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Composition and sources of aliphatic lipids and sterols in sediments of a tropical island, southern South China Sea: preliminary assessment

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Abstract Near-shore surface sediment was collected from five stations off Redang Island located on the eastern coast of Peninsular Malaysia. Freeze-dried sediments were Soxhlet extracted and then fractionated using column chromatography into aliphatic and polar fractions. Determination of these fractions was carried out using gas chromatography mass spectrometry. The concentration of total resolved aliphatic hydrocarbons in sediments ranged from 157 to 308 ng/g. The distribution of aliphatic fraction showed the presence of *n*-alkanes ranging from *n*C15 to *n*C33 with a minor odd-to-even predominance exhibiting carbon maximum, depending on station, at *n*C17, *n*C26, *n*C29 or *n*C31. Calculation of Carbon Preference Index (CPI) for CPI_{15-33} gave values ranging from 1.09 to 1.46. *n*-Alkanol in all sediment exhibits even-to-odd carbon predominance ranging from *n*C16 to *n*C28 and maximising at *n*C22. *n*-Fatty acids distribution ranged from *n*C14 to *n*C24 with a dominant maximum at *n*C16 and exhibiting high values of short chain fatty acids (≤*n*C20) to long chain fatty acids (>*n*C20) ratios. Unsaturated fatty acids, particularly *n*C16:1 and *n*C18:1 is also ubiquitous in all samples. Cholesterol is the most abundant compound amongst the sterol group ranging from 42.8 to 62.6 % of the total sterols. *β*-Sitosterol, brassicasterol and stigmasterol, are also present but of relatively lower amount. These observations suggest that the aliphatic lipids and sterols in the study area originate, mainly, from biogenic sources of marine microbial with minor contribution from epiticular waxes of terrestrial plants.

Keywords Marine sediment · Biogenic origin · *N*-alkanes · Polar aliphatic lipids · Biomarkers

Introduction

Organic matters in coastal marine environment can originate from various sources such as marine detritus, atmospheric fallout, terrigenous runoff and anthropogenic discharge from continental areas. Organic matter can also be produced in situ by current cycling or sedimentation processes. Composition of organic matter in marine sediment has received worldwide interest because of the need to assess fate and transport of organic matter in the environment. For this purpose, the biological markers, or biomarkers, are often used to trace the geological and environmental process, for instance, aliphatic lipids (homologous series of alkanes,

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acids and alcohols) are important biomarkers because they carry a strong carbon number predominance inherited from their biosynthesis and their presence in the environment have been used to indicate biogenic input from epicuticular waxes and higher plant-related lipids or marine organisms (Simoneit 2005).

Redang Island is one of the largest islands in the South China Sea off the east coast of Peninsular Malaysia. The island is approximately 45 km away from Kuala Terengganu coast and is one of popular tourism sites in Malaysia. The island is well known for its rich coral and other marine life. The main economic activities of Redang Island centred on fishing and tourism industries where major attractions include marine parks, corals, turtle sanctuary, snorkelling and diving sites. Tourism industry has grown rapidly over the years since the early 1990s (Lim 1997) and the quality of the marine environment of the Redang Island has undergone significant changes in line with the increase in tourism activities on the island. Even though seawater temperature has been known to play an important role in causing coral bleaching phenomenon on the island (Muhammad 2010), the presence of anthropogenic chemicals such as sunscreens in the water could also, potentially, cause similar event (Danovaro et al. 2008). Oil- and grease-related contaminants from untreated sewage discharges and boating (tourists and fishing vessels) operations could also affect the quality of the marine environment of the island. At present, information on the status of the marine environment in the Southern South China Sea, in particular with respect to organic geochemistry of the sediment, is still limited. Consequently, this study has been undertaken to determine the composition and assess the origin of aliphatic lipids and sterols found in the sediment in the study area. Information generated would provide an initial insight into the geochemistry of the organic matter in the marine environment of the eastern coast of Peninsular Malaysia.

Materials and methods

Sampling

Five surface sediments were collected from the southern coast (0–5 km from the shoreline) of Redang Island in Ogos, 2008, and transported to the laboratory in an ice chest. Approximate locations of the sampling sites are shown in Fig. 1 whilst the exact coordinates are provided in Table 1. Upon reaching the laboratory, all sediment samples were kept frozen (−20 °C) until further analysis. Samples were later freeze dried and then sieved using 500 μm sieve. Only the fine fractions (<500 μm) were used in analysis of the aliphatic lipids and sterols.

Extraction and fractionation

Freeze-dried sediments were Soxhlet extracted using hexane/dichloromethane (1:1 *v*/*v*) as solvent (UNEP 1992). Before extraction, internal standard consisting of predeuterated tetracosane ($n\mathrm{C}_{24}\mathrm{D}_{50}$) and 5α-androstane-3β-ol were spiked into the sediment for recovery assessment of the aliphatic and polar fraction, respectively. Sulphur content in the sediment was removed using mercury treatment (Beg et al. 2003). The concentrated extracts were then fractionated into subfractions on 5 % deactivated silica (230–400 mesh)–2 % deactivated alumina (70–230 mesh) columns. Twenty-five milliliter of *n*-hexane was used to elute the aliphatic fraction whilst 40 mL of 10 % methanol in DCM was used to elute the polar fraction. *N*,*O*-*bis*-(trimethylsilyl)-trifluoroacetamide plus 1 % of trimethylchlorosilane mixture was used to derivatise the polar fraction (Omar et al. 2009) in order to convert the compounds into trimethylsilyl derivatives prior to the injection into the gas chromatography–mass spectrometry (GC-MS).

GC-MS analysis

Identification and quantification of aliphatic lipids and sterols were carried out using Shimadzu-QP2010 GC-MS fitted with BPX-5 capillary column (30 m×0.32 mm i.d; 0.25 μm filmed thickness). Helium was used as a carrier gas set at a constant pressure of 14.2 kPa and the GC injection temperature was set at 290 °C using splitness mode while GC-MS interface was set at 300 °C. Column temperature for the analysis of the *n*-alkane and polar compounds was programmed as follows: hold for 2 min at 65 °C followed by a temperature ramp of 65–300 °C at 6 °C/min and then maintained at 300 °C for 20 min resulting in a total run time of approximately of 62 min. Verification of peaks was carried out based on key fragment ions, comparison of retention times to that of external standards. Confirmation of peaks was also carried out using the MS library.

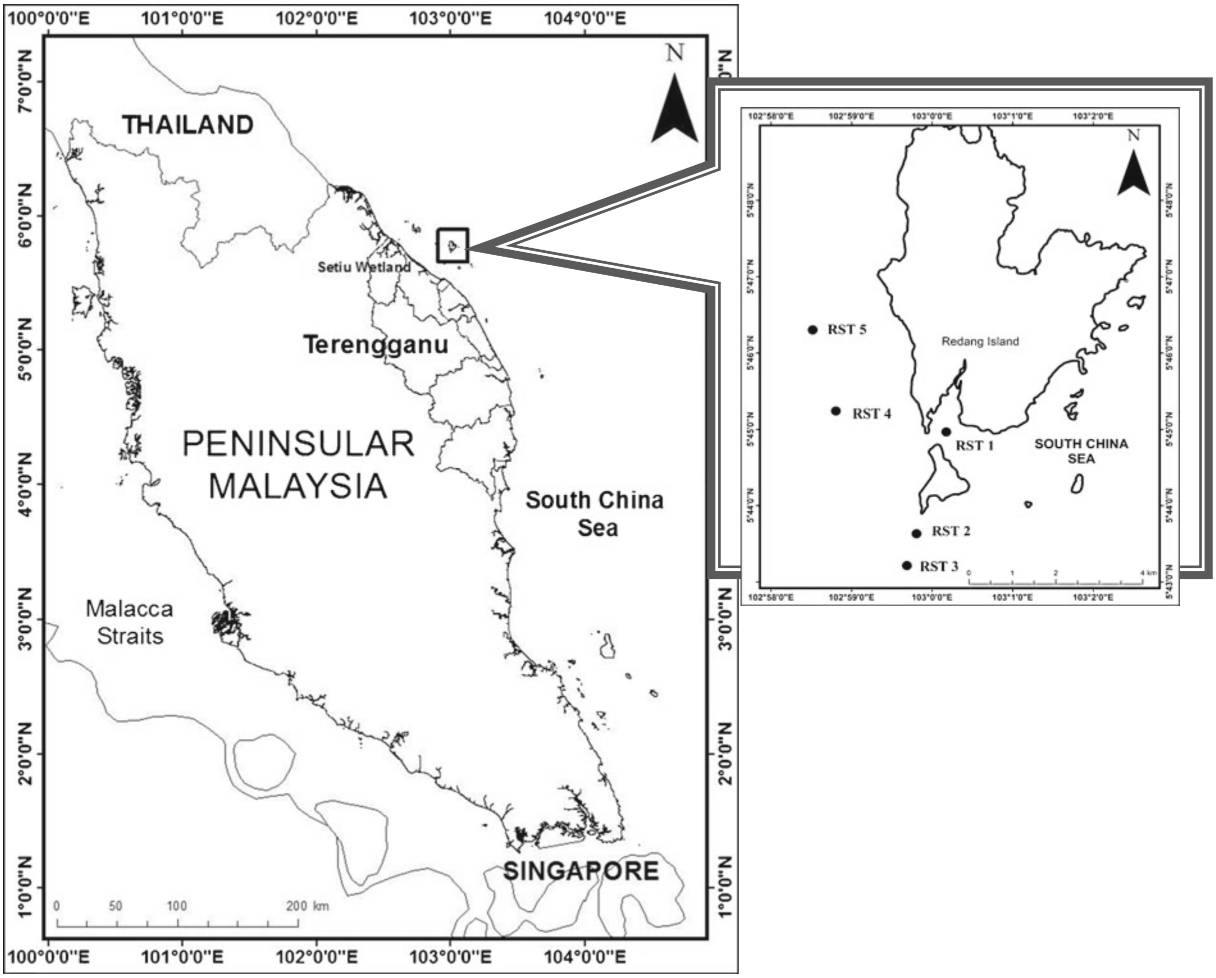

Fig. 1 Schematic representation of the sampling locations around Redang Island

Results and discussion

N-alkane

The concentration of total resolved aliphatic hydrocarbons (TRAH) ranged from 157 to 308 ng/g (dry weight; Table 2) with the highest and the lowest concentrations of TRAH found at RST 5 and RST 3, respectively. It has been reported that average biogenic hydrocarbons in coastal sediment is below in concentration 10 μg/g (UNEP 1992); based on this, it appears that the TRAH values observed at all stations monitored are within the natural range.

Table 1 Sampling stations in southern Redang Island coastal waters

Station	Latitude (°N)	Longitude (°E)
RST 1	5º 27' 01.80" N	103º 0' 08.28" E
RST 2	5º 26' 14.64" N	102º 35' 55.67" E
RST 3	5º 25' 59.16" N	102º 35' 52.43" E
RST 4	5º 27' 12.24" N	102º 35' 22.91" E
RST 5	5º 27' 59.04" N	102º 35' 13.20" E

Figure 2 shows a typical representative of total ion current chromatogram traces from GC-MS analyses for *n*-alkanes in sediments whilst Table 2 shows some other selected *n*-alkanes evaluation indices used in this study. A homologue series of *n*-alkanes ranging from *n*C15-*n*C33 was observed in all samples studied. With the exception of RST 5, all other samples exhibited a carbon maximum at C17 and a second carbon maximum at C29 or C31. In the case of RST5, a C-maximum was observed at *n*C26 with a second minor

Table 2 Selected *n*-alkane evaluation indices for Redang samples

	$CPI_{15–33}$	$CPI_{25–33}$	1st C_{max}	2nd C_{max}	TRAH (ng/g)	TARs	Ur:R	% Alk(Terr)
RST 1	1.19	1.17	C17	C29	249	0.843	2.35	21.4
RST 2	1.09	1.33	C29	C17	215	1.36	0.75	25.0
RST 3	1.35	1.90	C17	C31	157	0.583	1.22	19.9
RST 4	1.46	1.65	C31	C17	197	0.998	0.72	24.9
RST 5	1.15	1.18	C26	nil	308	1.56	1.00	22.9

CPI_{15-33}=0.5[(odd$\sum C_{15}-C_{33}$/even$\sum C_{14}-C_{32}$)+(odd$\sum C_{15}-C_{33}$/even$\sum C_{14}-C_{36}$]; CPI_{25-33}=0.5[(odd$\sum C_{25}-C_{33}$/even$\sum C_{24}-C_{32}$)+(odd$\sum C_{25}-C_{33}$/even$\sum C_{26}-C_{34}$]; *TARs*=(nC27+nC29+nC31)/(nC15+nC17+nC19); *Ur/R* unresolved over resolved *n*-alkane; *% ALK Terr*=sum of the nC27, nC29, nC31, nC33 to total *n*-alkane

maximum at *n*C29. Samples at RST 3 and RST 4 showed an odd over even carbon number predominance at low molecular homologues with carbon maximum (C_{max}) at *n*C17 and also at high molecular homologues with C_{max} at *n*C31. *n*-Alkanes with an odd over even carbon predominance observed at high molecular homologues, as reflected by their CPI value greater than 1 and coupled with C_{max} at either *n*C27, *n*C29 or *n*C31, is generally accepted as indicative of epicuticular waxes of higher (terrestrial) plants source (Mille et al. 1992). On the other hand, an odd over even carbon predominance in the low carbon number range (C15–C21) with CPI value greater than 1 and C_{max} at either *n*C15, *n*C17 or *n*C19 is generally associated with microbial hydrocarbons input (Clark and Blumer 1967) where a C_{max} at *n*C17 has been reported in the literature as indicative of contribution from bacterial lipids (Qiu et al. 1991; Goutx and Saliot 1980). RST 1 and RST 2 samples exhibited a similar pattern with odd over even carbon predominance at low carbon number range (C15–C21) with C_{max} *n*C17, with only marginal odd over even carbon predominance at high carbon homologues with C_{max} at C29. Calculation of CPI in carbon range C15–33 gave values ranging from 1.09 to 1.46 whilst CPI associated with mainly terrestrial derived alkanes (C25–33) gave values between 1.17 and 1.90; CPI values

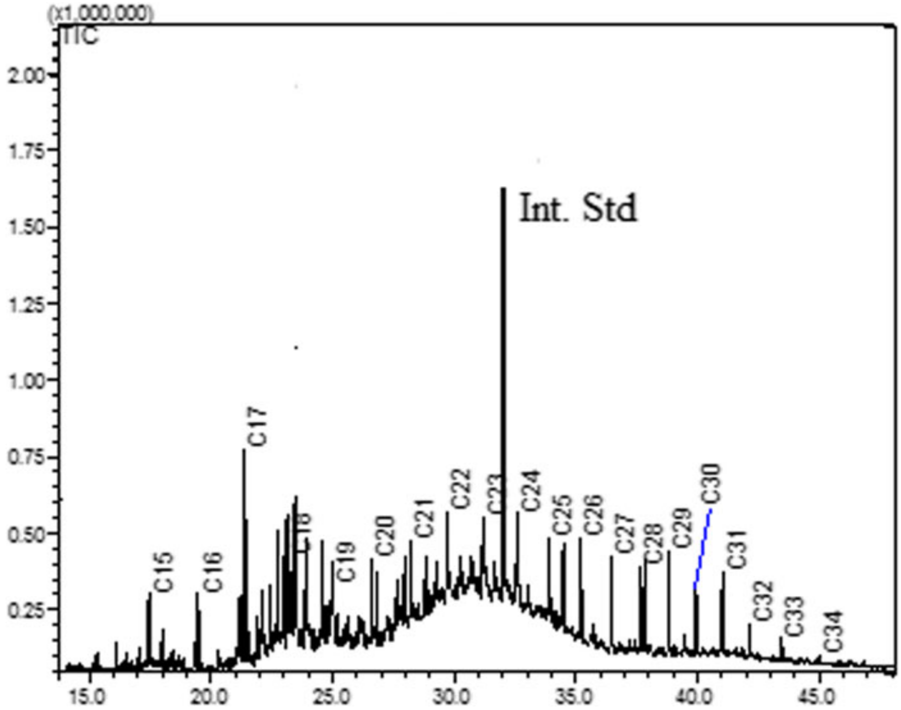

Fig. 2 Typical example of total ion current chromatogram of aliphatic hydrocarbons for RST 1. *Number above peaks* indicates carbon chain lengths, *Int std* internal standard

close to one are usually accepted as an indicator of petrogenic contamination, however, this could also be taken as an indicator of marine microbial lipids or recycled organic matter (Commendatore et al. 2000).

Apart from C_{max} and CPI values, other diagnostic parameters could also be used to further differentiate sources of alkanes found in the sediment or at least to confirm the findings based on the former two evaluation indices. For instance, terrestrial over aquatic hydrocarbon ratios (TARs) have been calculated; TARs greater than 1 suggest a slight dominance of terrestrial over the aquatic alkanes (Bourbonniere and Meyers 1996a, b). On the other hand, relative abundance of biogenic terrestrial alkanes (defined as sum of C27, C29, C31, and C33) over the total *n*-alkanes (% Alk (terr)) provides a measure of the relative importance of the terrestrial source over the other hydrocarbon sources (biogenic marine or anthropogenic sources) into the sediment (Colombo et al. 1996); % Alk (terr) found in this study ranged from 17.8 to 25.0 % of the total alkanes. This result suggests a relatively minor contribution of terrestrial plants alkanes into the area. For comparison, a similar study carried out in the Setiu wetland located in the coastal area of Setiu, on the Terengganu mainland (Mohd Tahir et al. 2009; Fig. 1) showed significantly higher contribution of terrigenous alkanes with % Alk (terr) values ranging from 30 to 65 % of total alkanes found in the area. This differences could be attributed to differences in terrestrial vegetation cover in the two areas; in the case of Redang Island, most of the forests and terrestrial plant cover are located on the northern and eastern side of Redang Island with limited cover on the southern side of the island whereas the Setiu wetland has significant cover of higher plant vegetation throughout the wetland as well as receiving riverine inputs from Setiu River basin.

Interestingly, another feature observed in chromatograms of RST 1, RST 2 and RST 5 is the presence of a relatively small hump (unresolved complex mixtures) which maximise in the region of C20–C26; the presence of a hump in this region could be indicative of possible petrogenic contamination; however, calculation of the unresolved area over the resolved area under the chromatogram (Ur/R) showed all stations gave values less than 2, except for RST 1 with value of 2.35, which suggest no significant contamination from petroleum or its degraded residues (Simoneit et al. 1991). Possible signs of contamination at RST 1 could be due to the relatively higher maritime activities in the area as the site is in the vicinity of the main jetty of the island and the marine park area.

N-alkanol and *n*-fatty acids

The distribution of *n*-alkanols observed was very similar among all stations, having carbon number ranging from *n*C16 to *n*C28 with significant even-to-odd distribution and carbon maximum at *n*C22OH. The precise source of *n*C22OH is seldom being reported; however, many literature cited *n*C22OH as from higher plants origins (Mudge and Norris 1997; Mohd and Mudge 2005; Treignier et al. 2006) since it is within the long chain alkanol carbon homologues (≥*n*C22OH). Volkman et al. (1999), on the other hand, reported that nC22OH is predominant in eustigmatophytes, phototropic marine and fresh/water microalgae while Jaffé et al. (2001) suggested that aquatic epiphytes which grow on sea grass as a possible source of the *n*C22OH. Since the ratios of short chain carbon (∑C9–C20) relative to long chain carbon homologue (∑C21–C28) calculated in this study ranged from 0.11 to 0.32, which indicate the predominance of long chain *n*-alkanols, it is likely that in this study area, these alcohols are mainly derived from terrestrial (vascular) plants lipids.

The distribution of *n*-fatty acids (*n*-alkanoic acids) found in all samples ranged from *n*C14 to *n*C18 with a maximum at *n*C16. The C_{max} at *n*C16 is the dominant lipid component in microorganisms (Meyers et al. 1980; Simoneit 1980) and it is ubiquitous in many marine organisms collected in Malaysian coastal waters such as marine sponges (*Xetospongia* sp. and *Aaptos aaptos*) (Habsah et al. 2009; Habsah et al. 2011), sea cucumber (*Stichopus chloronotus*) (Fredalina et al. 1999) and macrozoobenthos (*Telescopium telescopium*) as well as mangrove leaves (*Bruguiera gymnorhiza* and *Ridzophora apiculate*; Mohd Abdullah 2009). High abundance of short chain *n*-fatty acids and significant even-to-odd predominance reflects the important contribution of planktonic and bacterial inputs into the sediments (Azevedo 2003). At the same time, the presence of unsaturated acids of *n*C16:1 and *n*C18:1 in all samples, provide further evidence for relative contribution from bacteria (Mudge 2005; Duan 2000), marine planktonic and/or indicator of recent biogenesis (Azevedo 2003).

Sterols

Six dominant sterols, consisted of a series of C27 to C29 sterols, were identified in the sediment samples (Fig. 3). The C27 sterols were the most abundant sterols and accounted about 42.8–62.6 % of the total identified

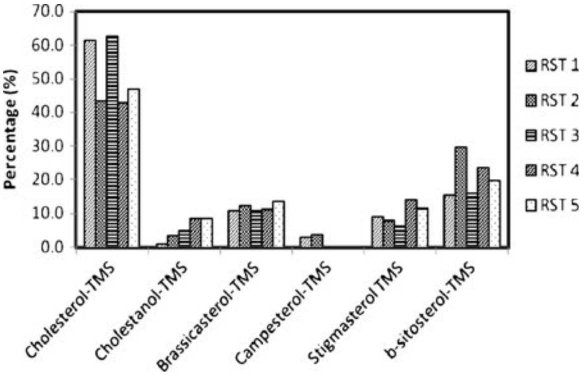

Fig. 3 The relative percentage of individual sterols that have been identified in each station

sterols found at these stations. Cholesterol (Cholest-5-en-3β-ol) is the major C27 sterols, whereas cholestanol (5α-cholestan-3β-ol) occur in relatively lower abundance. Since cholesterol is the major sterol present in animals, its presence in the sediment could not be use to relate to a specific animal source. Nonetheless, its presence in the marine environment could still be used as a general indicator of zooplankton or other marine fauna (such as phytoplankton, diatom and fish) input, since cholesterol is the major sterol for most marine animals (Volkman 1986). The origin for the cholestanol compounds can be traced back to either biological sources (e.g. biosynthesis of plankton organisms, e.g. dinoflagellates; Volkman 1986) or diagenetic transformation (from coprostanols) or hydrogenation (from unsaturated sterols) under anoxic environment.

β-sitosterol (24-ethylcholest-5-en-3β-ol) was the second most abundant sterol encountered at all stations; and along with stigmasterol (ethylcholest-5,22(E)-dien-3β-ol) which represented the C29 sterols, accounted about 21.9–37.7 % of the total identified sterols at these stations. Brassicasterol (24-methylcholesta-5,22-diene-3β-ol) and campesterol (24-methycholest-5-en-3β-ol) were the minor C28 sterols contributed only 10.8–16.0 % of the total identified sterols in these sediments. Campesterol, β-sitosterol and stigmasterol are common sterols in epicuticular waxes of vascular plants (Santos et al. 2008; Mudge and Norris 1997), but they could also be derived from some marine organisms such as algae (Volkman 1986). Brassicasterol is often used as a biomarker for algae lipids since it is present in most marine species (Volkman 1986; Duan 2000). C29/C27 ratios (i.e. *β*-sitosterol/cholesterol and stigmasterol/cholesterol) are also used as a useful indicator to differentiate terrestrial input of organic material from marine source (Mudge and Norris 1997). In general, the C29/C27 sterol ratios calculated for all stations are less than 1 (Table 3), providing further support to the dominance of marine biomass input into the sediments of Redang Island relative to the terrestrial plants source.

Conclusion

The distribution of aliphatic lipids and sterols present in five surface sediments collected from the southern region of Redang Island, South China Sea off the eastern Peninsular of Malaysia have been investigated. All sediment samples contained *n*-alkanes with an odd to even carbon predominance and carbon maximum reflective of biogenic sources corresponding to marine microbial and planktonic input with relatively minor contribution from terrestrial plant waxes. The total *n*-alkanes concentration is still within the United Nations Environment Programme (<10 μg/g) value suggested for biogenic hydrocarbons. The *n*-alkanol fraction is predominated with long chain carbon number homologous and exhibited an even to odd carbon predominance and carbon maximum at nC22, suggesting a significant contribution of vascular terrestrial waxes in this fraction. The presence of *n*-alkanoic acids predominating in short chain carbon homologue and *n*C16:1 and *n*C18:1 on the other hand reflects the important inputs of microorganisms' lipids component. Moreover, cholesterol was found to be the major sterol identified in all samples and together with the sterol diagnostic ratios provide further indication of the importance of marine biomass input (autocthonous input) to the overall organic matter found in sediments of Redang Island. In general, it can be concluded that marine biomass, and to a lesser extent the terrestrial plant waxes, are the main sources of the aliphatic lipids and sterols found in the sediment of southern Redang Island. It is recognised that more work is needed to study the sediment from the coastal areas of the whole island in order to obtain a clearer and more complete picture of the

Table 3 Selected sterols evaluation indices

	RST 1	RST 2	RST 3	RST 4	RST 5
β-Sitosterol/cholesterol	0.249	0.684	0.250	0.550	0.416
Stigmasterol/cholesterol	0.144	0.179	0.099	0.331	0.242

geochemistry of the aliphatic lipids and polar organic compounds in the surrounding area of this tropical island.

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