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## Trace Metals in *Thais clavigera* along Coastal Waters of the East Coast of Peninsular Malaysia

(Logam Surih dalam *Thais clavigera* di Sepanjang Perairan Pantai, Pantai Timur, Semenanjung Malaysia)

FUAD MOHD MISKON, NOOR AZHAR MOHD SHAZILI, FARIDAH MOHAMMAD & KAMARUZZAMAN YUNUS\*

### ABSTRACT

*The selected trace metals in the soft tissue of Thais clavigera from 11 sampling sites along the coastal waters of the east coast of Peninsular Malaysia were studied. Significant inter-spatial variations in trace metals were recorded. Sites with relatively high concentrations of the contaminant metals Hg, Cd, Pb and Zn are correlated to their close proximity to industrial and urban sites or to boating and aquaculture activities. This could possibly be contributed by the high growth of industrial activities like port and sewage release. Interspatial comparison with previous studies indicated lower measurement. Meanwhile, comparison with other studies around the world also designated lower values except for Zn. The metal accumulation patterns indicated an enrichment of essential metals over non-essential metals. Comparison of metal concentration with maximum permissible limits of toxic metals in food established in different countries, as well as Malaysian Food Act 1983 and Food Regulations 1985 Fourteen Schedule, indicated the values were well within safety levels.*

*Keywords: Coastal waters of the east coast of Peninsular Malaysia; food safety level; heavy metals; Thais clavigera*

### ABSTRAK

*Logam surih yang terpilih dalam tisu badan Thais clavigera di 11 lokasi persampelan di sepanjang pantai timur Semenanjung Malaysia telah dikaji. Perbezaan logam surih yang signifikan antara lokasi telah direkod. Lokasi yang secara relatifnya mempunyai kepekatan logam Hg, Cd, Pb dan Zn tercemar yang tinggi adalah berhubung kait dengan lokasi kawasan tersebut sama ada berdekatan dengan kawasan industri dan perbandaran atau aktiviti perkapalan dan akuakultur. Ini berkemungkinan disumbangkan oleh pertumbuhan tinggi dan aktiviti pengindustrian seperti pelabuhan dan pelepasan sisa. Perbandingan antara lokasi dengan kajian pada masa lepas menunjukkan pengiraan yang rendah. Sementara itu, perbandingan dengan kajian lain di seluruh dunia juga menunjukkan nilai yang rendah kecuali Zn. Corak pengumpulan logam menunjukkan pengkayaan logam perlu ke atas logam bukan penting. Perbandingan kepekatan logam dengan paras logam toksik maksimum yang dibenarkan di dalam makanan yang digubal di beberapa negara, serta Akta Makanan Malaysia 1983 dan Peraturan Makanan 1985 Jadual Empat Belas, menunjukkan bahawa nilai di dalam kajian masih di bawah paras selamat.*

*Kata kunci: Logam berat; paras keselamatan makanan; perairan pantai Pantai Timur Semenanjung Malaysia; Thais clavigera*

### INTRODUCTION

It has become a common practice to use aquatic organisms as bioindicators in many monitoring programs throughout the world. They are often published in response to concern about contamination on seafood or to employ marine organisms as biological monitors. Marine ecosystems may receive anthropogenic pollutants originating from various sources at the surrounding areas or from distant locations. Heavy metals might change into the persistent metallic compounds with high levels of toxicity which then bioaccumulated by the particular organisms, magnified in the food chain and lastly implicated the human health (Zhou et al. 2008). Aquatic invertebrates attempt to accumulate trace metals in their tissues whether or not

these metals are needed to their metabolism. Mollusks display greater spatial sensitivity, thus, are the most reliable tool for identifying sources of bioavailable contamination unlike sediments (Goldberg 1978; Hamed & Emara 2006; Thomson et al. 1984).

The use of mollusks bioindicators to study pollution in the Malaysian environment has received much attention including several studies on *T. clavigera* (Amin et al. 2006; Shazili et al. 1995; Yap et al. 2010). The information on the level of heavy metals pollution on the east coast of peninsular Malaysia is restricted to a small number of studies. Most of the economic activities are focused on the west coast of Peninsular Malaysia, thus most of the studies on heavy metals have been dedicated to this area

(Abdullah et al. 1999). The objectives of this study were to explore the bioavailability and extent of exposure of heavy metals in soft tissue of *T. clavigera* from different rocky shore sites along the coastal waters of the east coast of Peninsular Malaysia and provide the baseline data for the east coast region as well. Comparison with permissible limits in Malaysia and other countries could provide an insight in their safety levels for human and environment monitoring.

#### MATERIALS AND METHODS

Sampling sites (11 stations) included pristine, recreational sites and proximity of industrial and urbanized areas (Figure 1) as to provide a wider range of insight on bioaccumulation patterns related to environmental status were done. Only sites with the abundant numbers of *T. clavigera* population on natural rocky structures were selected. *T. clavigera* samples of relatively the same size were hand collected during low tide period in May and June 2009. All samples were placed in plastic bags, sealed, labeled and stored at 4-6°C during transportation to the laboratory where the samples were rinsed with running Mili-Q water (18.2 Ω) to remove sediment and salt particles prior to store frozen. After allometric parameter measurement and tissue extraction, extracted soft tissues were freeze dried before being weighed again in order to evaluate the water content and conversion factor. Freeze-dried samples were then pulverized to a homogenous powder and kept at room temperature until analysis.

The analytical procedure was based on Shazili et al. (2009) with little modification. Analysis was carried out using Inductive coupled plasma mass spectrometer (ICPMS) Perkin ELMER ELAN 9000. The results were blank corrected and expressed as  $\mu\text{g g}^{-1}$  dry weight. Mercury content was examined by direct measurement using the MA-2 Mercury Analyzer. Glassware used was immersed in 10% nitric acid ( $\text{HNO}_3$ ) solution in advance for contamination prevention. The quality of method used was confirmed in a separate comparative study using a standard reference material, Lobster Hepatopancreas TORT-2. Recoveries were as follows: 92.30% - Hg, 113.57% - Pb, 97.31% - Cd, 85.56% - Cu, 86.54% - Zn, 81.80% - Mn, 94.30% - Fe, 96.53% - Co, 88.59% - Se and 82.60% - Sr.

One-way analysis of variance (ANOVA) followed by Tukey's HSD post hoc test was employed for evaluation of variation and significant differences observed between metals in different sites. The strength and direction of the relationship between elements was elucidated using bivariate Pearson's correlation coefficients. All comparisons were made at least at the 95% ( $p < 0.05$ ) and 99% ( $p < 0.01$ ) levels of significance.

#### RESULTS AND DISCUSSION

Mean concentrations, expressed on a dry weight basis ( $\mu\text{g g}^{-1}$ ), for Hg, Pb, Cd, Cu, Zn, Mn, Fe, Co, Se and Sr from 11 sampling sites along the east coast of Peninsular Malaysia are presented in Table 1. The shell length of *T. clavigera* ranged between 2.69 and 3.58 cm in all sites.

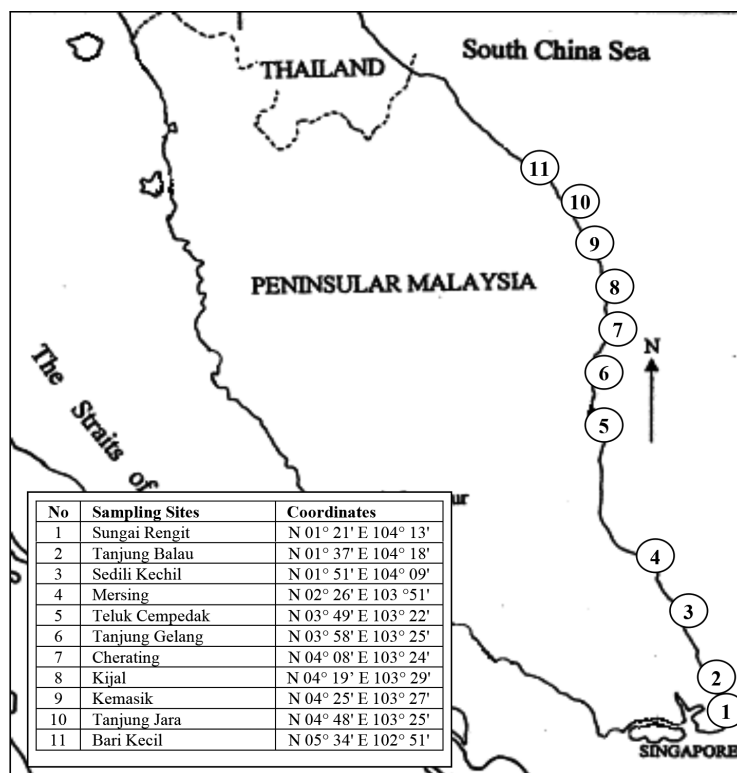


FIGURE 1. Map showing 11 sampling sites along the east coast of Peninsular Malaysia

The values of moisture content ranged between 64.13 and 84.69% while for conversion factor, ranged between 0.17 and 0.29. The positive significant correlations in the soft tissue were found for the metal pairs Hg-Zn ( $p<0.01$ ), Pb-Co ( $p<0.05$ ) and Fe-Sr ( $p<0.01$ ) while negative for Pb-Cd ( $p<0.05$ ) and Cu-Co ( $p<0.05$ ). No significant correlation was found for Mn-Se.

#### INTER-SPATIAL DISTRIBUTION

Metal concentrations in soft tissue from Tg. Balau has the highest concentration of Hg ( $0.44 \mu\text{g g}^{-1}$ ) and Zn ( $868.8 \mu\text{g g}^{-1}$ ); Tlk. Cempedak for Cd ( $5.74 \mu\text{g g}^{-1}$ ) and Se ( $9.50 \mu\text{g g}^{-1}$ ); Mersing for Cu ( $215.9 \mu\text{g g}^{-1}$ ) and Mn ( $6.60 \mu\text{g g}^{-1}$ ); and Cherating for Co ( $0.68 \mu\text{g g}^{-1}$ ), Fe ( $303.4 \mu\text{g g}^{-1}$ ) and Sr ( $101.8 \mu\text{g g}^{-1}$ ). The highest concentration of Pb was found in samples from Tg. Gelang ( $2.14 \mu\text{g g}^{-1}$ ), whereas for Zn was from Tg. Balau with a concentration almost doubled compared to other locations.

The accumulated elements in a bioindicator organism are a direct reflection of the total integrated bioavailability and contamination of the sampling locations. A comparison of metal concentrations among locations using Tukey's post hoc test (Table 1) lead to the conclusion that consistently high concentrations are generally found at Sg. Rengit, Tg. Balau and Tg. Gelang. By observation on the environment of the sampling locations, it is possible that the metals accumulated in studied species are derived

from anthropogenic sources by some activities at certain locations. It is comparative with the study from Shazili et al. (1995) which reported high concentration of Zn, Pb, Cu and Cd found at Tg. Gelang using rock oyster *Saccostrea* sp. as bioindicator. High concentration related to Tg. Gelang can be understood on the fact that it is located in the vicinity of industrial complexes. Effluents from the nearby domestic and industrial inputs flow through this area through large drains discharging directly to the sea. While in Sg. Rengit and Tg. Balau, Mashitah et al. (2012) reported that high degree of metal exposure found at the east coast area of Johor might have been resulting from shipping activities, oil and gas industries positioned to the south at Teluk Ramunia and Pangerang. Also, metal pollution problem in Tg. Balau could be the result of being adjacent to a resort vacation area and fishing landing jetty with heavy boat traffic. The use of Zn block in fishing vessels and Cu leachate from the antifouling paints of vessel with semi-enclosed geography of Tg. Balau would have resulted in enriched metal concentrations in its coastal waters. Commonly, high concentrations are often attributed to human activities as they are found at the vicinity of industrial complexes, urbanized area and resorts.

Interspatial variation of *T. clavigera* in this study are compared with the same locations from previous research at Sedili Kecil, Mersing, Teluk Cempedak and Bari Kecil (Shazili et al. 1995), indicating that Pb, Cd, Cu and Zn

TABLE 1. Concentrations of Hg, Pb, Cd, Cu, Zn, Mn, Fe, Co, Se and Sr ( $\mu\text{g g}^{-1}$  dry weight) in soft tissue of *Thais clavigera* (mean and standard deviation) from 11 sampling locations along the east coast of Peninsular Malaysia

Location	Hg	Pb	Cd	Cu	Zn	Mn	Fe	Co	Se	Sr
Sg. Rengit	0.17 $\pm 0.04$	0.43 $\pm 0.06^a$	3.73 $\pm 1.40$	192.2 $\pm 120.0$	308.8 $\pm 79.02^{a,b,c}$	2.86 $\pm 0.49$	225.31 $\pm 44.34$	0.29 $\pm 0.30^{a,b}$	5.88 $\pm 2.02$	34.98 $\pm 6.55$
Tg. Balau	0.44 $\pm 0.38$	0.21 $\pm 0.02^a$	4.15 $\pm 1.24$	148.1 $\pm 58.9$	868.8 $\pm 262.3^d$	4.30 $\pm 0.40$	257.37 $\pm 28.73$	0.31 $\pm 0.08^{a,b}$	7.69 $\pm 1.08$	48.71 $\pm 9.71$
Sedili Kecil	0.37 $\pm 0.07$	0.21 $\pm 0.06^a$	4.65 $\pm 1.82$	157.3 $\pm 71.6$	374.8 $\pm 106.0^{b,c}$	6.06 $\pm 3.71$	149.6 $\pm 88.35$	0.09 $\pm 0.07^a$	5.19 $\pm 3.08$	34.91 $\pm 30.02$
Mersing	0.31 $\pm 0.05$	0.49 $\pm 0.41^a$	5.00 $\pm 1.50$	215.9 $\pm 86.1$	238.1 $\pm 8.08^{a,b,c}$	6.60 $\pm 2.62$	157.91 $\pm 69.27$	0.10 $\pm 0.02^a$	7.16 $\pm 1.56$	42.11 $\pm 11.01$
Tlk. Cempedak	0.21 $\pm 0.02$	0.27 $\pm 0.03^a$	5.74 $\pm 1.77$	137.4 $\pm 94.9$	185.8 $\pm 35.53^{a,b,c}$	4.66 $\pm 0.87$	271.0 $\pm 196.0$	0.35 $\pm 0.14^{a,b}$	9.50 $\pm 3.49$	86.56 $\pm 107.0$
Tg. Gelang	0.13 $\pm 0.03$	2.14 $\pm 0.24^b$	2.67 $\pm 0.23$	107.1 $\pm 37.4$	386.1 $\pm 29.28^{b,c}$	6.25 $\pm 1.65$	162.4 $\pm 17.67$	0.34 $\pm 0.05^{a,b}$	4.34 $\pm 1.68$	22.51 $\pm 3.14$
Cherating	0.17 $\pm 0.02$	0.85 $\pm 0.68^a$	5.27 $\pm 1.55$	70.86 $\pm 64.71$	139.8 $\pm 16.9^{2a,b}$	2.38 $\pm 0.42$	303.4 $\pm 96.27$	0.68 $\pm 0.27^b$	6.49 $\pm 0.93$	101.8 $\pm 63.10$
Kijal	0.22 $\pm 0.04$	0.22 $\pm 0.02^a$	5.32 $\pm 1.55$	177.0 $\pm 30.9$	432.4 $\pm 66.12^c$	3.47 $\pm 0.20$	245.9 $\pm 159.8$	0.08 $\pm 0.03^a$	6.71 $\pm 2.69$	74.70 $\pm 73.91$
Kemasik	0.18 $\pm 0.08$	0.28 $\pm 0.01^a$	4.90 $\pm 1.93$	152.5 $\pm 100.7$	217.2 $\pm 85.82^{a,b,c}$	2.37 $\pm 0.63$	250.7 $\pm 105.0$	0.16 $\pm 0.11^a$	9.31 $\pm 3.15$	46.36 $\pm 13.59$
Tg. Jara	0.20 $\pm 0.03$	0.36 $\pm 0.04^a$	4.36 $\pm 1.21$	81.86 $\pm 36.28$	204.4 $\pm 5.29^{a,b,c}$	3.07 $\pm 0.87$	279.4 $\pm 64.09$	0.30 $\pm 0.33^{a,b}$	9.47 $\pm 4.51$	58.90 $\pm 36.46$
Bari Kecil	0.15 $\pm 0.03$	0.39 $\pm 0.03^a$	5.30 $\pm 0.26$	71.46 $\pm 5.94$	80.76 $\pm 8.30^a$	5.62 $\pm 1.28$	193.3 $\pm 104.75$	0.06 $\pm 0.02^a$	5.11 $\pm 0.45$	28.03 $\pm 5.10$

Tukey's Post hoc test comparisons of metal concentrations among sampling locations; means with different letters are significantly different ( $p<0.05$ )

values were lower than previous measurement, except for Cd at Mersing. Meanwhile, comparison with studies from different regions and countries (Blackmore 2000, 2001; Blackmore & Morton 2001; Cheung 2008; Han et al. 1996, 1997; Hung 2001; Jeng et al. 2000) as shown in Table 2, specified that Cd and Cu values are among the lower indicated values. Zn maximum concentration, however, indicated higher values compared with others and comparable with data from western coast of Taiwan. Unfortunately, the data available on metal concentrations of this species is very limited. No data was found on metal distribution differences among body parts of *T. clavigera* except within soft tissue. Most of studies on *T. clavigera* were mainly focused on tributyltin (TBT) monitoring as this species has been established as TBT bioindicator in coastal environment, which is monitored by the development of imposex and the imposition of male sex characteristics (Chan et al. 2008; Hung et al. 2001; Wang et al. 2010).

#### PATTERN OF METAL OCCURRENCES

Generally, the pattern of metal occurrences within the soft tissue of *T. clavigera* can be presented as Zn > Fe > Cu > Sr > Se > Cd > Mn > Pb > Co > Hg. The essential metals such as Zn, Fe, Cu, Se and Mn showed the highest accumulation rates over non-essential metals Hg, Cd and Pb. This is expected as in soft tissue, these metals has been

acknowledged as biologically essential for metabolism, (Amin et al. 2006; Joleey et al. 2004) playing a vital part as co-factors in enzymatic processes (Yap et al. 2010) and are a useful part of the respirational protein haemocyanin (Rainbow 2002). Later, these metals cannot be instantly excreted or detoxified as they are required to play roles in metabolism. Non-essential metals are among less accumulated metals as they are not needed in metabolism and would have no prerequisite minimum concentration. However, they are taken up by all organisms in proportion to the degree of environmental contamination levels.

The existence of Cu suggests the presence of haemocyanin, an oxygen-binding protein, as a major respiratory pigment. Confirmation has been found that *T. clavigera* has haemocyanin (Blackmore 2001). High Cu concentration supports the hypothesis that such respiratory pigments are present. It has been discovered that Cu and Zn are selectively localized in the tissue of *T. clavigera*. The highest concentration for Zn is being reached in hepatopancreas and gills while the highest Cu concentration is in the liver and haemocyanin in blood (Han et al. 1997). Such selectivity could explain the significance of Zn and Cu concentration within soft tissue of *T. clavigera* although specific tissues were not analyzed. There is an evidence suggesting that *T. clavigera* when exposed to low dissolved oxygen concentrations may yield

TABLE 2. Comparison of trace metals concentration data ( $\mu\text{g g}^{-1}$  dry weight) in soft tissue of *Thais clavigera* obtained in the present study with literature data from various regions all over the world

Species	Region	Year	Hg	Pb	Cd	Cu	Zn	References
<i>T. clavigera</i>	Taiwan coast	1991-1998	0.845	1.22	1.15	202	326	(Jeng et al. 2000)
	Taiwan coast	1994-1995				77.7		(Han et al. 1996)
	Western coast of Taiwan	1994-1998		2.3		492	768	(Hung et al. 2001a)
	Putai coast, Taiwan	1996				142-370	238-564	(Han et al. 1997)
	Cape d'Aguilar Hong Kong	1996-1997						
	- exposed site				8.28	183	313	(Blackmore 2000)
	- sheltered site				8.40	310	261	
	Cape d'Aguilar Hong Kong	1996						
	- exposed site				8.88	220	314	(Blackmore 2001)
	- sheltered site				10.33	327	246	
	Hong Kong coast	1996-1997			2.89-16.0	170-1853	204-523	(Blackmore & Morton 2001)
	Clearwater Bay, Hong Kong	2004-2005			2.80	357	237	(Cheung et al. 2008)
Butterfly Bay, Hong Kong	2004-2005			1.77	203	366		
<i>Thais sp.</i>	Bari Kecil, Terengganu	1995		0.95	28.6	155	187	(Shazili et al. 1995)
	Teluk Cempedak, Pahang			3.81	14.3	269	808	
	Mersing, Johor			12.9	2.86	287	529	
	Sedili Kecil, Johor			0.95	27.1	256	253	
<i>T. clavigera</i>	Coastal waters of the East Coast of Peninsular Malaysia	2009	0.13-0.44	0.21-2.14	2.67-5.74	70.86-215.87	80.76-868.78	This study

TABLE 3. Maximum permissible limits on heavy metals (in  $\mu\text{g g}^{-1}$ ) for food safety set by different countries

	Weight basis	Hg	Pb	Cd	Cu	Zn
Food category not specified						
Brazil	Dry		10 <sup>a</sup>	5 <sup>a</sup>	150 <sup>a</sup>	250 <sup>a</sup>
Thailand	Dry		6.67 <sup>b</sup>		133 <sup>b</sup>	667 <sup>b</sup>
Shellfish molluscs						
European Community	Wet		1.5 <sup>c</sup>	1 <sup>c</sup>		
Hong Kong	Wet		6 <sup>d</sup>	2 <sup>d</sup>		
Australia	Wet		2 <sup>e</sup>	2 <sup>e</sup>	30 <sup>f</sup>	
USA	Wet		1.7 <sup>g</sup>	3.7 <sup>g</sup>		
Malaysia	Wet	0.5 <sup>h</sup>	2 <sup>h</sup>	1 <sup>h</sup>	30 <sup>h</sup>	100 <sup>h</sup>
This study	Wet	0.05	0.13	1.10*	32.52*	73.70
	Dry	0.23	0.53	4.64	137.41	312.44

<sup>a</sup> Brazilian Ministry of Health (ABIA 1991)

<sup>b</sup> Ministry of Public Health, Thailand (MPHT 1986)

<sup>c</sup> European Community (EC 2006)

<sup>d</sup> Hong Kong Environmental Protection Department (HKEPD 1997)

<sup>e</sup> Food standards Australia New Zealand Authority (FSANZ 1996)

<sup>f</sup> Australian Government, 2006

<sup>g</sup> Food and Drug Administration of the United States (USFDA 1990)

<sup>h</sup> Malaysian Food Regulation Fourteen Schedule (1985)

\*Values that exceed the permissible limits set by Malaysian Food Regulation Fourteen Schedule

more haemocyanin to increase respiratory efficiency and consequently lead to increase in body concentration of Cu (Blackmore 2000).

#### COMPARISON WITH PUBLISHED PERMISSIBLE LIMITS

Metal concentrations in this study have been converted into wet weight basis using conversion factor for comparison with permissible limits set by Malaysia and other different countries (Table 3). Comparison made with the permissible limits set by the Malaysian Food Regulations 1985 Fourteen Schedule for Cd, Cu, Pb and Zn found that these metals are well below the safety limit. They were also below the endorsed guidelines for Cu and Zn set by the Brazilian Ministry of Health (ABIA 1991), the Ministry of Public Health of Thailand (MPHT 1986), the European Commission (EC 2006), the Hong Kong Environmental Protection Department (HKEPD 1997), the Food Standards Australia New Zealand Authority (FSANZ 1996), the Australian Government (2006) and the Food & Drug Administration of the United States (USFDA 1990). Cd and Cu values exceed the limits for a small number of the samples but the overall values did not exceed the safety limits. Although there is no report on human consumption of *T. clavigera* in Malaysia, constant monitoring should be taken as there is high potential of these metals to increase in the future, considering the levels are not far below safety limits in this study.

#### CONCLUSION

Generally, all samples were collected from the industrialized intertidal areas along the east coast of Peninsular Malaysia, thus the metal concentrations found in the studied species evidently indicate its bioavailability from anthropogenic

input along with the purely natural sources. Along with its wide distribution on rocky shore area along the east coast of peninsula, the present results of trace metals recorded in *T. clavigera* collected from 11 sites along the east coast of Peninsular Malaysia could serve as the baseline data for future reference.

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- Fuad Mohd Miskon  
Institute of Oceanography and Maritime Studies  
Kuliyyah of Science  
International Islamic University Malaysia  
25200 Kuantan, Pahang  
Malaysia
- Noor Azhar Mohd Shazili  
Faculty of Maritime Studies and Marine Science  
Universiti Malaysia Terengganu  
21030 Kuala Terengganu, Terengganu  
Malaysia
- Faridah Mohammad  
Department of Biological Science  
Faculty of Science and Technology  
Universiti Malaysia Terengganu  
21030 Kuala Terengganu, Terengganu  
Malaysia
- Kamaruzzaman Yunus\*  
Kuliyyah of Science, International Islamic University Malaysia  
25200 Kuantan, Pahang  
Malaysia

\*Corresponding author; email: kama@iiu.edu.my

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