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Oil Pollution in the Straits of Malacca, Malaysia: Application of Molecular Markers for Source Identification

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The Straits of Malacca is one of the world's busiest supertanker routes, and tanker-derived oil spills occur frequently. In addition, domestic oil spills have been increasing due to growing industrialization of Malaysia. The determination of the exact source of the spills is critically important. This paper examines the utility of biomarker compounds, triterpanes, to identify the source of the oil spills. Middle East crude oils (MECO), South East Asian crude oils (SEACO), tarballs, sediments, and mussels were analyzed. The most distinctive compositional features for the crude oils are that 17α , 21β (H) C_{29} norhopane and C_{31} – C_{35} homohopanes, especially C_{35} homohopanes, are depleted in SEACO. These remarkably different hopane compositions can be explained by the fact that MECO and SEACO are derived from carbonate (marine) and lacustrine/deltaic shale (nonmarine) source rocks, respectively. Two of the eight tarballs samples collected on the coast of Peninsular Malaysia were identified to be derived from Middle East petroleum based on their biomarker signatures. The results of oleanane analyses imply that a low concentration of oleanane does not always indicate a Middle East petroleum contribution although at high concentration oleanane can be a useful biomarker for South East Asian oil sources. Application of the source-identifier to sediment and mussel samples showed the Middle East oil signature. One possible explanation is that Middle East oil is used in formulating Malaysian lubricating oils which are the source of oil found in sediment and mussel samples. This is consistent with the analytical results for the lubricating oil used in Malaysia and the street dust samples.

Introduction

The Straits of Malacca is one of the world's busiest waterways (Figure 1a). In 1998 alone, there were approximately 32 000

different kinds of vessels passing through this narrow and shallow straits (1). Of those vessels, 31% were super-tankers originating from the Middle East, transporting crude oil and petroleum products to Singapore, currently Asia's largest oil refinery center, Japan, Hong Kong, Korea, and China. Major and minor oil spills in the Straits have been occurring very frequently in the past and have affected coastal ecosystems and marine fisheries. They are caused both by accidents due to the shallowness and narrowness of the Straits and by routine tanker operations (e.g. discharge of ballast waters) (1). For example, the latest major accidental spill occurred in late 1997 when the Chinese Tanker *An Tai*, carrying Middle East oil ran aground in the Straits spilling 235 tons of crude oil, threatening to destroy the remaining mangrove forest which lined the central coast of the Western part of Peninsular Malaysia. The Malaysian Marine Department has confirmed that the tanker was carrying Kuwaiti heavy oil. The oil spill also paralyzed the aqua-culture fisheries in the area for 2 months. The length of the coastline affected by the spill is approximately 250 km. In addition to the tanker-derived petroleum pollution, land-based oil input due to rapid urbanization and industrialization coupled with domestic petroleum production in Malaysia contributes to coastal oil pollution. It is therefore very important to determine the exact source of the spills. In this paper, we examine the utility of biomarker compounds to identify sources of the oil pollution in the Strait.

A broad range of biomarker compounds such as isoprenoid alkanes, steranes, hopanes, and polycyclic aromatic hydrocarbons (PAHs) have been proposed as molecular markers to identify the sources of oil pollution. Significance and utility of biomarkers in pollution studies is thoroughly reviewed in Volkman et al. (2). Blumer and co-workers (3) were among the earliest scientists who pioneered fingerprinting of marine pollutant hydrocarbons. Thereafter, numerous workers have extensively used molecular markers for identification of the sources of the pollutants (4–17). Two major classes of biomarkers used in petroleum geochemistry are hopanes (and other triterpanes) and steranes, which are trace oil components. They are persistent to environmental alterations (10, 11) and often have compositions characteristic for sources of petroleum and, therefore, can be used as source identifier of petroleum pollution. Hostettler and Kvenvolden (9) demonstrated the stability of terpanes and steranes in beached oil samples collected from Prince William Sound where *Exxon Valdez* had spilled oil, whereas dramatic disappearance of *n*-alkanes, isoprenoid, and many of PAHs in the oils was observed. The molecular distributions of terpanes and steranes as well as carbon isotope values were successfully applied to differentiate *Exxon Valdez*-derived oil from Californian oils derived from Miocene Monterey formation which had been introduced to the Alaskan region prior to the oil spill (8, 9, 12, 15). 18α (H)-oleanane has also been used for source identification of *Exxon Valdez* oil spill (15). Wang et al. (11, 14) identified oil residues weathered in environments for more than 10 years after oil spills using hopane and sterane fingerprints.

In South East Asia, there are large-scale oil fields and the organic geochemistries of the crude oils in the area (i.e., SEACO) have been extensively studied (e.g., 18–21). The oil fields together with heavy tanker traffic are potential sources of petroleum pollution to this area. However, there have been limited numbers of applications of biomarkers to oil pollution studies in this area. SEACO are characterized by biomarker compounds that include the presence of abundant plant-derived pentacyclic triterpanes (i.e., oleananes and bicadi-

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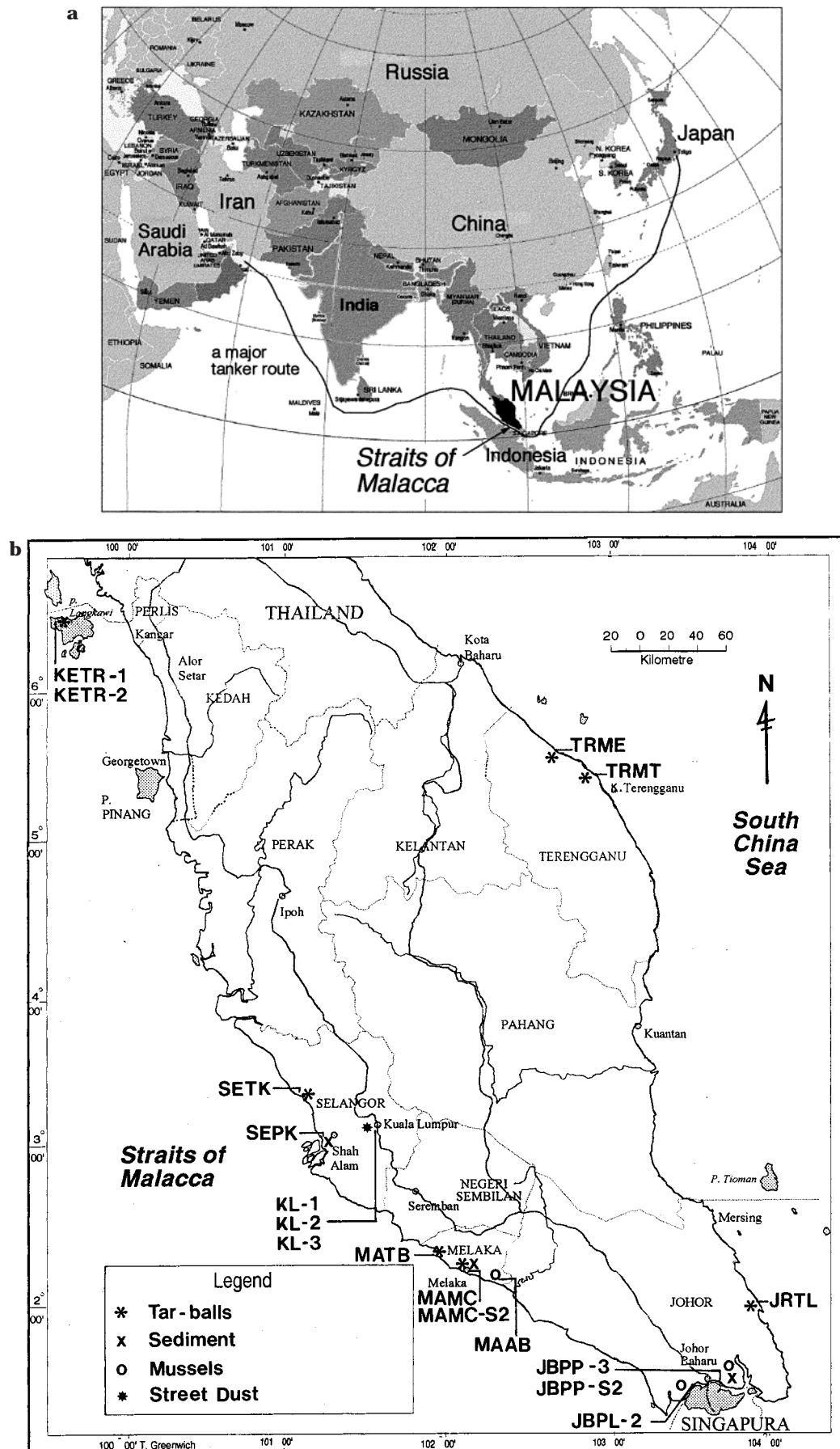


FIGURE 1. (a) Location of the Straits of Malacca, Malaysia. (b) Sampling locations for tar-ball, sediment, mussel, and street dust in Peninsular Malaysia.

TABLE 1. Sample Description for Tar-Balls, Sediments, Mussels, and Street Dust

station	sample code	date of sampling	description of site	location
Tar-Balls				
Tanjung Rhu, Langkawi	KETR-T1	Dec 25, 1997	remote area	West Coast
Tanjung Rhu, Langkawi	KETR-T2	Sep 09, 1998	remote area	West Coast
Tanjung Karang, Selangor	SETK	Dec 28, 1997	industrial area	West Coast
Malacca City, Malacca	MAMC-T2	Sep 23, 1998	industrial and urban area	West Coast
Tanjung Bidara, Malacca	METB	Sep 23, 1998	industrial area	West Coast
Merang, Terengganu	TRME	May 28, 1998	remote area	East Coast
Mengabang Telipot, Terengganu	TRMT	Sep 20, 1998	remote area	East Coast
Tanjung Leman, Johor	JRTL	May 30, 1998	remote area	East Coast
Sediment				
Malacca City, Malacca	MAMC-S2	Sep 22, 1998	industrial and urban area	West Coast
Pasir Puteh, Johor Bahru	JBPP-S2	Sep 23, 1998	industrial and urban area	West Coast
Port Klang, Selangor	SEPK	Sep 27, 1998	industrial and urban area	West Coast
Mussel (Green mussel; <i>Perna viridis</i>)				
Pasir Puteh, Johor Bahru	JBPP-3	Sep 23, 1998	industrial and urban area	West Coast
Pantai Lido, Johor Bahru	JBPL-2	Sep 23, 1998	industrial and urban area	West Coast
Anjung Batu, Malacca	MAAB	Sep 22, 1998	industrial area	West Coast
Street Dust				
Kuala Lumpur City 1	KL 1	Sep 24, 1998	heavy trafficked	City Center
Kuala Lumpur City 2	KL 2	Sep 24, 1998	heavy trafficked	City Center
Kuala Lumpur City 3	KL 3	Sep 24, 1998	heavy trafficked	City Center

nanes (19)). Currie et al. (22) proposed the utility of the triterpanes to distinguish tar-balls originated from South East Asian petroleum from those of Australian petroleum sources through their findings of oleanane and bicadinanes in coastal bitumens collected from Western Australian coast. However, the utility of the biomarkers for source identification of petroleum pollution occurring in the South East Asian coast has not been examined.

The main objective of the present study is to develop a molecular marker approach for source-identification of tar balls in the Straits of Malacca. The Middle East crude oils (MECO) and South East Asian crude oils (SEACO) and tar-balls collected on Malaysian beaches were analyzed for petroleum hydrocarbons. Our focus is triterpanes, including oleananes, due to their persistence and the source specific nature of their molecular distributions. The second objective is to apply the biomarker approach to sediments and biological samples (i.e., mussels) collected in Peninsular Malaysia.

Experimental Section

Sample Collection. The map of the sampling location and detail description of the sampling locations is described in Figure 1 and Table 1, respectively.

Source Petroleum. Crude oil samples including three Middle East oils (Marban, Arabian Light, and Ummu Shaif) and four South East Asian oils (Tapis, Labuan, Miri, and Sumatra) were kindly supplied by the Maritime Agency of Japan. Two lubricating oil samples were randomly purchased from the Malaysian market.

Tar-Ball Samples. Eight tar-ball samples were collected by hand using clean plastic gloves in the tidal zones from seven locations of the west and east coast of Peninsular Malaysia. Two samples MYKETR-T1 and MYKETR-T2 were taken from the same location but of two different dates. The samples were wrapped with aluminum foil, stored in clean plastic *Zip-Lock* bags, kept in a cooler box, transported to the laboratory, and stored at -18°C until analysis.

The Other Environmental Samples. Sediment samples were collected by Grab sampler from three locations in the coastal beaches and an estuary of Malaysia. The samples were stored in tight-sealed solvent-rinsed stainless steel containers and transported on ice to the laboratory. The sediment samples were stored at -18°C until further analysis.

Mussels were collected from jetties, navigational buoys, and aqua-culture cages from three different locations in the West Coast of Peninsular Malaysia. The mussels with shells were stored in *Zip-Lock* bags and stored on ice and transported to the laboratory. In the laboratory, the mussels were shucked with stainless steel scalpels, and whole body tissues were stored in tight-sealed solvent-rinsed stainless steel containers at -18°C until further analysis. Street dust samples were collected from three of the busiest streets of the city of Kuala Lumpur using a straw brush. The samples were stored in solvent-rinsed tight-sealed vial with Teflon-lined cap and transported to the laboratory and stored at -18°C until further analysis.

Analytical Procedure

Chemicals. Authentic standards of $n\text{-C}_{16}$, C_{18} , C_{20} , C_{22} , C_{24} , C_{26} , C_{28} , C_{32} , and C_{36} were purchased from Wako pure Chemical Ltd. $17\beta,21(\text{H})\beta$ -hopane, $17\alpha(\text{H})$ -22,29,30-trisnor-hopane (Tm), $17\beta(\text{H})$, $21\alpha(\text{H})$ -norhopane (C_{29} 17β), $17\alpha(\text{H})$, $21\beta(\text{H})$ -hopane (C_{30} 17α), $17\beta(\text{H})$, $21\alpha(\text{H})$ -hopane (C_{30} 17β), $18\alpha(\text{H})$ -oleanane, and $18\beta(\text{H})$ -oleanane were purchased from Chiron, Norway. A 100–200 mesh silica gel (F.C.923) from Davison Chemical Corp. was baked at 400°C for ~ 4 h, cooled, then activated at 200°C overnight, and deactivated with 5% (w/w) of distilled water. A 60–200 mesh silicic acid (SIL-A-200) from Sigma Chem. was rinsed with methanol and dichloromethane (DCM) using Soxhlet extraction, dried at 50°C , fully activated at 200°C overnight, and stored in dry n -hexane. Organic solvents were distilled in glass before use. All glassware was washed with detergents and tap water and rinsed successively with methanol, DCM, and distilled hexane.

Extraction and Fractionation. Crude oil and tar-ball samples (ca. 10 mg) were precisely weighted, dissolved in 1 mL of DCM/hexane (1:3, v/v), and transferred onto the top of 5% H_2O deactivated silica gel column (1 cm i.d. \times 9 cm). Hydrocarbons ranging from n -alkanes to PAHs with seven rings were eluted with 20 mL of DCM/hexane (1:3, v/v). The sample was then reduced in volume to ~ 5 mL where approximately 5 g of activated copper was added and allowed to stand overnight to react with elemental sulfur. The solution was passed through a glass funnel plugged with quartz wool, which trapped copper and the copper sulfide on it. The filtrates were rotoevaporated just to dryness, subsequently dissolved into 0.4 mL of n -hexane, and transferred onto the

fully activated silica gel column (0.47 cm i.d. \times 18 cm). Three fractions were eluted. The first fraction containing aliphatic and alicyclic hydrocarbons was eluted with 4 mL of hexane. The second fraction containing the alkylbenzenes and PCBs was eluted with 18 mL of hexane. The third fraction eluted with 7 mL of hexane/DCM (3:1, v/v) contained the higher molecular weight PAHs with 3–7 benzene rings. The first hexane fraction was evaporated and transferred to a 1.5-mL glass ampule. The hexane was evaporated just to dryness under nitrogen and redissolved in 50 μ L–1 mL of isooctane for subsequent instrumental analysis. The fractionation procedure is based on the method described in Takada et al. (23) and described in detail elsewhere (24).

Sediment samples were freeze-dried and Soxhlet extracted using DCM over 8 h. Wet mussel tissue (ca. 10 g) was macerated/extracted with DCM and prebaked sodium sulfate anhydrous in a glass centrifuge tube by Polytron (RT2000; Kinebatica). The sediment and mussel extracts were purified and fractionated using the same procedure as the oil samples. For the mussel samples, some coeluting materials in the alkane fractions were hydrolyzed with KOH/MeOH prior to alkane and hopane analyses.

Instrumental Analysis. Normal and isoprenoid alkanes were analyzed on Shimadzu 14 B gas chromatograph with flame ionization detector. A J&W Scientific Durabond DB-5, 30 m fused silica capillary column, 0.25 mm i.d. and 0.25 μ m film thickness was used with helium as the carrier gas at 200 kPa. The injection port was maintained at 300 $^{\circ}$ C, and the sample was injected with splitless mode followed by a 1 min purge after the injection. Column temperature was held at 70 $^{\circ}$ C for 1 min, then programmed at 30 $^{\circ}$ C/min to 150 $^{\circ}$ C, 5 $^{\circ}$ C/min to 310 $^{\circ}$ C, and held for 10 min. The detector temperature was held at 310 $^{\circ}$ C. Normal alkanes ranging from C₁₆ to C₃₆, pristane and phytane were determined. Identification of sample peaks was based on comparison of retention time of the authentic standard run on the same day and confirmed by GC-MS. Quantification was based on peak height, and the response factor was calculated through the standard run. Response factors for compounds with no corresponding standards were estimated by proportional allotment using standards which bracket the target peak on the chromatogram.

After the alkane analysis, the aliphatic and alicyclic hydrocarbon fraction was evaporated to dryness and redissolved into an appropriate volume (50–200 μ L) of isooctane containing 5 ppm of 17 β , 21 β (H)-hopane as an internal injection standard for triterpane analysis. Hopane analyses were made using a Hewlett-Packard 5972A quadrupole mass spectrometer integrated with a HP5890 gas chromatograph equipped with a J&W Scientific Durabond HP-5MS, 30 m fused silica capillary column, 0.25 mm i.d. and 0.25 μ m film thickness, using helium as the carrier gas on a constant flow rate mode at 1 mL/min. GC-MS operating conditions were 70 eV ionization potential with the source at 200 $^{\circ}$ C and electron multiplier voltage at \sim 2000 eV. The injection port was maintained at 300 $^{\circ}$ C, and the sample was injected with splitless mode followed by purge 1 min after the injection. Column temperature was held at 50 $^{\circ}$ C for 2 min, then programmed at 6 $^{\circ}$ C/min to 300 $^{\circ}$ C, and held for 15 min. A selected ion monitoring method was employed after a delay of 4 min, and triterpanes were quantified at m/z = 191. Peaks were identified by comparison of their retention times with those for the standards and their mass spectra, which were obtained on a different GC-MS run on Scan mode, with those in the literature (25, 26). Quantification was made by peak area. Response factor of 18 α (H)-22,29,30-trisnorhopane (Ts) was assumed to be the same as Tm, 17 α (H), 21 β (H)-30-norhopane (C₂₉ 17 α) as C₂₉ 17 β , and homohopanes ranging from C₃₁–C₃₅ of carbon number as C₃₀ 17 β . Because 18 α (H)-oleanane and 18 β (H)-oleanane cannot be separated on

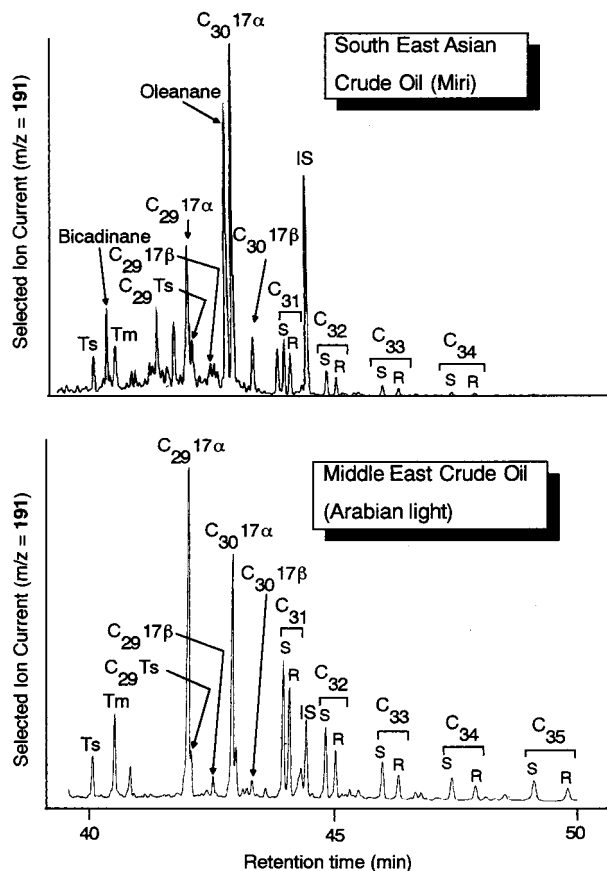


FIGURE 2. Gas chromatograms of triterpanes in South East Asian Crude Oil (SEACO) and Middle East Crude Oil (MECO). Ts: 18 α (H), 21 β (H), 22,29,30-trisnorhopane; Tm: 17 α (H), 21 β (H), 22,29,30-trisnorhopane; C₂₉ 17 α : 17 α (H), 21 β (H)-norhopane; C₂₉ 17 β : 17 β (H), 21 α (H)-norhopane; C₃₀ 17 α : 17 α (H), 21 β (H)-hopane; C₃₀ 17 β : 17 β (H), 21 α (H)-hopane; C₃₁ to C₃₅: homohopanes consisting of C₃₁ to C₃₅ carbons; S and R means stereoisomer at 22 carbon, respectively. IS: Internal Injection Standard (17 β (H),21 β (H)-hopane).

the gas chromatogram, an average of response factors of both isomers obtained from independent runs was applied to the samples. Differences in response factors of both oleanane isomers were within 10%, and, therefore, using the averaged factor it has not caused significant errors. For another set of stereoisomers (C₃₀ 17 α and C₃₀ 17 β), the differences in their response factors were within 20%. These suggest that the expected differences in response factors for the other set of stereoisomers (e.g. Ts and Tm, C₂₉ 17 α and C₂₉ 17 β) are within 20%.

Reproducibility of the whole analytical procedure was checked through the triplicate analysis of an oil sample. Relative standard deviations of individual alkanes and triterpane concentrations were less than 11% and 13%, respectively. Recoveries were determined by spiking a mixture of the standards listed above to the oil samples followed by the entire analytical procedure. Recovery of the spiked standards was more than 82% for alkanes and over 87% for triterpanes.

Results and Discussion

Figure 2 shows typical gas chromatograms of pentacyclic triterpanes monitored by the mass selective detector of Middle East crude oil (MECO) and South East Asian crude oil (SEACO). For both crude oils, 17 α , 21 β (H) C₃₀ hopane (C₃₀ 17 α) and 17 α , 21 β (H) C₂₉ hopane (C₂₉ 17 α) are dominant with stair-step progression of C₃₁ to C₃₅ homohopanes. The

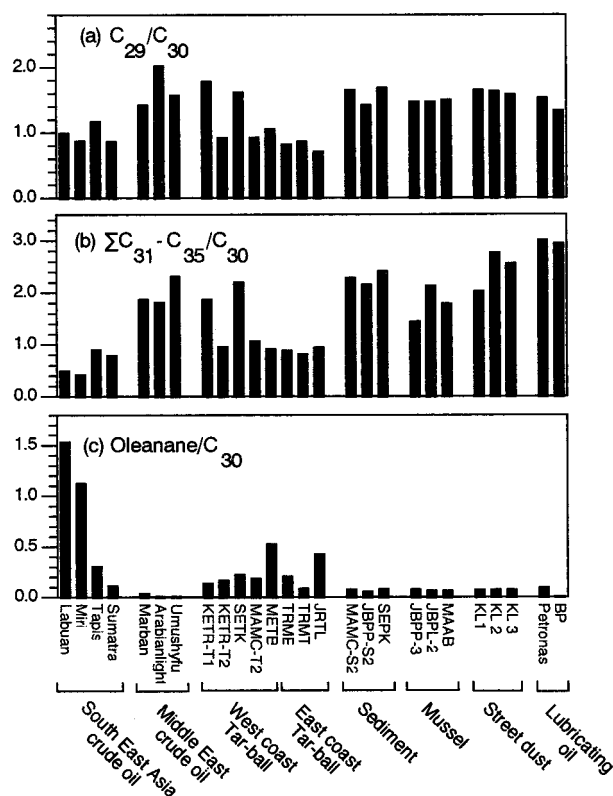


FIGURE 3. Triterpane compositions in crude oils, lubricating oils used in Malaysia, tar-ball, sediment, mussel, and street dust samples collected from coasts of Peninsular Malaysia. (a) C_{29}/C_{30} : a ratio of 17α 21β (H) C_{29} hopane to 17α 21β (H) C_{30} hopane. (b) $\Sigma C_{31}-C_{35}/C_{30}$: a ratio of sum of homohopanes ranging from C_{31} to C_{35} to 17α 21β (H) C_{30} hopane. (c) Oleanane/ C_{30} : a ratio of oleanane to 17α 21β (H) C_{30} hopane.

most distinctive compositional features are that C_{29} 17α is dominant over C_{30} 17α in MECO, and homohopanes ranging from C_{31} to C_{35} are depleted in SEACO. Similarities and differences in these chromatographic patterns can be described by molecular ratios normalized by C_{30} 17α , that is the C_{29}/C_{30} ratio and the $\Sigma C_{31}-C_{35}/C_{30}$ ratio. Figure 3 and Table 2 summarize the ratios for the crude oil samples. A consistent pattern of higher C_{29}/C_{30} ratio (1.41–2.01) and higher $\Sigma C_{31}-C_{35}/C_{30}$ ratio (1.80–2.30) were observed for Middle East crude oil, with lower ratios for South East Asian crude oils (0.85–1.16 and 0.41–0.89, respectively). These characteristics can be explained by a difference in geological conditions during petroleum formation. Middle East petroleum is derived from marine carbonate source rocks and abundant in C_{29} 17α hopane and $\Sigma C_{31}-C_{35}$ 17α (H), 21β (H), $22S$ and $22R$ homohopanes (2). Most oils of South East Asian origin are derived from nonmarine (i.e. lacustrine, fluvio-deltaic) shale source rocks (27, 28) which are characterized by low content of tricyclic terpanes and homohopanes (20, 21) and by lower C_{29}/C_{30} ratios (18). The differences we found for the analyzed oils (i.e. C_{29}/C_{30} and $\Sigma C_{31}-C_{35}/C_{30}$ ratios) are reasonable and, therefore, are candidates for source-identifiers of oil spill in the Straits of Malacca.

As previously indicated by Sosrowidjojo et al. (19), oleanane was abundant in some of the SEACO, while there is no significant oleanane in the MECO (Figure 3). This is because SEACO received higher plant contributions which are related to lacustrine deposition environment and MECO are of marine origin. Biscadinanes, another indicator of higher plant contribution (19), were also tentatively identified (Figure 2). However, oleanane contents in SEACO varied with oleanane/ C_{30} ratio ranging from 0.11 to 1.53 (Table 2, Figure

3). The large variation in oleanane/ C_{30} ratio has been reported by Okui et al. (20, 21). This can be explained by spatial heterogeneity of higher plant inputs, and/or the oleanane/ C_{30} ratio can be affected by thermal maturation of petroleum (20, 21). Both the C_{29}/C_{30} ratio and $\Sigma C_{31}-C_{35}/C_{30}$ ratio, on the other hand, are more consistent within SEACO, probably because the ratios are related to geological parameters which are common for wider areas than input/maturity parameters (i.e., oleanane/ C_{30} ratio). The C_{29}/C_{30} and $\Sigma C_{31}-C_{35}/C_{30}$ ratios could be more reliable source-identifiers.

Table 2 includes some other possible source parameters. The homohopane index percentage of C_{35} homohopanes ($22S + 22R$) relative to the sum of C_{31} to C_{35} homohopanes has been applied as an indicator of redox condition of marine sediments during diagenesis in the field of petroleum geology (25). A higher homohopane index is interpreted as an indication of highly reducing marine depositional conditions (25). The homohopane index clearly distinguishes MECO (9.2%–11.2%) from SEACO (0%–2.9%). Tm/Ts (17α - $22,29,30$ -trisorhopane/ 18α - $22,29,30$ -trisorneohopane) and $C_{31}S/(S+R)$ ($17\alpha,21\beta$ (H)-homohopane ($22S$)/ $17\alpha,21\beta$ (H)-homohopane ($22S$) + ($22R$)) were applied to some oil spill studies (9, 11, 14). Both parameters, however, do not show consistent differences between MECO and SEACO. This is partially due to these parameters being maturity parameters as well as source parameters. Probably both groups (MECO and SEACO) consist of oils with different stages of maturation.

Among the eight tar-ball samples, tar-balls taken from Langkawi (KETR-T1) and Tanjung Karang (SETK) showed higher C_{29}/C_{30} ratio (1.77 and 1.61) and $\Sigma C_{31}-C_{35}/C_{30}$ ratio (1.86 and 2.19) than the others (0.81–1.04, 0.80–1.05, respectively, Figure 3 and Table 2). This indicates that these two tar-balls were derived from Middle East petroleum. These patterns are expressed by grouping on a diagram using the two ratios, as shown in Figure 4a. Plots for the crude oil samples on C_{29}/C_{30} vs $\Sigma C_{31}-C_{35}/C_{30}$ diagram are categorized into two distinctive groups: one for the Middle East and another for South East Asian. Plots for the tar-ball samples clearly fall into the two categories on the diagram (Figure 4a). SETK and KETR-T1 come under the Middle East oil category. This compelling evidence indicates that the tar-ball had originated from the Middle East oil. Homohopane index gave supportive evidence for this estimation. The index was higher for SETK (12.4%) and KETR-T1 (8.7%) than the others (2.1%–6.2%). These estimations are consistent with the Tanjung Karang sample (SETK) which was collected just after the oil spill accident caused by the Chinese tanker carrying Middle East oil (Kuwaiti heavy oil). As for KETR-T1, the sampling location is situated on the beach which is directly exposed to the prevailing ocean currents (North East Monsoon) as well as the entry point into the Straits of supertankers originating from Middle East. Oils spilled from these tankers may have been deposited by currents to the beaches. However, further studies have to be done to confirm this suggestion. KETR-T2, on the other hand, was collected in different date and was found to be of domestic origin.

The rest of the tar-ball samples are plotted within the South East Asian oil category, indicating they are from local sources (Figure 4a). This is also consistent with the geographical situations that all the east coast samples (i.e. TRME, TRMT and JRTL) show the SEACO signature. Malaysian east coast has much less traffic of super tankers from Middle East and has some off-shore oil wells. The tar-balls found on the east coast are probably derived from some spills from the petroleum platforms. The east coast normally experience an intense North East Monsoon from November to January which can strand tar-balls from the off-shore oil platforms onto the beaches although there is no such study to support this evidence. Some samples from the west coasts (i.e., the Straits of Malacca) have probably received spillage from

TABLE 2. *n*-Alkanes and Biomarker Compounds for Middle East and Malaysian Crude Oils, Lubricating Oil, and Tar-Balls

sample	description/ origin	alkanes (FID trace)					triterpanes (<i>m/z</i> = 191)						
		Pr/Ph ^a	L/H ^b	CPI ^c	ΣAlk ^d (mg/g)	ΣAlk/ ΣHop ^e	ΣHop ^f (μg/g)	C ₂₉ /C ₃₀ ^g	ΣC ₃₁ –C ₃₅ / C ₃₀ ^h	Tm/Ts ⁱ	C ₃₁ S/ (S+R) ^j	oleanane/ C ₃₀ ^k	homohopane index ^l
Middle East Crude Oil (MECO)													
Marban	U.A.E.	0.94	3.92	0.93	49.0	147	333	1.41	1.86	0.35	0.576	0.03	9.2
Arabian Light	Saudi Arabia	0.64	4.60	0.96	24.9	15	1683	2.01	1.80	2.31	0.563	0	10.1
Umm Shaif	U.A.E.	0.97	3.35	0.96	37.9	42	899	1.56	2.30	0.76	0.569	0	11.2
mean		0.90	3.96	0.90	37.2	68	972	1.66	1.99	1.14	0.569	0.001	10.2
South East Asian Crude Oil (SEACO)													
Labuan	Malaysia	4.01	2.43	1.15	98.9	130	760	0.98	0.48	2.20	0.589	1.53	0
Miri	Malaysia	4.03	2.45	1.34	52.1	34	1535	0.85	0.41	1.49	0.553	1.12	1.5
Tapis	Malaysia	3.66	4.52	1.12	127.9	204	626	1.16	0.89	1.43	0.508	0.30	2.0
Sumatra	Indonesia	2.91	1.62	1.07	109.1	141	773	0.85	0.78	0.52	0.582	0.11	2.9
mean		3.65	2.75	1.17	97.0	127	923	0.96	0.64	1.41	0.558	0.77	1.6
Lubricating Oil													
Petronas	Malaysia	0.34	0.48	1.39	2.1	0.53	3959	1.52	3.00	0.70	0.551	0.09	9.8
BP	Malaysia	0	0.29	1.13	3.4	0.62	5362	1.32	2.93	1.00	0.570	0	11.5
Tar-Balls													
KETR-T1	West Coast	1.60	1.59	0.99	11.1	31	357	1.77	1.86	1.97	0.566	0.13	8.7
KETR-T2	West Coast	2.36	1.24	1.07	100.7	133	757	0.83	0.94	0.59	0.570	0.16	6.2
SETK	West Coast	0.42	1.72	1.03	4.4	12	373	1.61	2.19	1.59	0.590	0.22	12.4
MAMC-T2	West Coast	1.14	0.21	1.09	53.7	24	2217	0.91	1.05	0.70	0.570	0.18	5.9
METB	West Coast	4.14	1.07	1.09	174.9	113	1550	1.04	0.90	2.11	0.513	0.52	2.1
TRME	East Coast	2.19	0.97	1.08	20.1	69	290	0.81	0.87	0.52	0.576	0.20	4.8
TRMT	East Coast	2.10	1.31	1.05	108.1	59	1849	0.85	0.80	0.58	0.561	0.08	3.7
JRTL	East Coast	1.70	0.75	1.05	16.4	110	150	0.70	0.93	0.71	0.570	0.42	5.7

^a Pr/Ph = a ratio of pristane to phytane. ^b L/H = sum of *n*-C₁₆-*n*-C₂₆ alkane relative to sum of *n*-C₂₇-*n*-C₃₆ alkane. ^c CPI = 1/2[(*n*-C₂₅ + *n*-C₂₇ + *n*-C₂₉ + *n*-C₃₁ + *n*-C₃₃)/(*n*-C₂₄ + *n*-C₂₆ + *n*-C₂₈ + *n*-C₃₀ + *n*-C₃₂) + (*n*-C₂₅ + *n*-C₂₇ + *n*-C₂₉ + *n*-C₃₁ + *n*-C₃₃)/(*n*-C₂₆ + *n*-C₂₈ + *n*-C₃₀ + *n*-C₃₂ + *n*-C₃₄)]. ^d ΣAlk = sum of *n*-alkane ranging from C₁₆ to C₃₆. ^e ΣAlk/ΣHop = a ratio of ΣAlk to ΣHop. ^f ΣHop = sum of triterpanes identified in Figure 2. ^g C₂₉/C₃₀ = a ratio of 17α,21β(H)-30-norhopane to 17α,21β(H)-hopane. ^h ΣC₃₁-C₃₅/C₃₀ = a ratio of sum of 17α,21β(H)-C₃₁ homohopane to 17α,21β(H)-C₃₅ homohopane relative to 17α,21β(H)-hopane. ⁱ Tm/Ts = 17α-22,29,30-trinorhopane/18α-22,29,30-trisnorhopane. ^j C₃₁S/(S+R) = 17α,21β(H)-C₃₁ homohopane(22S)/17α,21β(H)-C₃₁ homohopane(22S)+(22R). ^k Oleanane/C₃₀ = a ratio of oleanane to 17α,21β(H)-hopane. ^l Homohopane index = a percentage of 17α,21β(H)-C₃₅ homohopane to sum of 17α,21β(H)-C₃₁ homohopane to 17α,21β(H)-C₃₅ homohopane.

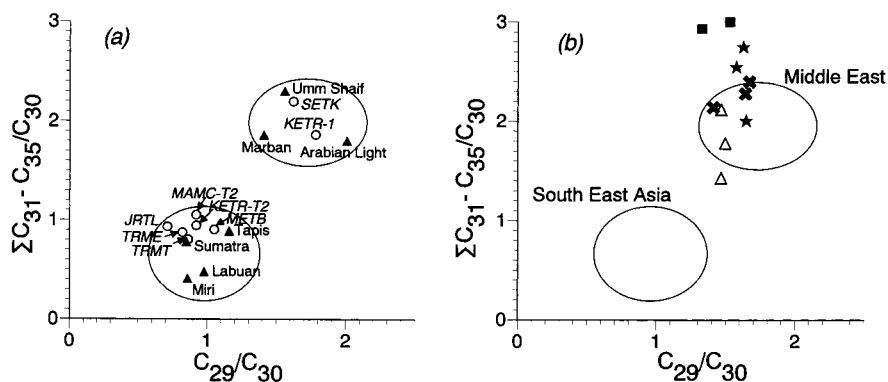


FIGURE 4. C_{29}/C_{30} vs $\Sigma C_{31}-C_{35}/C_{30}$ diagrams: (a) the crude oil samples (closed triangle) and the tar ball samples (open circle) and (b) the sediment (cross), mussel (open triangle), street dust (star), and lubricating oil (closed square) samples. The circles are drawn based on the data for the crude oils in panel (a).

domestic oil. For example, the tar-ball sample MAMC-T2 whose hopane composition shows SEACO signature was collected from Malacca city where there are oil refineries. Oil seeps may be another possible contributor to tar-balls having SEACO signature. However, there is no oil seep occurring in the Straits of Malacca (Boo, F., Marine Environmental Research for Petronas, Malaysia, personal communication, 1999).

Classification by oleanane does not conflict with that based on C_{29}/C_{30} and $\Sigma C_{31}-C_{35}/C_{30}$ ratios. SETK and KETR-T1, which are categorized in the MECO origin by the hopane ratios, have only trace amounts of oleanane. Two of the tar-ball samples (METB and JRTL) in SEACO category based on the hopane ratios have also a higher oleanane/C₃₀ ratio (Figure 3). However, the other samples in the SEACO category show only trace amounts of oleanane. This is probably due

to the variability in oleanane contents in SEACOs, as shown in Figure 3. The results imply that low concentration of oleanane does not always indicate Middle East petroleum contribution although at high concentration oleanane can still be a useful biomarker for South East Asian oil sources.

Results of GC-FID analysis (i.e. *n*-alkanes and isoprenoid alkanes) for the crude oils and the tar-balls gave supportive evidence for the source-identification by triterpanes and provide information on sources and weathering. In all the tar-ball samples *n*-alkanes were predominant, and the familiar "hump" of the unresolved complex mixture (UCM) indicating the oil has undergone weathering and degradation (2) is noticeably absent. This may suggest that the tar-balls collected have a short residence time on the sea which is not enough for extensive weathering and have just been deposited on the beach. However, relative enrichment of wax com-

TABLE 3. *n*-Alkanes and Biomarker Compounds for Sediments, Mussels, and Street Dust

sample	description/ origin	alkanes (FID trace)						triterpanes (<i>m/z</i> = 191)				
		Pr/Ph ^a	CPI ^c	ΣAlk/ ^d (μg/g)	ΣAlk/ ΣHop ^e	ΣHop/ ^f (ng/g)	C ₂₉ /C ₃₀ ^g	ΣC ₃₁ –C ₃₅ / C ₃₀ ^h	Tm/Ts ⁱ	C ₃₁ S/ (S+R) ^j	oleanane/ C ₃₀ ^k	homohopane index ^l
Sediments												
MAMC-S2	West Coast	2.76	2.04	8.17	3	2799	1.64	2.28	0.84	0.534	0.07	10.2
JBPP-S2	West Coast	3.06	2.58	4.67	2	2404	1.41	2.14	0.82	0.553	0.05	9.8
SEPK	West Coast	2.67	2.89	0.69	7	98	1.67	2.40	0.89	0.443	0.07	8.9
Mussels												
JBPP-3	West Coast	4.45	1.19	0.655	7	91	1.46	1.43	0.57	0.548	0.07	7.2
JBPL-2	West Coast	12.00	1.30	0.498	22	23	1.46	1.66	0.56	0.547	0.06	7.1
MAAB	West Coast	9.25	1.60	0.191	3	55	1.49	1.78	0.71	0.471	0.06	5.7
Street Dust												
KL 1	City Center	1.39	1.10	460	22	21259	1.64	2.47	1.12	0.554	0.07	12.3
KL 2	City Center	2.13	1.89	65	8	6745	1.62	2.75	1.41	0.558	0.07	13.8
KL 3	City Center	0.59	1.17	50	10	5034	1.57	2.55	1.33	0.554	0.07	14.0

^{a,c–l} See legend of Table 2.

^{a,c–l} See legend of Table 2.

ponents (i.e. *n*-alkanes) in tar in ballast waters discharged from tankers may have occurred due to fractionation during cooling of the oil cargoes (29). The observed depletion of UCM (i.e., enrichment of *n*-alkanes) may also indicate that the tar-balls are derived from ballast waters from tankers (domestic and international). To examine both possibilities (weathering vs ballast water origin) future determination of some other molecular markers that can provide information on weathering will be helpful. Good candidates are PAHs, some of which are susceptible to photodegradation, and their molecular distribution may be used as an index of weathering.

MECO tend to be more abundant in lower molecular weight alkanes than SEACO. This trend is expressed by L/H ratio, that is a ratio of sum of C₁₆–C₂₆ normal alkanes to sum of C₂₇ to C₃₆ alkanes. L/H ratio for MECO was 4.0 ± 0.6 on average, while 2.8 ± 1.2 for SEACO with an exceptional higher value (4.52) for Tapis crude oil (Table 2). Due to the environmental alterations, low molecular weight hydrocarbons were depleted in tar-ball samples, with L/H ratio ranging from 0.21 to 1.72. SETK and KETR-T1, both of which are suspected as MECO origin using triterpane ratios, have relatively higher L/H ratio (1.72 and 1.59) than the others (Table 2).

A ratio of two isoprenoid alkanes (i.e., pristane/phytane ratio) has been used in petroleum geochemistry as an indicator of redox potential in the source rock, although thermal maturity and source input could interfere with the estimation (25). High pristane/phytane ratios (> 3.0) and low values (< 0.6) indicate terrestrial organic matter input under oxic and hypersaline anoxic conditions, respectively, whereas intermediate values are not so distinctive due to the interference (25). Pristane/phytane ratios were found to be distinctive of MECO (0.64–0.97) and SEACO (2.91–4.03) as indicated in Table 2. This seems to be consistent with the above explanations that MECO were generated in marine deposit under reducing conditions and SEACO receive terrestrial input under oxic conditions. However, the pattern for the tar-ball samples was found to be not so distinctive. SETK and KETR-T1 have slightly lower ratios (0.42–1.60) than the others (1.14–4.14). This ambiguousness is probably due to different stages of maturity of source petroleum.

Finally, we tried to apply the source-identifier (C₂₉/C₃₀ ratio and ΣC₃₁–C₃₅/C₃₀ ratio), which was successful for the tar-ball samples, to the sediment and mussel samples. As shown in Figure 3 and Table 3, mussels and sediments samples had considerably higher values of C₂₉/C₃₀ and ΣC₃₁–C₃₅/C₃₀ ratios and homohopane index and trace amounts of oleanane. All these characteristics indicate that hydrocarbons in the sediments and mussels are of Middle East sources. On

the C₂₉/C₃₀–ΣC₃₁–C₃₅/C₃₀ diagram the sediment and mussel samples fall into Middle East category (Figure 4b). This seems to be a little bit strange. For example, the sediment sample SEPK was collected from an upper estuarine station which is somewhat difficult to receive any spilled oil from tankers carrying MECO. One possible explanation is that Middle East oil is imported and used in the making of Malaysian lubricating oils which are the source of the petroleum found in the sediments and mussel samples. One of the reasons why the imported petroleum instead of domestics is used for Malaysian lubricating oil could be the waxy nature of Malaysian crude oil. This scenario is consistent with higher C₂₉/C₃₀ and ΣC₃₁–C₃₅/C₃₀ ratios for the lubricating oil used in Malaysia and the street dust samples (Figure 3). These biomarker compounds in the street dusts might have come from lubricating oils of the motor vehicles. The compounds may have reached and accumulated in sediments and mussels via street runoffs. For mussels, however, the possibility of differential uptake of individual hopanes should be examined, because their high-molecular-weight and bulky nature may interfere the passing through the cell membranes. Also, the number of sediment and mussel samples was limited, and therefore more samples should be analyzed to examine the above scenario in future study.

Overall, the analytical results in the present study clearly demonstrated the utility of C₂₉/C₃₀ ratio, ΣC₃₁–C₃₅/C₃₀ ratio, and homohopane index as molecular tools to distinguish the source of petroleum (i.e. Middle East vs South East Asian petroleum) in the Straits of Malacca. South East Asian crude oils are characterized by lower C₂₉/C₃₀ ratio, ΣC₃₁–C₃₅/C₃₀ ratio, and homohopane index, and they are based on organic geochemical background concerning the petroleum formation (i.e. their lacustrine/deltaic shale source rocks). Two of the five tar-ball samples collected along the West Coast of Peninsular Malaysia are derived from MECO, based on the biomarker ratios. Results of *n*-alkanes and isoprenoid alkanes analysis with GC-FID for crude oils and tar-balls gave additional supportive evidence for the source-identification by triterpanes and provide background information on weathering. On the other hand, due to high variability of oleanane contents in SEACO, it was concluded that oleananes are not definitive markers but supportive markers for the case of the Straits. High concentrations of oleanane indicated their South East Asian origin, but low concentrations do not always indicate Middle East origins. We found that application of the source-identifiers to the sediment and mussel samples were not as successful as that of the tar-ball samples and the characteristics of biomarker compounds are indicative of Middle East sources. One possible explanation is that Middle

East oil is used for the formulation of lubricating oil in Malaysia. For mussel and sediment samples, alternative molecular markers should be developed. Because domestic oil pollution is sometimes derived from land-based sources, concurrent determination of anthropogenic markers of land-based pollution (e.g. linear alkylbenzenes and coprostanols, (30, 31)) may be useful to distinguish domestic oil pollution from that of the tanker-derived pollution. PAHs could be a useful clue for source-identification of petroleum pollution as well as for index of weathering. We are examining the utility of these approaches as well as applying the triterpane markers to a larger number of tar-ball samples collected all over the Malaysian coasts for their source-identification.

Acknowledgments

We are grateful to Mr. Ali Mashinchian for his cooperation in collecting samples and shucking the mussels. We thank Maritime Agency of Japan for providing crude oil samples for our analysis, Dr. A. Okui for providing information on organic geochemistry of South East Asian petroleum, and Dr. N. Ogura for valuable comments, suggestions and encouragement. We also thank Nissan Foundation of Japan for financial support for this project. This study was also supported by a Grant-in-Aid from the International Scientific Research Program of the Ministry of Education of Japan (Mussel Watch: Marine Pollution Monitoring in Asian Waters; Project No.09041163). Several graduates and undergraduates in our laboratories provided welcome assistance with the fieldwork.

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Received for review August 13, 1999. Revised manuscript received January 3, 2000. Accepted January 5, 2000.

ES9909500