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APPLICATION OF PENTACYCLIC TRITERPANES AS A BIOMARKER IN IDENTIFYING THE SOURCE OF OIL POLLUTION IN THE EAST COAST OF PENINSULAR MALAYSIA

Mohamad Pauzi Zakaria[1]*, Suhaimi Suratman[2], Nor Sayzwani Sukri[3], Vahab Vaezzadeh[4], Chui Wei Bong[4, 5]

[1]Associate member, Institute of Ocean and Earth Sciences (IOES), University of Malaya, 50603 Kuala Lumpur, Malaysia

[2]Institute of Oceanography and Environment, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia

[3]Faculty of Earth Science, Universiti Malaysia Kelantan, Kampus Jeli, Beg Berkunci No. 100, 17600 Jeli, Kelantan, Malaysia

[4]Institute of Ocean and Earth Sciences (IOES), University of Malaya, 50603 Kuala Lumpur, Malaysia

[5]Laboratory of Microbial Ecology, Institute of Biological Sciences, University of Malaya, Kuala Lumpur, Malaysia

*Corresponding author: drpauzi@gmail.com

ABSTRACT

South China Sea is a major shipping route for oil transportation from Middle East to Northeast Asian countries. A study was carried out to determine the source of oil pollution in the east coast of Peninsular Malaysia. Pentacyclic triterpanes (hopanes) were used as molecular markers to fingerprint the source of petroleum hydrocarbon pollution. Ten core samples were collected and extracted using soxhlet extraction procedure. Samples were separated and fractionated using 2-steps silica gel column chromatography and injected into gas chromatography-mass spectrometry. The Σhopanes ranged from 2.12 ng/g to 1331.74 ng/g dry weight. The sources of petroleum hydrocarbons were identified by diagnostic ratios of C_{29}/C_{30} hopanes and ΣC_{31}-C_{35}/C_{30} hopanes. Based on the ratios, seven of the sediment samples were originated from South East Asia Crude Oil, while the rest were originated from the Middle East Crude Oil. Hopanes are powerful markers for oil source identification.

Keywords: *Pentacyclic triterpanes, Sediment core, Diagnostic ratios, South China Sea, Petroleum pollution.*

1.0 INTRODUCTION

Hydrocarbon is an organic compound consisting entirely of hydrogen and carbon. With relation to chemical terminology, aromatic hydrocarbons or arenes, alkanes, alkenes and alkyne-based compounds composed entirely of carbon or hydrogen are referred to as pure hydrocarbons, whereas other hydrocarbons with bonded compounds or impurities of sulfur or nitrogen, are referred to as impure hydrocarbons. Naturally, hydrocarbons are found in crude oil, where decomposed organic matter provides an abundance of carbon-hydrogen bonds. Generally, hydrocarbons can be either derived from natural sources such as organisms and natural seeps or human activities such as industries, agriculture, accidental oil spills, surface run-offs and atmospheric inputs. These activities will bring micro-organic pollutants to aquatic ecosystems and finally deposit in form of sediment [1]. Organisms produce diverse compounds on Earth that are not known from inorganic origins, and therefore are regarded as biomarkers [2].

Biomarkers are also known as molecular fossils meaning they are derived from organisms. Biomarkers are defined as groups of compounds with distinctive structure which can be related to the compound generation by living organisms through reasonable transformation pathways. Biomarkers occur widely in most petroleum and petroleum products [3]. A large number of compounds have been identified in crude oil. The organic matter in sedimentary rocks and oil consists of a highly complex mixture of compounds covering a wide range of structures and molecular weights. The name of hopane comes from the Hopea which is a tree species from which hopane was first isolated in 1958. Hopanes are produced by ferns, mossed, lichens, fungi and some higher plants. Generally, hopanes are the types of triterpanes which are found ubiquitously in almost all ancient sediments and crude oils. Hopane consists of various homolog and stereo-isomers and their compositions vary among the crude oils and petroleum products depending on the origins and the maturation stages of crude oils and on the refinery process. Hopanes are present in high boiling point products of petroleum and cannot be found in gasoline and diesel fuels [4]. They are derived from oxygenated analogues such as bacteriohopanetetrols found in most bacteria and cyanobacteria. They are among the last products of biohopanoids in the process of diagenesis [5]. Generally, hopanes are non-toxic with no health impacts on human. Nevertheless, hopanes are utilized as molecular markers to identify the sources of oil pollution and some petroleum product (such as lubricating oil, heavy residual oil, asphalt and tires) since they are persistent in the environment [6] and have composition characteristics for source identification of petroleum. Hopanes are very resistant to chemical, photochemical and microbial degradation, however, they can still be biodegraded in natural environment condition and laboratory.

South China Sea is a large marine ecosystem located in the east coast of Peninsular Malaysia with abundant natural resources such as oil and natural gas. The South China Sea has also active offshore oil production [7]. Increasing activities such oil exploration and transportation of crude oil by supertankers in the South China Sea can increase the incidence of oil pollution. Indeed, the South China Sea is one of the most important routes for oil tankers, however, the data on the sources of the spills on beaches are not well documented [8]. There is a variety of potential sources of petroleum in the coastal areas. Land-based pollution from anthropogenic activities is another major source of pollution underlining the importance of public awareness on environmental issues [9]. Fast growth of industrialization and urbanization in Peninsular Malaysia intensifies the release of land-based petroleum hydrocarbons. Studies on sources of petroleum pollution have been dominantly performed in the west coast of Peninsular Malaysia and petroleum pollution in the east coast has remained largely unknown [10-13]. Therefore, source identification of petroleum hydrocarbons in the east coast of Peninsular Malaysia is of high priority. This research aims to identify sources of petroleum pollution in sediments of the east coast of Peninsular Malaysia using hopanes as biomarkers. The concentrations of hopanes will be also determined in sediments.

2.0 METHODOLOGY

2.1 Study Area and Sample Collection

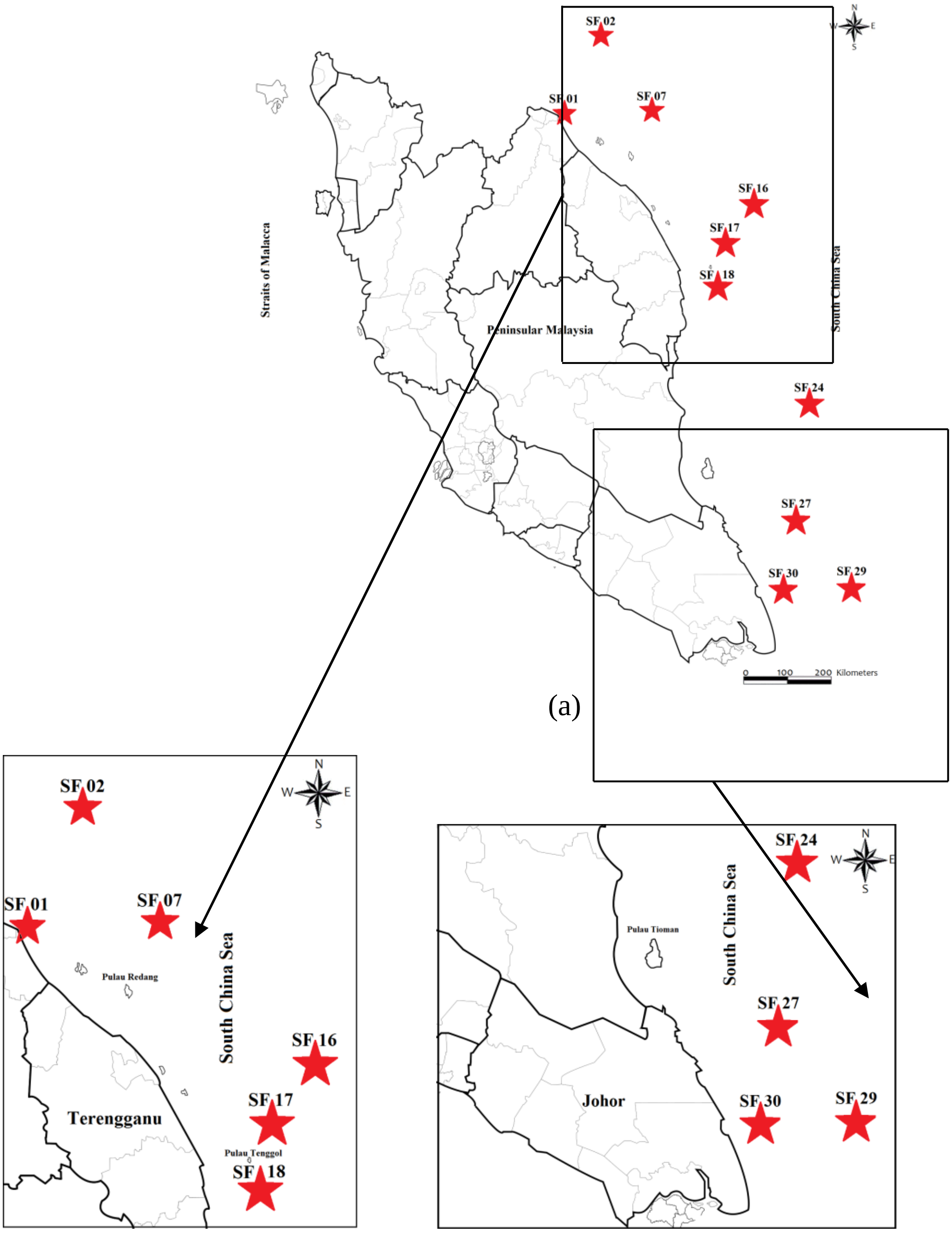
Samples were collected in January 2013. The map of the sampling locations and the detailed description is illustrated in Figure 1a to Figure 1c. Sampling was carried out using the Universiti Malaysia Terengganu's *RV Discovery*. Ten core sediment samples were collected with different depth using an Eckman Dredge Sediment Sampler (Table 1). The sediment was then cut into 3 different layers as shown in Table 2. The sediment was transferred into the stainless steel container with Teflon-lines up cap to avoid any cross-contamination and kept in iceboxes (less than 4 °C) to be transferred to the laboratory. The samples were stored at -18 °C in the laboratory before analysis.

2.2. Chemical Analysis

A procedure established previously by Zakaria *et al.* [14] was used for analyzing hopanes in sediment. All the glassware was washed with detergents, rinsed three times with tap water and distilled water, respectively. Finally, glassware was rinsed three times with methanol, acetone and distilled hexane, successively. Then, the glassware was wrapped with aluminum foil and baked at 60 °C for two hours to get rid of all organic contaminants and cooled at room temperature.

Blank samples were also analyzed in this experiment to detect possible contamination from other sources. A total of 20 g of the wet sediment samples was accurately weighed and added with enough anhydrous Na_2SO_4 (to absorb water) to dry the sediment samples. The dried sediment was placed into the cellulose thimble and extracted using Soxhlet apparatus with 250 ml dichloromethane (DCM) as solvent for 8 hours. Activated copper were added into each extract and left overnight. Activated copper removes sulfur element from the samples which can cause interference in chromatograms. The extract volume was reduced using rotary-evaporator. The next step was 1st Step Column Chromatography to clean the organic compounds from impurities in order to ensure a smooth analysis. The glass column

(0.9 mm internal diameter) was packing with 5% H_2O deactivated silica gel. A volume of 20 ml hexane: DCM (3:1 v/v) was used to separate organic compounds from polar compounds. All samples were rotary evaporated to 1 ml and transferred to 2nd Step Column Chromatography. Glass column in this step with an internal diameter of 4.7 mm was packed with 18 cm of 100% activated silica gel. In this step, the separated hydrocarbon compounds.



(b) (c)

Figure 1. (a), (b) and (c): Map of the sampling sites.

Table 1. Coordinates, depth and distance of sampling sites.

Stations	Longitude (E)	Latitude (N)	Depth (m)	Distance (NM)
SF01	102 19.00'	06 13.99'	13	2.7
SF02	102 47.04'	06 50.04'	46.5	50
SF07	103 01.00'	06 10.00'	45	40
SF16	104 12.60'	05 18.50'	60	56
SF17	103 42.98'	04 54.12'	54	17
SF18	103 49.98'	04 28.14'	40	20
SF24	104 36.00'	03 32.08'	62	70
SF27	104 16.97'	02 16.94'	30	19.5
SF29	104 41.97'	02 00.55'	46	35
SF30	104 15.03'	01 48.04'	14	4.5

3.0 RESULTS AND DISCUSSION

3.1 Total Hopanes

A total of 10 core sediment samples with 3 different layers from each core were examined in the east coast of Peninsular Malaysia. Table 4 lists dry weight of the sediment samples according to the layers. Gas chromatography-mass spectrometry (GC-MS) was used to analyze hopanes in sediments. Figure 2 shows the distribution of total hopanes in the study area. The total hopanes in sediments of selected areas in the east coast of Peninsular Malaysia ranged from 2.14 to 1331.74 ng/g dry weight. The highest total hopanes was found at SF02A. SF02A is located near major oil fields and also many fisheries, recreational and marine activities take place in the coastal area of this sampling point which can be the potential sources of petroleum hydrocarbons giving rise to the concentrations of total hopanes. Furthermore, the oil pollution can also come from the municipal, residential and shipping activities in this area. The lowest total hopanes was detected at SF02B (2.14 ng/g) which is at the same area with the highest total hopanes but the intermediate layer of the core sediment. It can be explained by the fact that surface sediment (SF02A) is highly influenced by recent anthropogenic activities as mentioned earlier, whereas intermediate layer of sediment is representative of the time before the area was developed when these anthropogenic activities were absent. However, other factors such as degradation can play significant roles in the concentrations of hopanes in different layers of sediments. In this study, the highest total

Table 2. The layer data of the sampling point at South China Sea.

Stations	**Layer (cm)**
SF 01A	2-4
SF 01B	26-28
SF 01C	44-45
SF 02A	0-6
SF 02B	38-44
SF 02C	83-86
SF 07A	0-2
SF 07B	14-16
SF 07C	24-26
SF 16A	0-2
SF 16B	12-14
SF 16C	28-30
SF 17A	0-2
SF 17B	10-12
SF 17C	18-20
SF 18A	19-24
SF 18B	23-34
SF 18C	34-39
SF 24A	0-4
SF 24B	68-71
SF 24C	131-136
SF 27A	0-4
SF 27B	22-24
SF 27C	46-48
SF 29A	0-2
SF 29B	14-16
SF 29C	35-38
SF 30A	0-4
SF 30B	53-56
SF 30C	101-106

A, the surface of sediment layer; B, the intermediate of surface layer; and C: the bottom of sediment layer.

Table 3. The GC-MS parameters for hopane analysis

Parameter	Setting for hopane analysis
Carrier gas	Helium (1ml/min)
Oven temperature (°C)	300
Interface temperature (°C)	300
Column-1 flow cal.	1.0
Ms source	230
Ms Quad	150
Det-f temperature (°C)	280

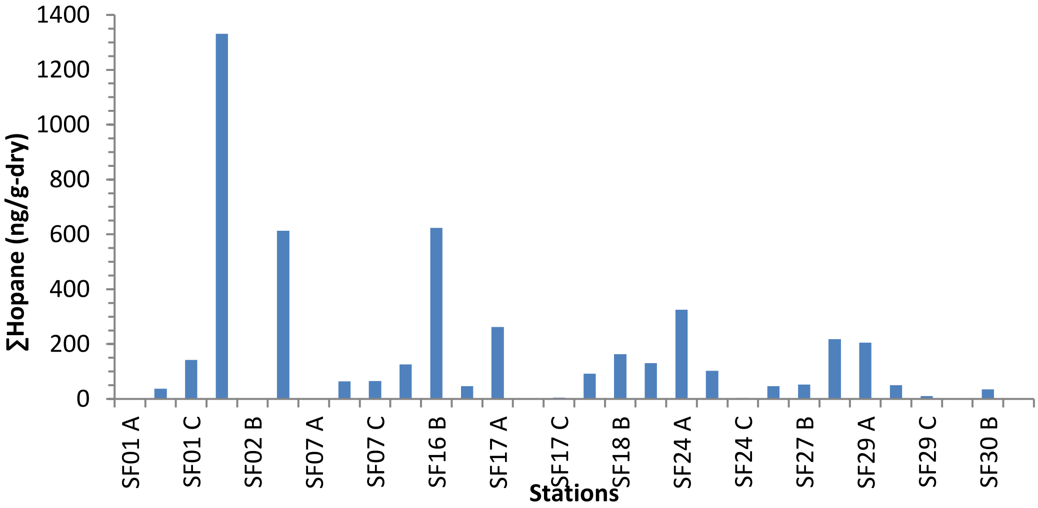


Figure 2. Total hopanes in sediment; (A, the surface of sediment layer; B, the intermediate of surface layer; and C: the bottom of sediment layer).

Hopanes were detected in different layers of core sediments. The highest total hopanes were detected in the surface sediment of SF02, SF17, SF24 and SF29 core samples which can be due to severe current release of oil pollution from various anthropogenic activities in these areas. On the other hand, the highest total hopanes in SF16, SF18 and SF30 were detected in the intermediate sediment layer. Furthermore, SF01, SF07 and SF27 showed the highest total hopanes in the bottom layer of sediment. This can be due to lower current inputs of petroleum hydrocarbons and also degradation of hopanes. Table 4 shows the description of the data from hopanes in sediments of the east coast of Peninsular Malaysia.

Table 4. Hopanes concentrations and ratios in sediments from east coast of Peninsular Malaysia

Stations	Triterpanes (m/z = 191)					
	∑Hopane (ng/g-dry)[a]	Dry weight (g)	C_{29}/C_{30}[b]	$\sum C_{31}$-C_{35}/C_{30}[c]	Tm/Ts[d]	Oleanane/C_{30}[e]
SF01 A	nd	9.35	nd	nd	nd	nd
SF01 B	36.91	12.41	0.56	4.04	0.16	0.24
SF01 C	142.38	11.39	1.43	1.49	1.91	0.17
SF02 A	1331.74	10.63	0.45	3.67	1.37	0.14
SF02 B	2.14	11.37	0.42	2.31	0.02	0.06
SF02 C	613.16	12.00	0.45	4.20	1.11	0.13
SF07 A	nd	8.03	nd	nd	nd	nd
SF07 B	64.31	12.32	0.62	2.28	1.35	0.20
SF07 C	65.04	13.04	0.68	2.85	0.43	0.05
SF16 A	125.88	13.38	0.59	3.03	0.09	0.06
SF16 B	623.46	13.54	0.46	3.60	0.76	0.13
SF16 C	46.59	14.12	0.65	6.45	0.77	0.33
SF17 A	262.65	8.04	0.79	2.20	0.24	0.15
SF17 B	nd	12.08	nd	nd	nd	nd
SF17 C	4.25	13.05	0.71	1.57	0.04	0.13
SF18 A	91.62	15.18	0.57	3.11	0.86	0.19
SF18 B	163.67	14.36	2.96	2.36	1.12	0.30
SF18 C	130.14	13.10	0.74	2.53	1.00	0.14
SF24 A	324.73	13.77	0.50	1.71	0.33	0.13
SF24 B	102.24	13.87	0.69	3.65	0.12	0.28
SF24 C	3.96	14.02	0.77	4.53	0.53	1.00
SF27 A	47.18	15.24	0.78	4.46	0.18	0.15
SF27 B	52.97	15.07	0.24	4.70	0.65	0.40
SF27 C	217.90	14.63	0.53	2.61	0.71	0.19
SF29 A	205.43	13.27	2.06	2.76	0.45	0.37
SF29 B	50.63	13.41	0.70	7.33	0.10	0.35
SF29 C	10.20	12.47	0.70	2.03	0.23	0.08
SF30 A	2.27	11.68	1.26	2.59	3.79	0.30
SF30 B	34.75	12.05	1.06	1.20	0.37	0.07
SF30 C	nd	12.14	nd	nd	nd	nd

[a] Sum of pentacyclic triterpanes

[b] Ratio of 17α,21β(H)-30 norhopane to 17α,21β(H)-hopane

[c] Ratio of sum 17α,21β(H)-31 homohopane to 17α,21β(H)-C_{35}homohopane relative to 17α,21β(H)-hopane

[d] Ratio of 17α-22,29,30-trisnorhopane relative to 18α-22,29,30-trisnorhopane

[e] Ratio of Oleanane relative to 17α,21β(H)-hopane

nd, not determine; (A, the surface of sediment layer; B, the intermediate of surface layer; and C: the bottom of sediment layer).

3.2 C_{29}/C_{30} Ratio and $\sum C_{31}$-C_{35}/C_{30} Ratio

The outcomes of this study showed that the major crude oil in the study area were the Middle East Crude Oil (MECO) or South East Asian Crude Oil (SEACO). In both crude oils, 17α, 21β(H) C_{29} hopane (C_{29} 17α) is dominant with stair- step progression of C_{31} to C_{35} homohopanes. The most significant distinctions are that C_{29} 17α is dominant over C_{30} 17α in MECO and the homohopanes ranging from C_{31} to C_{35} are depleted in SEACO. Therefore, the sources of crude oils can be distinguished by molecular ratios including C_{29}/C_{30} ratio and the $\sum C_{31}$-C_{35}/C_{30} ratio which are both normalized by C_{30} 17α [15-16]. The combined ratio of the selected pair of triterpanes, especially the ratio of C_{29}/C_{30} is apparently independent of weathering effect in most cases and can be extremely useful in the identification of oil sources and oil matching. The characteristics of the SEACO and MECO can be explained by the difference in geological conditions during petroleum formation. The lower values of the C_{29}/C_{30} ratio and the $\sum C_{31}$-C_{35}/C_{30} ratio were observed for South East Asian Crude Oil (SEACO), while higher ratios were detected for Middle East Crude Oil (MECO). MECO oil is derived from the marine carbonate source rocks, whereas SEACO oil is derived from the nonmarine (such as lacustrine, flurio-deltaic) shale source rocks [17].

Figures 3 and 4 show the C_{29}/C_{30} ratio and the $\sum C_{31}$-C_{35}/C_{30} ratio, while Figure 5 shows the comparison of C_{29}/C_{30} ratio and $\sum C_{31}$-C_{35}/C_{30} ratio in sediment samples of the east coast of Peninsular Malaysia. From the Figure 3, C_{29}/C_{30} ratios show a remarkably constant pattern ranging from 0.42 to 1.43 except for SF18B and SF29A showing the highest values at 2.96 and 2.06, respectively. Figure 4 summarizes the $\sum C_{31}$-C_{35}/C_{30} ratios showing constant values ranging from 1.20 to 4.70 expect for SF16C and SF29B which show the highest values at 6.45 and 7.33, respectively. Figure 5 shows the comparison of C_{29}/C_{30} ratio and $\sum C_{31}$-C_{35}/C_{30} ratio where all the 30 sediment samples fell within the range of SEACO except for two sediment samples which fell within the range of MECO (SF16C and SF29B). SEACO is the domestic crude oil from Southeast Asian oil fields, therefore, abundance of SEACO in sediments of the east coast of Peninsular Malaysia comes of no surprise. Spillage of oil from oil tankers transferring SEACO from east Peninsular Malaysia oil fields can be a possible source of petroleum pollution. Tanker ballast water and tanker washing discharges can also contribute to oil spills. Moreover, oil spills as a result of drilling and other oil production activities in numerous oil platforms of east Peninsular Malaysia besides natural oil seeps can be other sources of SEACO in sediments [7-8]. However, the sampling points with predominance of MECO were probably influenced by spillage of MECO from oil tankers coming from the Middle East. However, this statement needs further study to prove the accidental spillage of oil from these oil tankers.

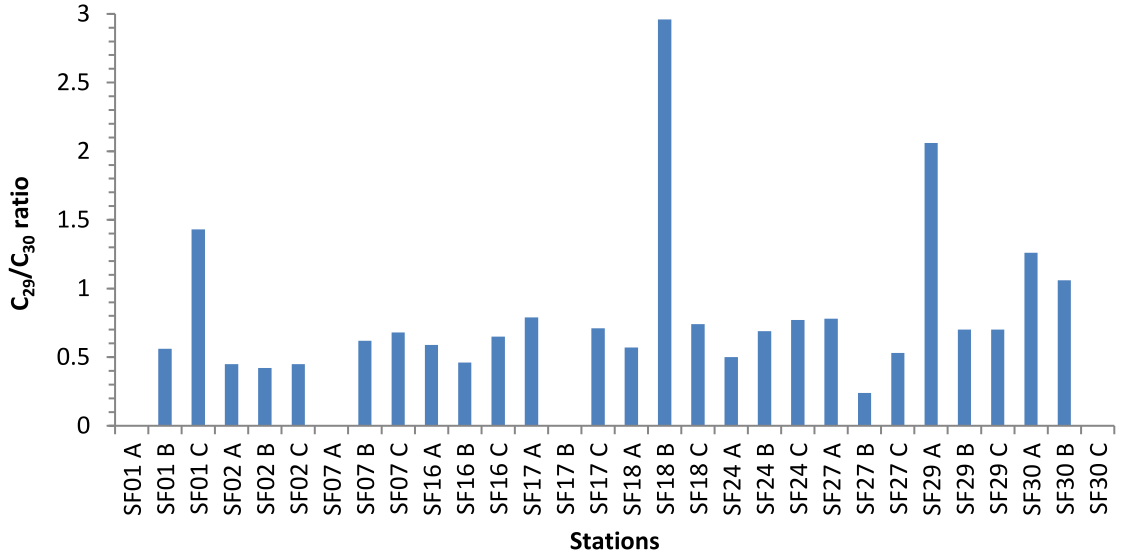


Figure 3. The C_{29}/C_{30} ratio in sediment; C_{29}, 17α, 21β (H)-30 norhopane; C_{30}, 17α, 21β(H)-hopane; (A, the surface of sediment layer; B, the intermediate of surface layer; and C: the bottom of sediment layer).

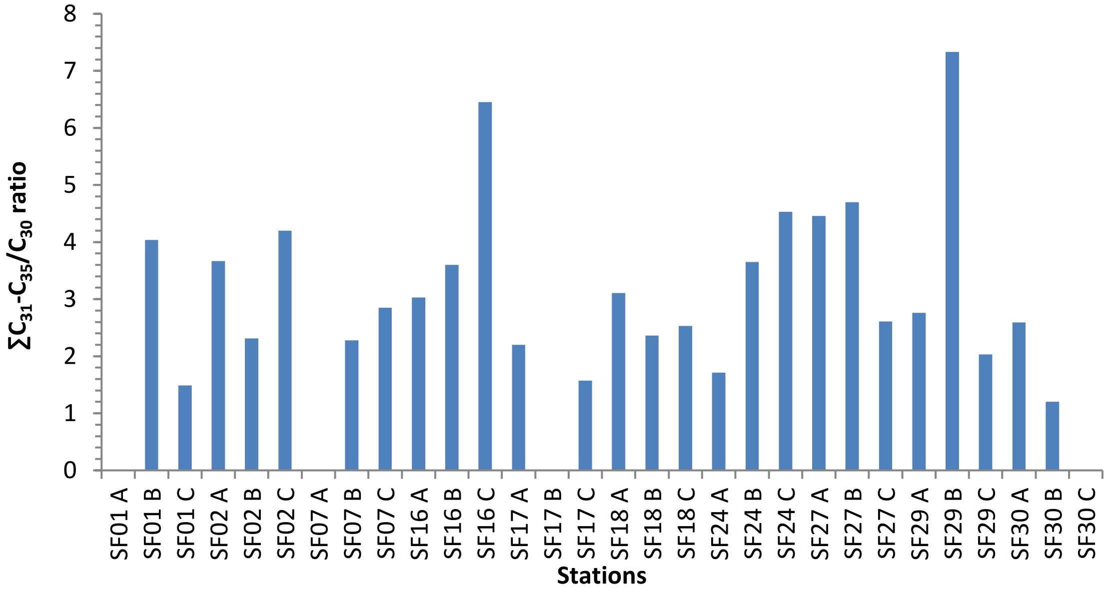


Figure 4. $\sum C_{31}-C_{35}/C_{30}$ ratio in sediment; $\sum C_{31}-C_{35}$, sum of 17α,21β(H)-31 homohopane to 17α,21β(H)-C35 homohopane; C_{30}, 17α,21β(H)-hopane; (A, the surface of sediment layer; B, the intermediate of surface layer; and C: the bottom of sediment layer).

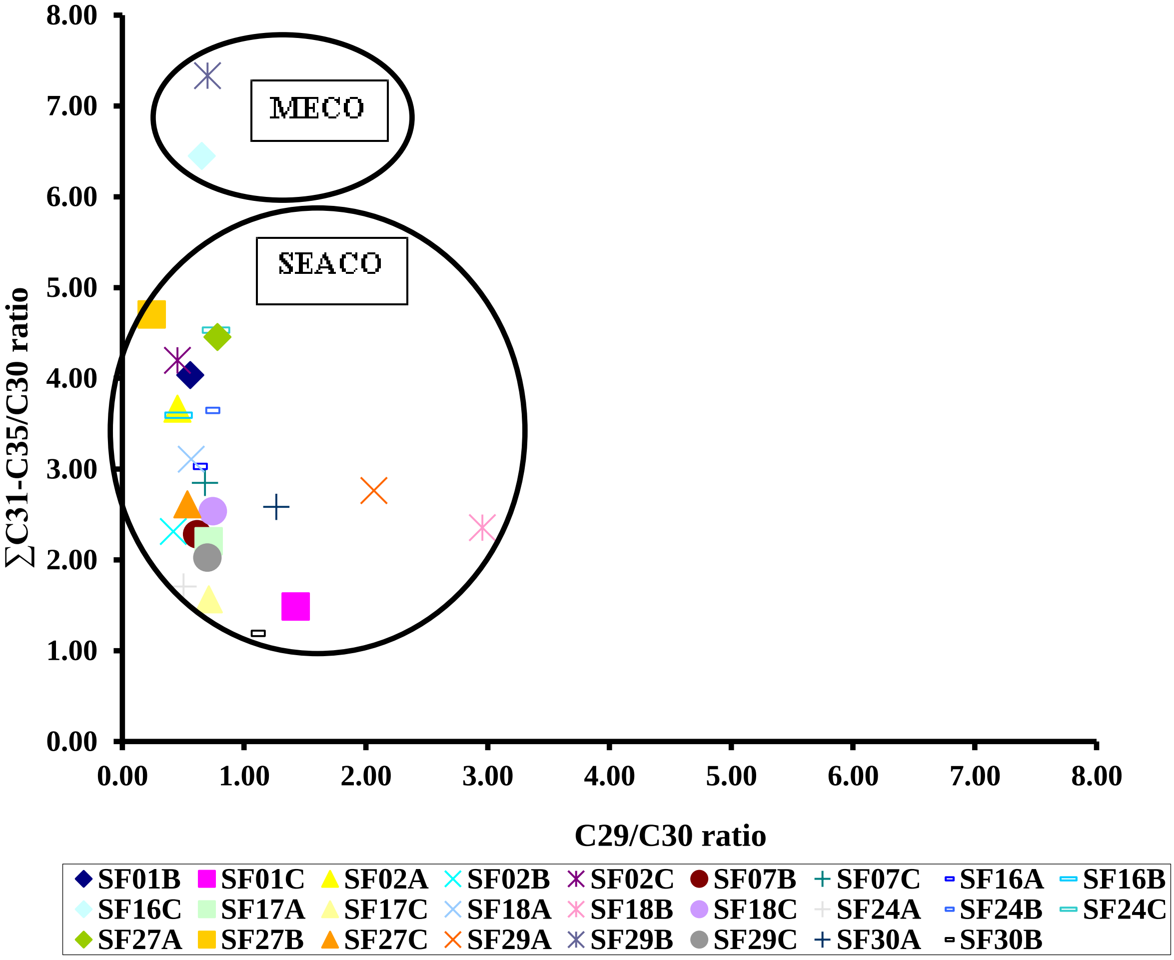


Figure 5. C29/C30 vs. ∑C31-C35/C30 in sediment; (A, the surface of sediment layer; B, the intermediate of surface layer; and C: the bottom of sediment layer).

3.3 Tm/ Ts Ratio

Tm stands for 17α-22,29,30-trisnorhopane, while Ts stands for 18α-22,29,30-trisnorhopane. The ratios of these two C_{27} hopanes can be used as an indicator for the thermal maturation when comparing hydrocarbon from the same source. According to Peter and Moldowan [18] the ratio varies considerably in oil and often provides a good parameter for environmental study. The oil from the carbonate source rock (MECO) appears to have low Tm/Ts compared to those generated from shale (SEACO). Tm/Ts ratios ranged from 0.02 to 3.79 in sediments of the east coast of Peninsular Malaysia. As shown in Figure 6, the highest value of Tm/Ts ratio at 3.79 was found in SF30A. Tm/Ts ratio is both maturity and source dependent. Tm/Ts ratio is a sensitive tool and should be used with caution since this ratio does not show consistent differences between MECO and SEACO [14]. This is partially due to the fact that Tm/Ts ratio is a maturity parameter as well as a source parameter as mentioned earlier. Both

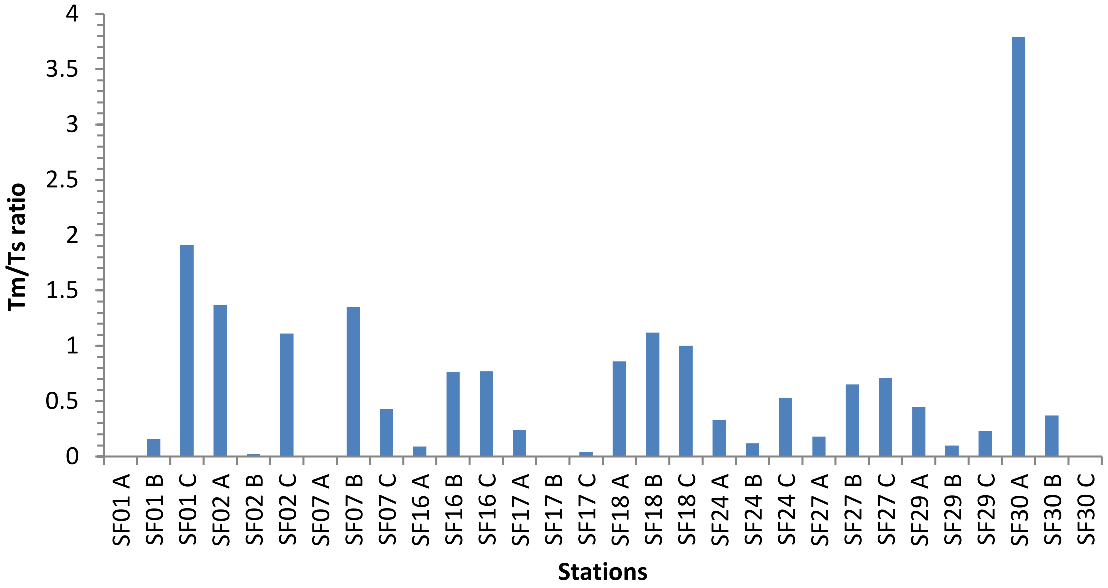


Figure 6. The Tm/Ts ratio in sediment: Tm, 17α-22,29,30-trisnorhopane; Ts, 18α-22,29,30-trisnorhopane; (A, the surface of sediment layer; B, the intermediate of surface layer; and C: the bottom of sediment layer).

MECO and SEACO can consist of oil in different stages of maturation. Tm and Ts commonly co-elute with tricyclic or tetracyclic terpanes on the *m/z* 191 mass chromatograms, resulting in superiors Tm/Ts ratio. Therefore, petroleum hydrocarbons with SEACO origin can still have low Tm/Ts ratios. Considering other hopanes diagnostic ratios, lower amounts of the Tm/Ts ratios in SF16C and SF29B confirm MECO origin in sediments of these two sampling points.

3.4 Oleanane/C_{30}

Oleanane/C_{30} hopane (oleanane index) measured at *m/z* 191 chromatograms is highly specific for higher plant inputs from the Createaceous period or earlier [18]. Oleananes appear as single peaks in *m/z* mass chromatograms eluting before the C_{30} 17α (H), 21β (H)-hopane on nonpolar capillary column and are rarely more abundant than the hopanes [19]. Although both 18α (H) and 18β-oleanane are found in immature oil, the latter is thermally less stable. Equilibrium between the 18β-isomer probably occurs before peak oil generation. Thus, the sum of 18α (H)- and 18β-isomer should be used in the oleanane/ C_{30} hopane ratio for purposes of correlation. Oleanane is more abundant in some of the South East Asian petroleum, while there is no significant oleanane in the Middle East petroleum. This is because South East Asian petroleum receives plant contributions related to lacustrine deposition environment, whereas Middle East petroleum is of marine origin.

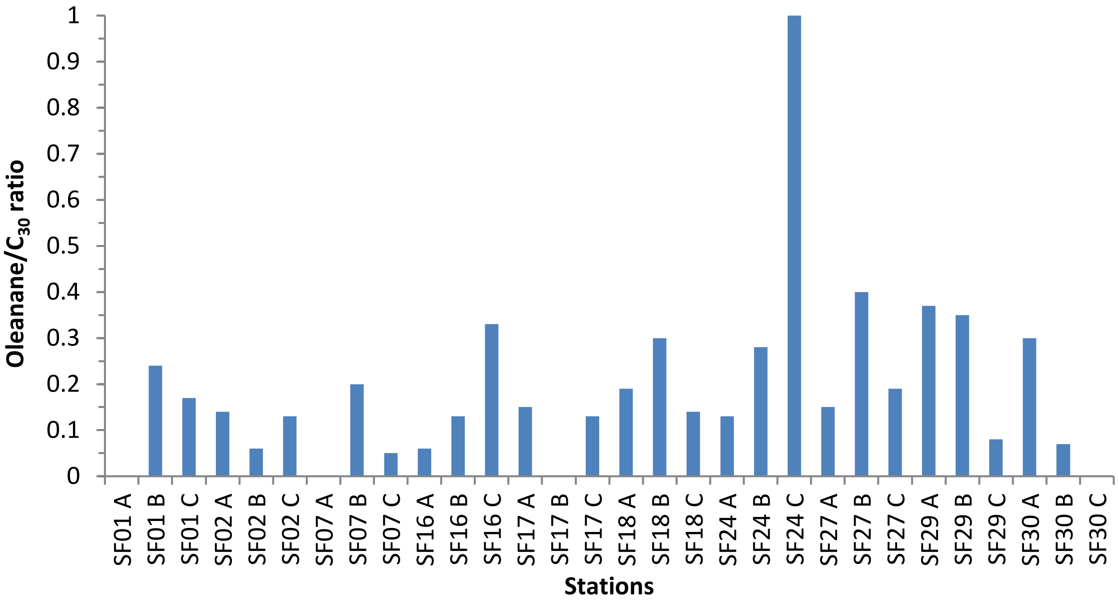


Figure 7. The Oleanane/C_{30} ratio in sediment: C_{30}, 17α,21β(H)-hopane; (A, the surface of sediment layer; B, the intermediate of surface layer; and C: the bottom of sediment layer).

Oleanane/C_{30} ratio is within the range of 0.05 to 1.00 as shown in Figure 7. This distinctive pattern of the ratio can be explained by spatial heterogeneity of higher plant inputs. In addition, the ratio can be affected by thermal maturation of the petroleum. Although SF16C and SF29B were categorized under the MECO, trace amounts of oleanane were detected in these two sampling points. Higher levels of oleanane were detected in other sampling points as an indication of SEACO origin hydrocarbons. The results imply that high concentration of oleanane does not always indicate SEACO petroleum contribution, although at low concentrations oleanane can still be a useful biomarker for MECO oil sources.

4.0 CONCLUSIONS

The South China Sea is rich with natural resources and is a major route for oil tanker transportation, while the east coast of Peninsular Malaysia which faces to South China Sea is important for fisheries, recreational and marine activities, tourism and maintaining the bio-diversity in the tropical area. Due to quick increase of industrialization and urbanization, large amounts of land-based petroleum hydrocarbons are also released to the aquatic environment of the east Peninsular Malaysia. Thus, source identification of petroleum hydrocarbons is of high priority in this region. The total hopanes in sediments of selected areas in the east coast of Peninsular Malaysia ranged from 2.14 to 1331.74 ng/g dry weight. The outcomes of this study showed that hopanes are powerful molecular makers for sources identification of oil pollution. Application of the source identifiers, C_{29}/C_{30} and $\sum C_{31}\text{-}C_{35}/C_{30}$

ratio, clearly suggested that the oil contaminations in sediments are mostly originated from SEACO except for SF16C and SF29B that had MECO origin. Moreover, SF18B and SF29A showed high ratios of C_{29}/C_{30} which can be a sign of MECO origin. This indicated that petroleum hydrocarbons in sediment samples predominantly originated from the domestic oil and oleanane/C_{30} and Tm/Ts gave supportive evidence for this statement. Further studies are recommended on sediment of the east coast of Peninsular Malaysia to acquire more details on distribution and sources of oil pollution in the environment of the east coast of Peninsular Malaysia and improve the current dataset of oil pollution in this area.

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