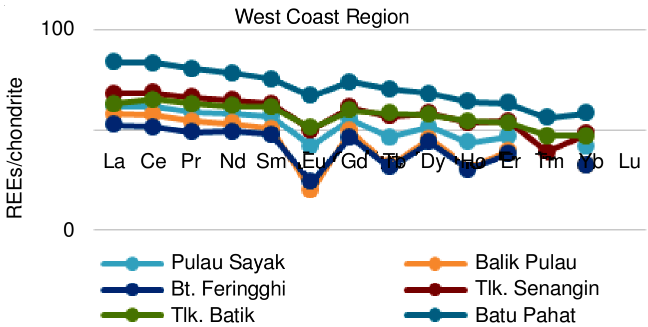

Fig. 1. Chondrite-normalized plots for the REEs in the soft tissue of *S. cucullata*

trations. All sites clearly show negative Eu anomaly. However for Ce, which was expected to deviate as a result of being redox element displaying a little and almost no anomaly.

Table-5 presenting indicator ratios of Ce/Ce*, Eu/Eu* and $(La/Yb)_N$ in the soft tissue of *S. cucullata*. Through the calculation of indicator ratio, results indicated that all sites were displaying positive Ce anomaly. Indicator of Eu/Eu* across sites displayed identical ratios with all sites presenting ratios less than unity, indicating the monopoly of negative Eu anomaly. $(La/Yb)_N$ ranging from between 4.38 to 10.89, displaying a large variation of LREE enrichment within sites. Balik Pulau revealing highest $(La/Yb)_N$ while Tlk. Batik site was the lowest. In general, the soft tissue of *S. cucullata* was characterized by Ce/Ce* higher than unity and Eu/Eu* less than unity.

Cerium and Eu in the chondrite-normalized REEs pattern in *S. cucullata* display a range of anomalies. There is tendency fora positive Ce anomaly and a negative Eu anomaly within

TABLE-5
INDICATOR RATIOS OF Ce/Ce*, Eu/Eu* AND (La/Yb)N IN SOFT TISSUE OF *S. cucullata*

Site	Ce/Ce*	Eu/Eu*	(La/Yb)N
P. Sayak	1.20	0.27	6.25
Blk. Pulau	1.24	0.06	10.89
Bt. Feringghi	1.09	0.12	6.59
Tlk. Senangin	1.25	0.32	6.22
Tlk. Batik	1.31	0.41	4.38
Bt. Pahat	1.30	0.49	10.56

soft tissue of *S. cucullata* are derived from particulate matter of sediment and might be related to the deviation in seawater. Weltje *et al*. [17] clarified that water sample possibly included suspended particulate particles < 0.45 µm, *i.e.*, colloids. It is evident that colloids in seawater are closely related with rock oyster feeding behaviour as experimented by Wisely and Reid [20]. Certainly, it was exposed by other reports that the colloidal material is preferentially enriched in Ce in river water and second most concentrated REE in the dissolved particulate matter in lake water [17]. Whereas in seawater, the certain process by oxide particles which take the insoluble Ce(IV) with them giving the results of positive Ce anomalies [16]. The chondrite-normalized pattern in this study strongly suggests that the REEs accumulated by *S. cucullata* was derived mostly from indigenous rocks. Granite is the dominant rock of the east coast of peninsular Malaysia and thus was the main natural source of REEs supplying the South China Sea [21].

Assessment with maximum permissible concentrations: A report on maximum permissible limits of REEs was reported by Sneller *et al*. [22], yet, it was limited to Y, La, Ce, Pr, Nd, Sm, Gd and Dy, with no limits set for the marine organism by regulation. Thus, comparisons with permissible limits are quantified by comparing with metal data as REEs concentrations seems to be lower compared with these metal value. Metal chosen includes Hg, Pb and Cd due to their lowest limits value compare to other metals. For a valid comparison with maximum permissible limits for food safety, the concentrations of species in this study have been converted into wet weight basis using conversion factor. Such procedure was executed due to most of the safety levels established were expressed on a wet weight basis.

Table-6 displayed maximum permissible limits on selected heavy metals (µg g^{-1}) for food safety set by different countries whereas Table-7 presented a comparative analysis of mean values of REEs concentration (µg g^{-1}) in wet and dry states of the soft tissue of *S. cucullata*. In comparison with the permissible limits set by the Malaysian Food Regulations 1985 Fourteen Schedule for Hg (0.5 µg g^{-1} wet weight), Pb (2.0 µg g^{-1} wet weight) and Cd (1.0 µg g^{-1} wet weight), the mean concentrations of REEs in *S. cucullata* on a wet weight basis across the series are lower than the limits, except for Ce and Nd. These elements presented values higher than Hg limits, however, do not exceed Pb permissible limit. With regard to dry weight basis, Ce concentration is also slightly higher than limit sets for Cd, but still lower than limit sets for Pb. Still, this is not a case for Nd. With the exception of Ce and Nd, the REEs levels across series are lesser than the endorsed guidelines set by by the Brazilian Ministry of Health, the Ministry of Public

TABLE-6
MAXIMUM PERMISSIBLE LIMITS ON SELECTED HEAVY METALS (µg g^{-1}) FOR FOOD SAFETY SET BY DIFFERENT COUNTRIES

Limit values	Weight basis	Hg	Pb	Cd	Ref.
Brazil	Dry		10	5	[23]
Thailand	Dry		6.67		[24]
European Community	Wet	0.5	1.5	1	[25]
Hong Kong	Wet		6	2	[26]
Australia	Wet		2	2	[27]
USA	Wet	1	1.7	3.7	[28]
Malaysia	Wet	0.5	2	1	[29]

TABLE-7
MEAN VALUES OF RARE EARTH ELEMENTS CONCENTRATION (µg g^{-1}) IN WET AND DRY STATES OF SOFT TISSUE OF *S. cucullata*

Element	Wet	Dry
La	0.35	1.88
Ce	1.02*	5.44
Pr	0.14	0.76
Nd	0.59*	3.14
Sm	0.13	0.71
Eu	0.02	0.15
Gd	0.14	0.74
Tb	0.02	0.12
Dy	0.08	0.46
Ho	0.01	0.08
Er	0.03	0.19
Tm	0.00	0.03
Yb	0.02	0.10
Lu	0.00	0.01

Health of Thailand, the Commission Europe, the Hong Kong Environmental Protection Department, the Food Standards Australia New Zealand Authority, the Australian Government and the Food and Drug Administration of the United States.

The results of the comparative analysis indicate that Ce and Nd concentrations in rock oyster *S. cucullata* are higher than the food security limits set. *S. cucullata* is one of important food sources consumed in Malaysia [30]. However, the consideration whether this oyster is unsafe for consumption is debatable as other elements across REEs series did not pose high levels. Though there is no report on occurrences of human poisoning over marine organisms food chain.

Potential anxieties concerning effects of constant exposure to low levels of REEs on human health have been ascending. It has been reported that REEs are significantly accumulated in blood, brain and bone after entering human body [31-34]. Regarding the effects of REEs to the human body, studies reported by Arvela *et al*. [35,36] and Zhu *et al*. [37] indicated that high exposure levels might be connected to health problems, for instance, liver function declining. It can be concluded that the consumption of studied organisms should not result in an excessive volume that might be damaging to human health, nevertheless, the long-time effects of REEs on human beings should be taken into account.

Conclusions

From the present study, the followings can be concluded:

• The bivalve mollusc, *S. cucullata* possessed high potential as cosmopolitan bioindicators for REEs due to its feeding behaviour that relates well to REEs sources. The samples taken from this study could be considered representative for background concentration in the Malaysian rocky shore environment

• Consistent chondrite-normalized patterns suggested that light REEs and heavy REEs fractionation in coastline marine environment produces more light REEs and less heavy REEs. Correspondingly, the enrichment of LREE in selected species is consistent throughout the rocky shore areas. Ce and Eu show consistent anomalies behaviour from the other REEs as a result of their redox chemistry. The REEs fractionation patterns normalized to chondrite and shale were remarkably similar indicating a common source of the REEs.

• The consumption of studied organisms should not result in an excessive volume that might be damaging to human health, nevertheless, the long-term effects of REEs on human beings should be taken into account, as species in this study are related with human consumption.

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