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DISTRIBUTION OF FATTY ALCOHOLS IN SURFACE SEDIMENTS OF THE ENDAU RIVER, JOHOR

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ABSTRACT

The distribution and concentrations of extractable fatty alcohols in surface sediments of the Endau River were determined to identify their sources and variations. A total of 18 surface sediment samples were taken from Sungai Endau, Johor. Samples were then extracted and analyzed using a gas chromatography-mass spectrometer (GC-MS). Total fatty alcohol concentration ranged from 0.62 to 34.54 ngg^{-1} dry weight sediment. Generally, the study area is dominated by long chain fatty alcohols which are indicator for terrestrial organic matter. This is also supported by the ratio of short chain/long chain fatty alcohol and alcohol source index (ASI). This study showed the fatty alcohols detected in the study area originated from various sources with inputs from terrestrial being the more dominant sources.

Key words: Endau River, fatty alcohols, sediments, source determination, alcohol source index

INTRODUCTION

Biolipid marker refers to the use of lipid compounds as biological markers to determine the content, distribution, source and movement of organic matter particularly in aquatic environment (Mudge & Norris, 1997; Yoshinaga *et al.,* 2008). Additionally, biolipid markers are also often used to identify contamination that occurs in aquatic environment especially sewage contamination (Fahl & Stein, 1999; Bull *et al.,* 2002; Froehner *et al.,* 2008; Pratt *et al.,* 2008). Fatty alcohol is one of the lipid compounds that has potential as biolipid marker to differentiate organic matter input that originates from marine, terrestrial sources or a result of action by bacteria on the aquatic environment (Mudge & Norris, 1997; Mudge & Seguel, 1999; Seguel *et al.,* 2001; Zhang *et al*., 2014).

Fatty alcohol distribution in the environment is widespread, encompassing those contained in each organism in the layer of deep sea sediments and sedimentary column. This involves direct input by organisms or a result of the decomposition process of compounds that contain fatty alcohol components (Belanger *et al.,* 2008). Fatty alcohols are derived naturally from oils or fats of plants and animals as its compounds are found in most organisms (Mudge, 2005). Parameswaran *et al* (1994) stated that in general, the main source of fatty alcohols in aquatic environment is from marine zooplankton and ester wax that is found in phytoplankton and terrestrial plants.

Short chain fatty alcohols ($\leq C_{20}$) are indicators with input from marine organisms especially phytoplankton and zooplankton (Grimalt & Albaigés, 1990; Mudge & Norris, 1997; Mudge & Seguel, 1999; Volkman *et al.,* 1999; Seguel *et al.,* 2001; Treignier *et al.,* 2006). Long chain fatty alcohols ($>C_{20}$) represent input from terrestrial plants into the aquatic environment (Grimalt & Albaigés, 1990; Mudge & Norris, 1997; Mudge & Seguel, 1999; Seguel *et al*., 2001; Wakeham & McNichol,

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2014). The presence of branched-chain fatty alcohols (*-iso* and *–anteiso*) shows bacterial metabolic process on straight-chain compounds with even-numbered carbon atoms (Mudge & Norris, 1997; Seguel *et al*., 2001; Mudge & Duce, 2005). Content of this compound will increase when there is sewage disposal into the aquatic environment because of the presence of bacterial colony together with the sewage waste or the presence of bacterial colony together with organic matter to be decomposed in the environment (Mudge & Norris, 1997; Seguel *et al.,* 2001).

This study was conducted in Endau River, Mersing, Johor, which is a mangrove swamp area with villages located at the edge of Endau River. The main activity of the local people is the fisheries sector which is actively carried out along the coastal area located near the mouth of the river. Therefore, the organic compound sources in this area include anthropogenic sources comprising of waste from the local people's activities, natural sources in the form of plant residue particularly mangroves and biological activities of other organisms either aquatic or terrestrial organisms. Endau River was found suitable as a study site of fatty alcohol distribution because of the existence of various fatty alcohols. This study aimed at examining the fatty alcohol distribution in the study area based on its content in the surface sediments as well as identifying the sources of fatty alcohols in the area.

MATERIALS AND METHODS

Sampling

The study site is located at Endau River bordering between the state of Johor and Pahang; it includes the coastal area at the river mouth until Kampung Londang Batu. Surface sediment sampling activities and *in-situ* parameter measurements were conducted at 18 sampling stations (Figure 1; Table 1). Surface sediment samples were obtained using PONAR grab and placed in glass bottles. Samples were stored with ice

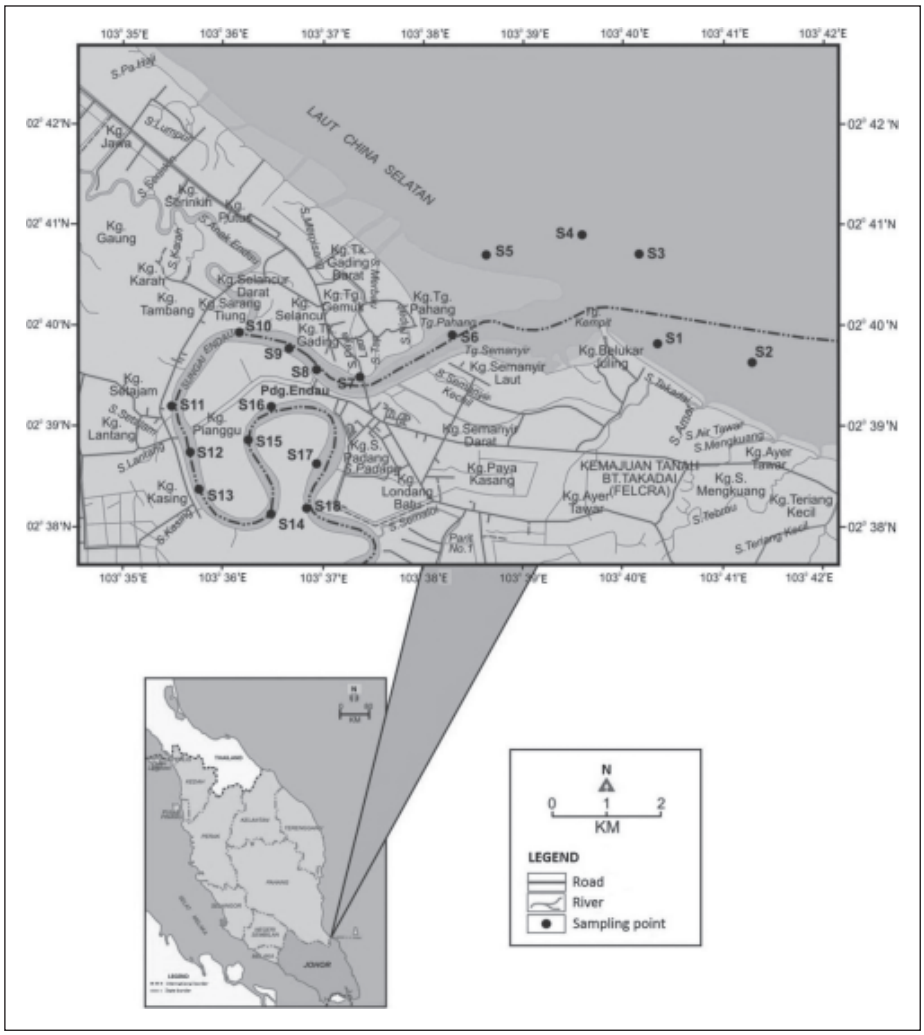

Fig. 1. Study site and sampling stations.

Table 1. Coordinate and *in-situ* parameter of sampling stations

Sampling station	Coordinate		Depth (m)	Water salinity (psu)
	Latitude °N	Longitude °E		
1	02°41'31.0	103°40'39.9	2.6	33.4
2	02°39'62.3	103°41'31.0	2.9	33.8
3	02°40'79.7	103°40'10.7	3.2	29.8
4	02°40'99.8	103°39'62.5	5.0	28.2
5	02°40'60.3	103°38'68.7	3.9	26.8
6	02°39'97.7	103°38'31.3	4.6	20.0
7	02°39'72.7	103°36'65.8	6.0	11.5
8	02°39'45.2	103°37'29.6	6.4	13.9
9	02°39'59.3	103°36'95.7	6.7	11.9
10	02°39'95.5	103°36'13.8	8.9	12.1
11	02°39'13.9	103°35'36.3	6.5	11.9
12	02°38'75.2	103°35'63.0	4.7	9.2
13	02°38'25.0	103°35'48.0	8.1	9.6
14	02°38'15.6	103°36'42.7	6.0	7.8
15	02°38'83.5	103°36'11.9	12.5	7.2
16	02°39'19.9	103°36'43.0	8.8	6.2
17	02°38'68.0	103°36'85.5	3.5	5.9
18	02°38'12.1	103°36'83.2	7.9	3.4

in a cool box and stored at freezing temperature once the samples arrived at the laboratory until the next step of analysis was carried out.

Fatty alcohol extraction

Extraction was carried out using procedure adapted from Mudge and Norris (1997) and Masni and Mudge (2006). The method involved three main phases, namely reflux, liquid-liquid separation, and derivatives before analysis using gas chromatography-mass spectrometry (GC-MS).

Some amount of surface sediment samples was also dried in the oven at temperature of 60°C. The dried samples were weighed every day until they achieved constant weight. Wet weight and dry weight of samples were used to calculate the dry weight of each sample extracted because the fatty alcohol concentration data obtained as a result of the GC-MS analysis were measured in sediment dry weight unit.

A total of 30-40 g wet weight of sediments was hydrolyzed using 50 mL of 6% potassium hydroxide solution in methanol for 4 hours. Samples were then left to cool down before centrifuged at 4000 r.p.m. for 3 minutes. Formed supernatant was placed in separating funnel and 20 mL of hexane and 10 mL of de-ionized distilled water were added. Samples were then shaken vigorously until two layers were formed, that is the top layer containing the non-polar lipid compounds with sterols and fatty alcohols and the bottom layer containing polar lipid compounds. The samples containing the non-polar lipid were placed again in the round flask and the procedure above was repeated to maximize the extraction result.

The samples were then evaporated using rotary evaporator at a temperature of 40°C and re-dissolved in 2-3 mL of hexane before transferred into the vial. anhydrous sodium sulphate was added to the samples to absorb water molecules and compounds still present in the samples. The sample solution was next filtered using filter-paper and dried using nitrogen gas.

About 2-3 drops of bis(trimethylsilyl)-triflouroacetamide (BSTFA) were added into the samples before heated in the heating block at a temperature of 60°C for 10 minutes. BSTFA replaces the active hydrogen atom with the trimethylsilylgroup, $-Si(CH_3)_3$ making the compound more volatile and stable for GC injection. Samples were dried using nitrogen gas once more and re-dissolved with 1 mL of hexane. Samples were kept at a temperature of -20°C until analysed using GC-MS.

Gas chromatography-mass spectrometer (GC-MS)

Perkin Elmer's Gas Chromatography-Mass Spectrometer (GC-MS) (Clarus 500) was used to analyse the fatty alcohol compounds found in the samples. DB-5HT column with the length of 30 m, internal depth of 0.25 mm and thickness of 0.10 μm was used to separate the fatty alcohol compounds in the samples into individual components. Pure helium gas (99.999%) was the carrier gas for the samples when analysed using GC-MS. The temperature programme started at 80°C and was increased at a rate of 15°C min^{-1} until it reached the temperature of 300°C. Temperature was then increased further to 350°C at a rate of 5°C min^{-1} and maintained at the temperature for 10 minutes (Ali *et al*., 2015).

The spectrum of each fatty alcohol compound present in the samples was recorded directly into the GC-MS computer programme, Turbo Mass, to identify the compounds' spectrum based on the diagnosis of mass for each compound. Fatty alcohol compound concentration was determined using calibration curve of octadecanol standard solution in the form of ether-TMS with concentration range of 1-10 µg/g. Detection limit value for the fatty alcohol compound analysis in this study was obtained by using the octadecanol-TMS standard solution, student t-value and standard deviation. Detection limit value for fatty alcohol compound was 0.15 µg/g.

Quality control

Quality control was implemented throughout the study period to guarantee the quality of the research carried out at the laboratory analysis stage and also the GC-MS analysis. All the glass apparatus used during analysis should be soaked overnight in Decon-90 solution, rinsed using de-ionised distilled water as well as dried and rinsed using organic solvent such as hexane.

Quality control during analysis using GC-MS involved calibration procedure using blank solution and standard solution. Blank solution used in this study was hexane solution which was the main solvent in the fatty alcohol compound extraction. Analysis of each set of sample began with blank solution analysis, followed by standard solution and then 5 actual samples. The procedure was repeated for each sample set. Results of the blank solution analysis showed no contamination occurred that was capable of changing the results of the fatty alcohol analysis. In addition, micro syringes to inject samples into the GC were cleaned using dichloromethane in methanol after each sample was analysed.

RESULTS AND DISCUSSION

Fatty alcohol distribution

A total of 20 fatty alcohol compounds were present (C_{12}-C_{25}) including branched chain compounds (*-iso* and *–anteiso*) that were identified with concentration range of 0.62-67.57 ngg^{-1} sediment dry weight. Fatty alcohol concentration for each sampling station is listed in Table 2. Generally, the study site recorded long chain fatty alcohols (C_{12}-C_{20}) which were 48% of the total fatty alcohols, followed by short chain fatty alcohols (27%) and the rest were branched compounds (Figure 2). Figure 3 shows the distribution for the three categories of fatty alcohols for each sampling station. A total of 15 sampling stations were dominated by long chain fatty alcohols, while 3 sampling stations, namely Station 6, 13 and 18 were dominated by short chain fatty alcohols.

In addition to the evaluation based on fatty alcohol concentration, calculation of alcohol source index (ASI) and ratio of short chain/long chain fatty alcohols [(C_{12}-C_{20})/(C_{21}/C_{25})] was also used to identify the dominant source of fatty alcohols in the study area. The ratio of short chain/long chain fatty alcohols showed that the fatty alcohol input came from various sources. Data of short chain/long chain fatty alcohol ratio from this study showed that most of the sampling stations were dominated by long chain compounds (Figure 4). Ratio value of <1.0 is significant for freshwater systems that contained long chain fatty alcohols more than short chain fatty alcohols (Mudge & Norris, 1997; Treignier *et al.,* 2006). ASI calculation involved the 4 main fatty alcohol compounds which are C_{14} and C_{16} compounds representing short chain fatty alcohols while long chain fatty alcohols are represented by C_{22} and C_{24} compounds. ASI data in this study showed that most of the sampling stations were dominated by C_{22} and C_{24} long chain fatty alcohols compared to C_{14} and C_{16} short chain fatty alcohols with value >1.0 (Figure 5 and 6).

Based on the fatty alcohol concentration, both ASI and short chain/long chain fatty alcohol ratio data, it was found that fatty alcohol content produced by terrestrial sources were higher compared to fatty alcohols produced by marine sources. This is due to the location of the sampling stations that were situated near to terrestrial sources particularly mangroves that are found abundantly along Endau River. Forest area, palm oil plantation and rubber plantation activities in the surrounding area of the study site also contribute to the long chain compound input in the aquatic environment. The findings recorded that long chain compounds were present with concentration range of 0.59-65.03 ngg^{-1} sediment dry weight with C_{24} compound being the primary long chain compound at the study site. Long chain fatty alcohols (C_{21}-C_{25}) that are produced by terrestrial plants are the indicator for terrestrial organic matter (Seguel *et al.,* 2001; Treignier *et al.,* 2006; Zhang *et al.*, 2014; Wakeham & McNichol, 2014, Tahir *et al.*, 2015). Nevertheless, this compound is also produced by several aquatic organisms such as macrophyte and microalgae but at lower concentrations (Ficken *et al.,* 2000; Volkman *et al.,* 1998; Volkman *et al.,* 1999).

Short chain fatty alcohols (C_{12}-C_{20}) are indicators for marine organisms especially phytoplankton and zooplankton (Seguel *et al.,* 2001; Treignier *et al.,* 2006; Kumar, 2013; Wisnieski *et al.*, 2014). This compound is present at concentration range of 0.62-67.57 ngg^{-1} sediment dry weight with C_{16} compound being the primary short chain compound in the study area. A total of

Table 2. Fatty alcohol concentration for each sampling station

Sampling station	Fatty alcohol concentration (ngg^{-1} dry weight of sediment)																			
	C_{12}	$_bC_{13}$	C_{13}	C_{14}	$_iC_{15}$	$_aC_{15}$	C_{15}	C_{16}	$_iC_{17}$	$_aC_{17}$	C_{17}	C_{18}	C_{19}	C_{20}	C_{21}	C_{22}	C_{23}	C_{24}	$_bC_{25}$	C_{25}
1	1.02	n.d.	n.d.	0.69	18.14	1.76	1.69	4.42	11.22	n.d.	0.79	3.10	1.75	2.66	2.97	5.15	1.36	9.69	19.03	34.54
2	1.12	n.d.	n.d.	1.15	10.68	1.53	1.10	3.93	5.88	0.89	1.17	3.20	n.d.	1.95	3.43	4.47	12.37	60.30	7.58	7.28
3	1.24	n.d.	n.d.	1.02	29.60	1.46	0.83	4.42	24.37	0.75	0.74	2.77	1.52	2.81	1.34	5.73	13.10	65.03	28.23	19.21
4	1.57	n.d.	n.d.	1.57	3.33	0.96	n.d.	1.73	0.66	n.d.	0.85	1.26	0.62	0.96	0.89	8.99	2.90	0.59	1.75	2.39
5	1.03	n.d.	n.d.	1.03	0.88	0.88	n.d.	1.69	n.d.	n.d.	0.62	2.33	1.04	n.d.	3.39	6.33	7.73	37.41	8.75	3.73
6	4.53	n.d.	10.35	2.78	24.85	3.82	1.16	67.57	n.d.	1.30	1.70	49.70	5.26	23.55	4.11	9.23	2.19	2.63	6.43	8.32
7	1.05	n.d.	n.d.	1.00	13.87	1.12	n.d.	3.40	7.70	2.93	0.78	3.99	1.06	3.13	3.02	6.50	16.43	3.41	8.62	10.62
8	1.37	n.d.	n.d.	3.33	3.33	0.84	n.d.	1.80	1.83	n.d.	n.d.	1.69	n.d.	1.07	n.d.	1.26	1.55	11.18	7.40	4.37
9	1.41	n.d.	n.d.	1.59	0.98	0.62	n.d.	0.62	n.d.	n.d.	n.d.	2.24	n.d.	n.d.	n.d.	7.22	n.d.	10.15	8.64	14.05
10	1.03	n.d.	n.d.	1.03	1.94	1.75	n.d.	6.19	n.d.	n.d.	n.d.	3.86	0.79	n.d.	0.89	2.09	n.d.	16.87	12.03	26.64
11	1.76	n.d.	n.d.	1.10	1.76	1.10	n.d.	0.84	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	7.75	0.94	9.55	2.12	0.81
12	0.97	n.d.	n.d.	1.38	1.38	n.d.	n.d.	8.26	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.85	1.36	13.79	2.69	4.56
13	1.29	1.48	n.d.	2.28	11.01	n.d.	1.32	11.80	2.32	1.60	1.02	9.82	2.12	5.73	1.50	1.69	0.98	0.68	6.67	7.42
14	1.10	1.10	n.d.	0.92	2.70	1.82	0.75	4.95	n.d.	n.d.	0.88	4.86	0.94	1.98	0.89	5.12	n.d.	18.44	2.10	0.95
15	1.48	n.d.	n.d.	4.94	4.94	0.90	n.d.	1.81	n.d.	n.d.	0.75	3.49	0.76	1.83	n.d.	3.03	n.d.	16.17	10.02	11.75
16	1.08	n.d.	n.d.	0.94	1.98	1.98	n.d.	5.15	n.d.	n.d.	0.83	2.46	1.08	0.77	n.d.	3.16	0.66	38.94	7.29	13.32
17	3.31	n.d.	n.d.	1.45	1.45	1.45	n.d.	3.33	n.d.	n.d.	n.d.	2.99	0.75	1.46	0.62	8.91	1.36	39.00	4.26	3.42
18	1.26	n.d.	n.d.	2.44	4.47	2.82	n.d.	12.39	0.99	0.76	n.d.	7.24	1.65	2.43	1.14	1.84	1.81	5.18	2.35	1.41

n.d. – not detected or below the limit of detection

b – branched; *i*- iso, *a* – anteiso

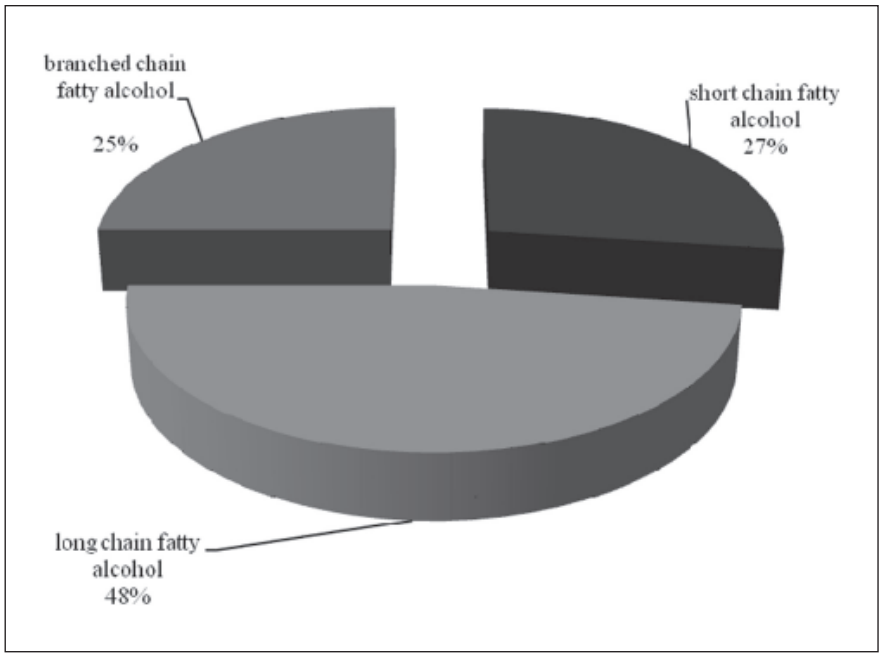

Fig. 2. Percentage of fatty alcohols identified present in the study area.

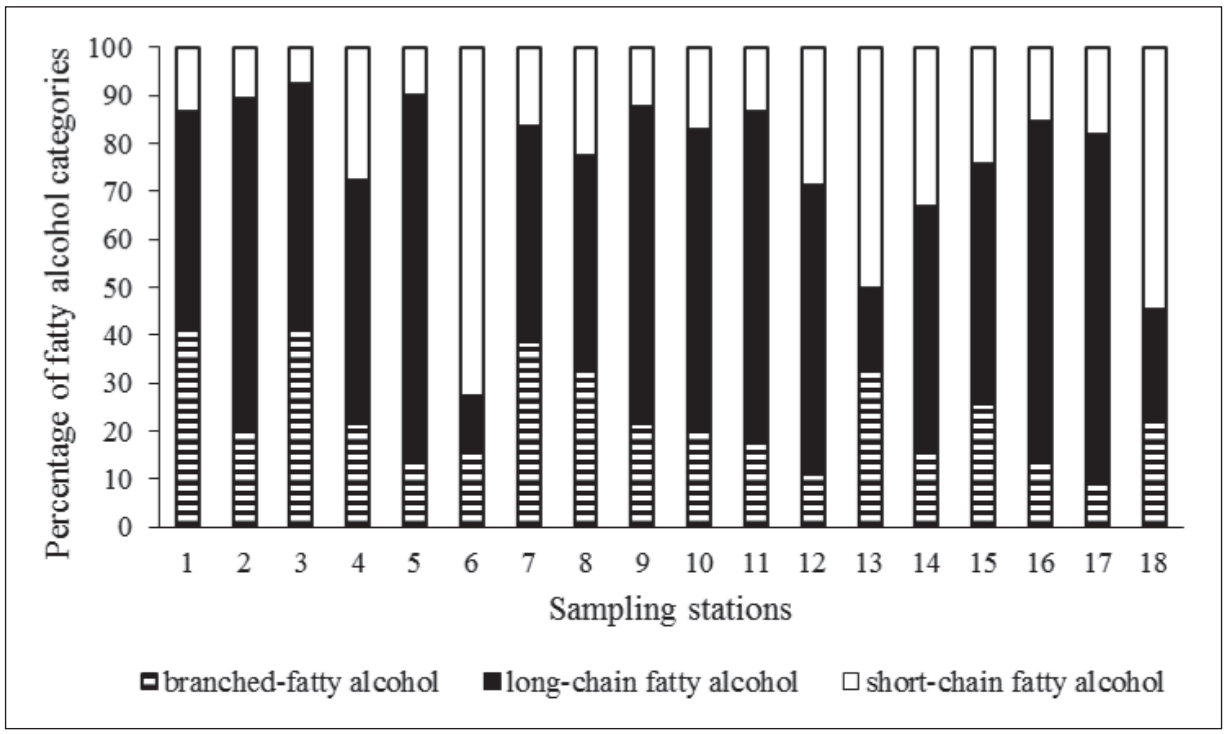

Fig. 3. Percentage of fatty alcohol categories for each sampling station.

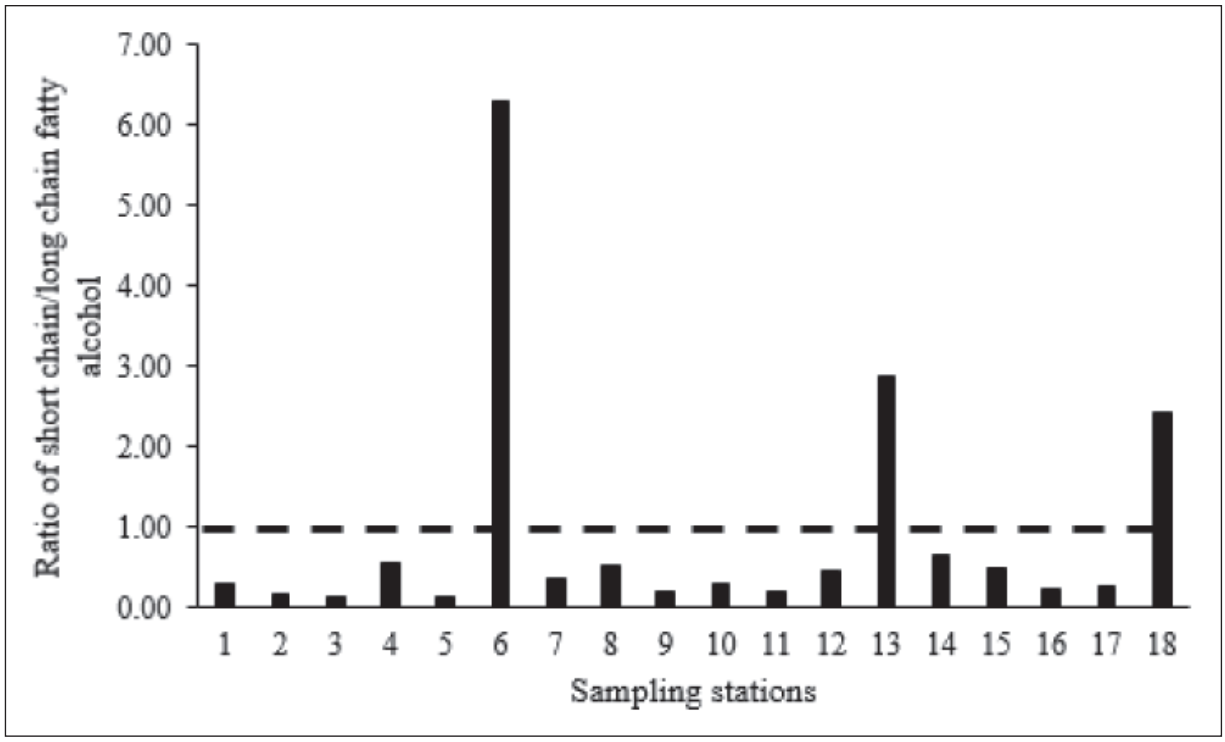

Fig. 4. Ratio of short chain/long chain fatty alcohols for each sampling station.

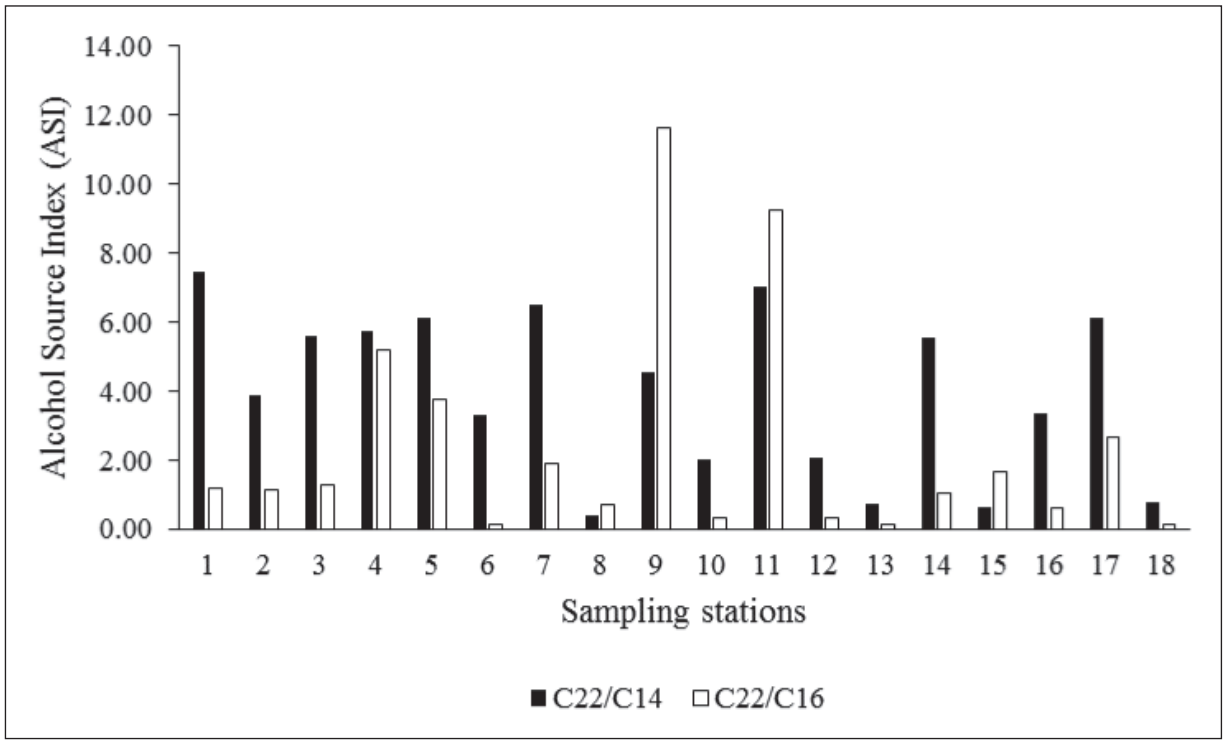

Fig. 5. Alcohol Source Index for C_{22}/C_{14} and C_{22}/C_{16} for each sampling station.

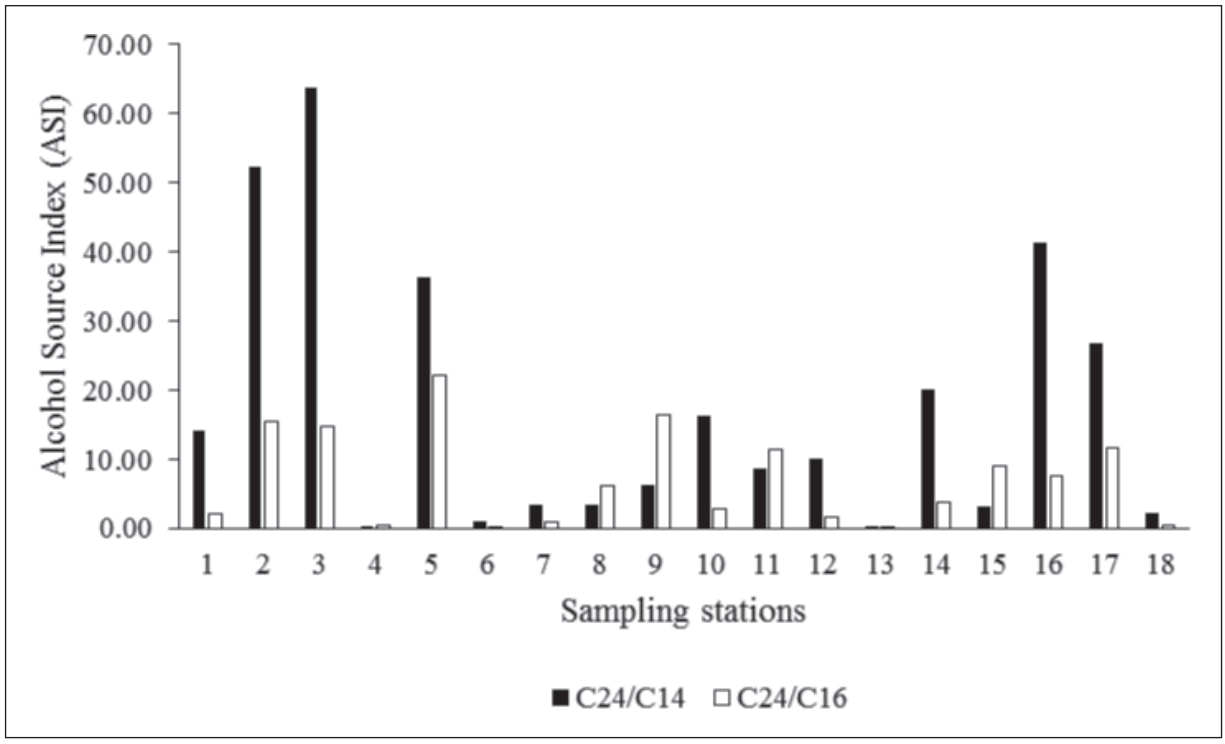

Fig. 6. Alcohol Source Index for C_{24}/C_{14} and C_{24}/C_{16} for each sampling station.

3 sampling stations were dominated by short chain fatty alcohols, namely Station 6, 13 and 18. Station 6 which is located at the river mouth with salinity value of 20.0 psu recorded the highest short chain fatty alcohol content compared to the other sampling stations with its main compound being C_{16} fatty alcohol. Marine organisms particularly phytoplankton are the primary source of short chain fatty alcohols at the river mouth area. Ogura *et al* (1990) discovered that C_{16} compound is the main compound found in phytoplankton. Logan *et al* (2001) stated that phytoplankton's habitat is more concentrated in the river mouth area even though it can inhabit freshwater environments. This explains the high C_{16} compound content in Station 6 in comparison to other sampling stations. Station 13 and 18 are river environment dominated by short chain compounds. The presence of short chain fatty alcohols in river environments is normally not influenced by input from marine organisms because there are several other organisms that can produce this compound such as freshwater organisms and terrestrial plants but at lower concentrations from those produced by marine organisms (Treignier *et al.,* 2006; Yunker *et al.,* 1995).

Branched-chain fatty alcohol compound can be used as bacterial indicator because these compounds are produced from bacterial metabolism on even-chain length compounds (Parkes, 1987). Branched-chain fatty alcohol compounds (-*iso* and –*anteiso*) detected in this study comprised of four main compounds which are *iso*-C_{15} ($_iC_{15}$), *anteiso*-C_{15} ($_aC_{15}$), *iso*-C_{17} ($_iC_{17}$) and *anteiso*-C_{17} ($_aC_{17}$) as well as two branched compounds C_{13} and C_{25}. Branched-chain fatty alcohols are 25% of the total fatty alcohols with concentration range of 0.62–29.60 ngg^{-1} sediment dry weight.

Iso-C_{15} is formed from C_{14} straight-chain with the addition of one methyl group at carbon 13. Therefore, the ratio between the even-chain and odd-chain precursor of methyl derivatives can be used to show the stage of bacterial metabolism in the sample (Mudge & Norris, 1987). In general, low value for both ratio $\Sigma C_{15}/C_{14}$ and $\Sigma C_{17}/C_{16}$ was recorded in this study except for sampling stations 1, 2, 3, 6 and 7 (Figure 7). All sampling stations obtained higher $\Sigma C_{15}/C_{14}$ ratio compared to $\Sigma C_{17}/C_{16}$ ratio. Mudge *et al* (2008) stated that the difference in value between the two ratios might be caused by the presence of different bacterial groups in the environment that is also influenced by the presence of oxygen and organic matter input that entered the aquatic environment. Sewage waste is the organic matter that could influence the presence of bacterial colony in the environment because sewage waste contains nutrient needed for bacterial metabolism (Mudge & Norris, 1997; Reeves & Patton, 2005).

CONCLUSION

Fatty alcohol variation in the surface sediment showed that the organic matter input in the study

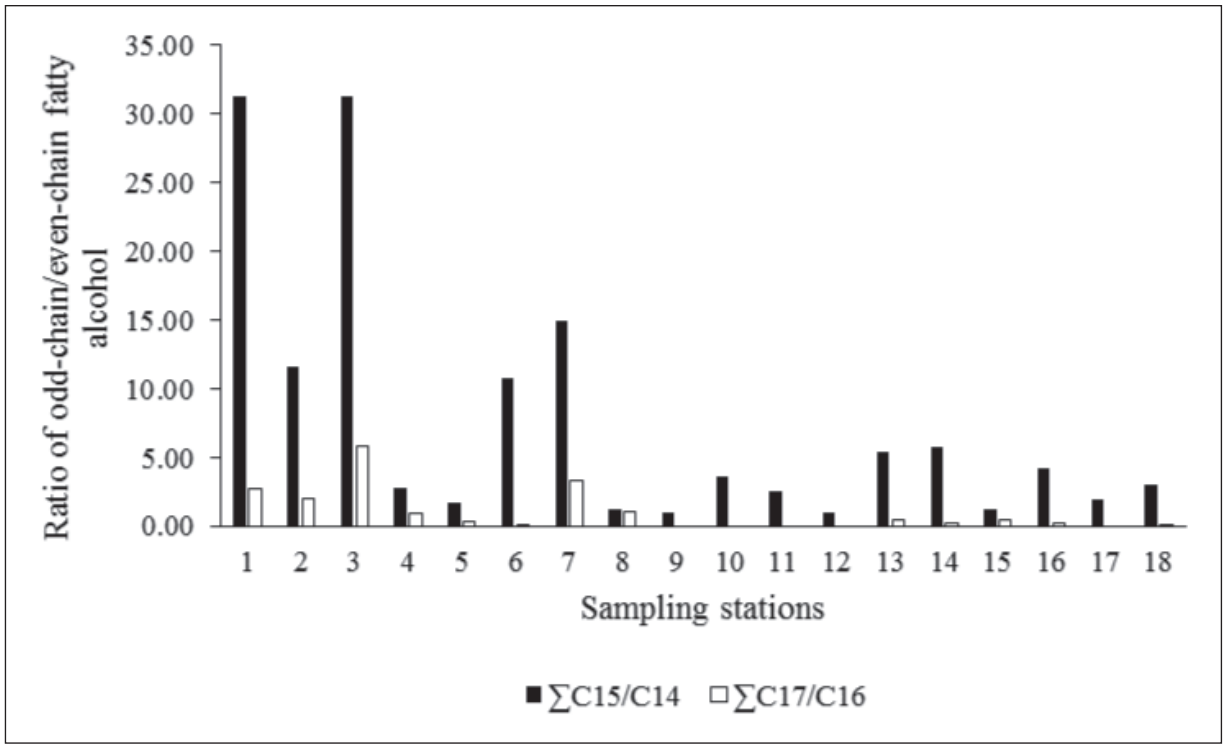

Fig. 7. Ratio of odd-chain/even-chain fatty alcohols for each sampling station.

site originates from various sources particularly terrestrial plants, marine organisms as well as bacterial colony. Long chain fatty alcohol compounds that dominate the study site represents 48% of the total fatty alcohols originating from mangroves, forest area, palm oil and rubber tree planting activities around the study area. Data of short chain/long chain fatty alcohol ratio and alcohol source index (ASI) also showed high long chain compound content in the study site. Branched-chain fatty alcohol content that represents the presence of bacterial colony was found to be low which might be due to the lack of organic matter input particularly sewage waste that could influence the presence of the bacterial colony in the environment.

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