

Fatty Alcohols in Surface Sediment from Pulau Tinggi, Johor, Malaysia

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Abstract

Lipid biomarkers are components derived from the cells of organisms. They can be used to assess their own contribution to aquatic sediment and relate back to their sources. In this study, lipid biomarkers, such as fatty alcohols, were focused on and measured in the surface sediment samples collected from Pulau Tinggi. After which, the compounds were extracted before being analyzed by GC-MS. Results suggest that mixed inputs coming from various sources with short chain fatty alcohols were the predominant compounds, followed by branched compounds and long chain fatty alcohols. These constituted 76%, 13% and 11% of the total fatty alcohols respectively. The ratio between short and long chain fatty alcohols, for most of the samples, also reflected a high amount of short chain compounds which was shown through their having a ratio greater than 1, with the exceptions of two sampling stations (KP3 and KP9), which had a value of 1, thus indicating equal concentrations of both compounds. It can therefore be concluded that this study area is dominated by short chain fatty alcohols which relates to the major contribution of marine sources in locations such as Pulau Tinggi, which is located in the middle of open sea.

Keywords: Lipid biomarker, fatty alcohol, surface sediment

1. Introduction

The role played by fatty alcohols, such as lipid biomarkers, to evaluate aquatic environments is still sparse compared to sterols and fatty acids. However, the application of lipid biomarkers in Malaysia itself is in the early phase compared to other countries such as the US, Australia and those in European,

where this has been applied since the 1970s. Lipid biomarkers are predominantly produced in the cell walls of biology entities such as phytoplankton, zooplankton, terrestrial plants, bacteria, higher animals and various other organisms (Thoumelin et al., 1997; Logan et al., 2001; Mudge, 2005). Lipids, particularly sterols, fatty acids and fatty alcohols, have been widely used as biomarkers for terrigenous and marine-derived organic matter in a range of aquatic and sedimentary environments (e.g. Mudge & Norris, 1997; Seguel et al., 2001; Treignier et al., 2006; Froehner et al., 2008). Lipids have unique characteristics, a particular one being their specificity of biosynthesis from each of their sources and stability in sediments (Saliot et al., 1991). Their insolubility in water leads them to adsorb to particulate matter and have a tendency to settle with sediment (Mudge, 2005; Froehner et al., 2008).

Fatty alcohols are naturally derived from plant or animals oils and fats as they occur in all living organisms from bacteria to humans (Mudge, 2005; Mudge et al., 2008). Fatty alcohols are used widely in the industrial sector in detergents, pharmaceutical, cosmetic and personal cleansing products (Mudge, 2005; Belanger et al., 2008). However, despite there being less attention focused on fatty alcohols such as lipid biomarkers, fatty acids have been largely accepted as a reliable indicator to assess the marine, terrigenous and bacterial contribution to aquatic sediments (Mudge & Norris, 1997; Logan et al., 2001; Mudge & Duce, 2005; Treignier et al., 2006). Fatty alcohols can themselves be divided into three categories; short chain, long chain and branched (*-iso* and *-anteiso*) compounds based on their main sources. Short chain ($\leq C_{20}$) fatty alcohols are derived from marine organisms including plankton and microalgae, while long chain fatty alcohols ($>C_{20}$) are associated with terrigenous inputs, particularly terrestrial plants. Branched compounds, with odd carbon numbered, are produced by bacterial activity (Mudge & Seguel, 1999; Seguel et al., 2001; Shi et al., 2001; Mudge, 2005; Mudge & Duce, 2005).

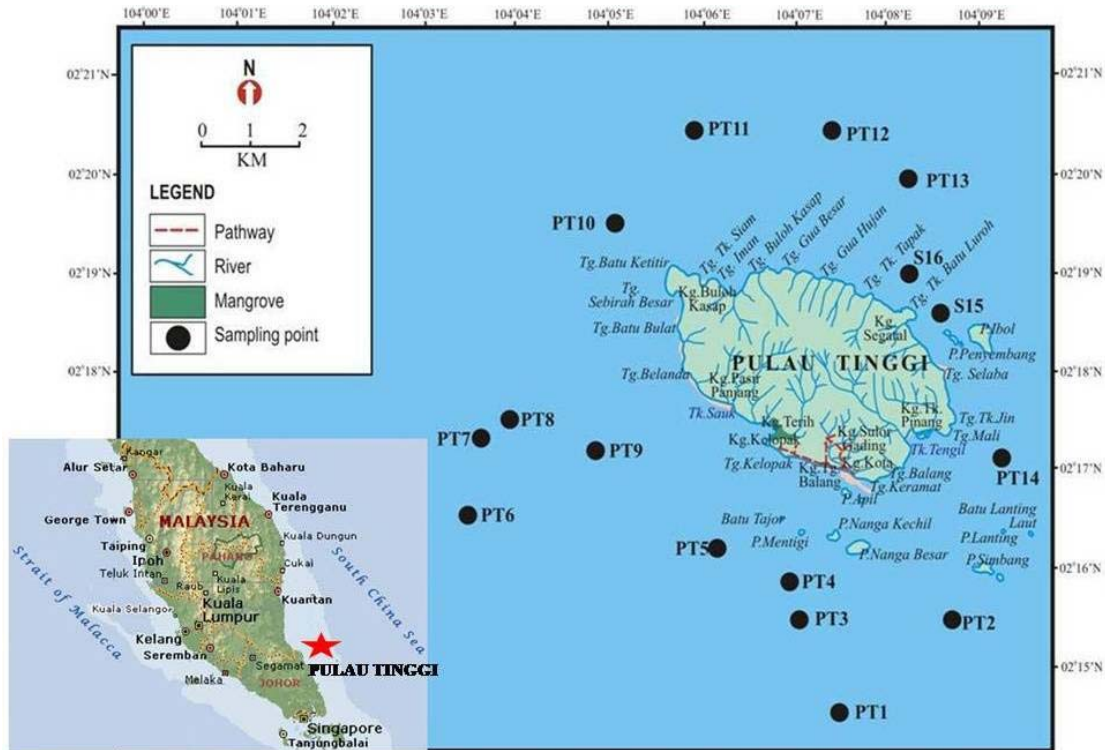
This paper outlines an assessment of fatty alcohol distribution in samples of surface sediment from Pulau Tinggi, Johor, Malaysia and makes use of the compounds as tracers for various sources of organic matter in the study area.

2. Research Method

2.1. Study Area

Pulau Tinggi is one of Malaysia's tropical islands and was gazetted as a Malaysian Marine Park in 1994. It is located about 37 km southeast of Mersing and the northeast coast of the State of Johor. The island is mostly covered with secondary lowland dipterocarp rainforest and has the highest residential population along the east coast of the Johor islands.

A total of sixteen sampling stations were established randomly in the coastal area of Pulau Tinggi (Fig.1 and Table 1). Surface sediments were then collected from each sampling station using a PONAR grab. Samples were stored in a freezer at -4°C whilst awaiting further analysis.

Figure 1: Study Area**Table 1:** Sampling Stations

Station	Latitude (N)	Longitude (E)
PT1	02° 14' 32	104° 07' 33
PT2	02° 15' 32	104° 08' 39
PT3	02° 15' 33	104° 07' 01
PT4	02° 15' 51	104° 07' 59
PT5	02° 16' 09	104° 06' 09
PT6	02° 16' 29	104° 03' 29
PT7	02° 17' 14	104° 04' 43
PT8	02° 17' 19	104° 04' 59

Station	Latitude (N)	Longitude (E)
PT9	02° 17' 05	104° 04' 56
PT10	02° 19' 27	104° 05' 03
PT11	02° 20' 34	104° 05' 55
PT12	02° 09' 39	104° 00' 43
PT13	02° 11' 24	104° 00' 43
PT14	02° 12' 00	104° 02' 05
PT15	02° 18' 37	104° 08' 34
PT16	02° 19' 00	104° 08' 09

2.2. Total Organic Carbon (TOC) Analysis

The TOC content in the sediment samples was analyzed by first heating the samples in a furnace (Heiri et al., 2001). Sediment samples were dried in the oven at 60°C for about 2-3 days until a consistent weight was gained. They were then crushed in dry mortar with a porcelain pestle. Once again samples were oven-dried at 105°C for 24h, followed by combustion in a furnace at 550°C for 4h. The TOC content was then calculated using the following equation;

$$\% \text{ TOC} = \frac{DW_{105} (\text{g}) - DW_{550} (\text{g})}{DW_{105} (\text{g})} \times 100$$

Where DW_{105} represents the dry weight sample at 105°C and DW_{550} the dry weight of the samples after heating to 550°C.

2.3. Fatty Alcohol Analysis

The methods used for preparation and analysis followed the extraction procedures given in the literature of Mudge and Norris (1997), and Masni and Mudge (2006). Approximately 30-40g wet weight of sediment was hydrolyzed with 50ml of 6% potassium hydroxide in methanol. The samples

were refluxed for 4h and centrifuged at 2500 r.p.m for 5 minutes. The supernatant was then funneled into a separating flask.

Non-polar lipids were extracted from the supernatant by liquid-liquid separation. A total of 20ml of hexane and 10ml of double distilled water were added to the supernatant. The mixture was then shaken vigorously. After being shaken, the cap of the separating flask was loosened to release the pressure inside. The non-polar fraction was collected and transferred into a florentine flask. The whole procedure was repeated to ensure maximize extraction. Samples were evaporated at 40°C in a rotary evaporator, redissolved in 2-3ml of hexane and then transferred into a 14ml vial. Anhydrous sodium sulphate was added to remove any water and polar compounds left in the samples. The remaining solution was filtered through filter paper and blow-dried under oxygen free nitrogen (OFN).

Samples derivatisation had to be undertaken in order to permit the analysis of compounds with the Gas Chromatograph (GC). Approximately 2-3 drops of bis-(trimethylsilyl) trifluoroacetamide (BSTFA) were added to the samples and then heated in a heating block for 10 minutes at 60°C. Finally, they were evaporated to dryness under OFN and then redissolved in 1ml of hexane.

A computerized gas chromatography-mass spectrometry (GC-MS) (Perkin Elmer Clarus 500) was used to analyze the sterols in the samples. The temperature program used started at 80°C, increasing at 15°C min⁻¹ to 300°C, then at 5°C min⁻¹ to a maximum of 350°C for 10 minutes. Calibration was carried out using an octadecanol-TMS solution in order to quantify the peaks obtained from the analysis. All results are relative to bulk sediment and are expressed on a dry-weight basis.

2.4. Quality Assurance Procedures

Standard methods and techniques were adopted during this work. In the laboratory, analyses were carried out in Decon-90 washed glassware. The efficiency of the whole extraction process was confirmed by the repeat reflux of some sediment samples; whereby no further fatty alcohol compounds could be detected in these later extractions. Blanks and calibration standards were used throughout the GC injections. A blank was injected first and followed by the calibration standard. Five samples were injected afterwards, and followed by the blank and calibration standard again. Random samples were extracted three times to test the reproducibility of the extraction. The reproducibility of the extraction was found to be greater than 90%. Procedural blanks were also analyzed and no compounds of interest were measured in any sample. All glassware and Teflon-lined caps used in these analyses were rinsed with organic solvents prior to work.

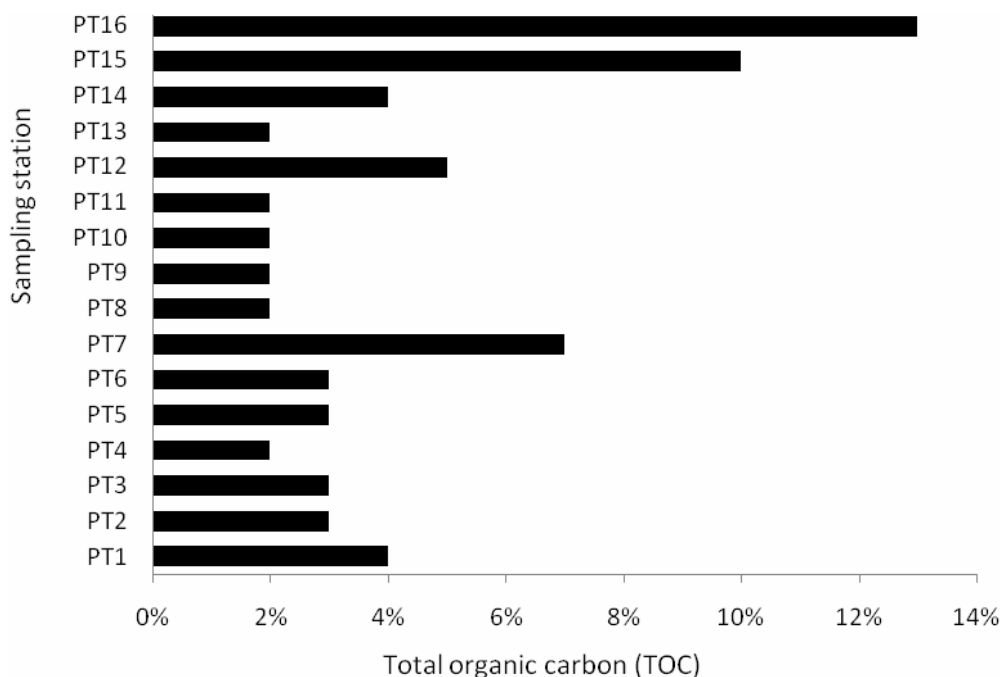
3. The Results Discussion

3.1. Total Organic Carbon (TOC)

It was observed that total organic carbon (TOC) values for the surface sediment samples from Pulau Tinggi ranged from a minimum of 2% to a maximum of 13%, with a mean value of 0.04% (Figure 2). The highest content of TOC was found at station PT16 followed by station PT15 (10%), whilst the rest were within the range of 2-7%. Both station PT16 and PT15 received input from the nearby river, being located at the river mouth (Figure 1) where mangroves grow along the riverside. Consequently, both these sampling stations were found to have high amounts of terrestrial organic matter while the other sampling stations are most likely to have received organic carbon from marine organisms. The input of terrestrial organic matter into the aquatic environment is directly affected by the role of river and atmosphere that transport components produced by terrestrial plants (Seki et al., 2006). In addition, TOC is also naturally produced by phytoplankton and diatom, specifically in high productivity environments (Pagani et al., 1999; Méjanelle & Laureillard, 2008) where photosynthesis is the main process that produces carbon dioxide and converts it into organic carbon (Tolosa et al., 2008). The size of sediment samples also plays an important role in determining TOC. This is particularly so where fine-grained sediments have a high amount of TOC due to the high surface area to adsorbed organic

matter in comparison to sand which has large sized grains (Hyun et al., 2002; Ramaswamy et al., 2008). Sediment samples from station PT15 and PT16 consisted of a mixture of muddy and sand sediments, whilst the other sediment samples were only sand. This could explain the high amounts of TOC at these stations.

Figure 2: TOC at each sampling station



3.2. Distribution of Fatty Alcohol

The results obtained in this investigation give a variation of thirteen fatty alcohols in sixteen surface marine sediment samples as shown in Table 2. The sum of the fatty alcohol concentration ranged from 3.03 to 1990.79 ng g⁻¹ dry weight sediment (d.w). The data reflected a mixture of compounds derived from various sources of marine and terrigenous organisms, although short chain fatty alcohol (C₁₂-C₂₀) is the most abundant compound accounting for 76% of total fatty alcohol, followed by branched fatty alcohol (13%) and long chain fatty alcohol (C₂₁-C₂₄) (11%) (Figure 3). Individually, the two major compounds quantified are C₁₃ and C₁₂. C₁₃ fatty alcohol constitutes 32% of total fatty alcohol, with a mean concentration of 1015.99 ng g⁻¹ d.w. with the values ranging within 222.6 ng g⁻¹ d.w at station PT5 and 1011.79 ng g⁻¹ d.w at station PT14. The next most abundant compound is C₁₂ which constitutes 30% of total fatty alcohol, with the minimum and maximum concentration also at station PT5 (602.5 ng g⁻¹ d.w) and station PT14 (1990.79 ng g⁻¹ d.w) respectively with a mean value of 965.42 ng g⁻¹ d.w). Therefore, total concentrations for the remaining fatty alcohols determined, constitute a total of 38% of fatty alcohol, including branched compounds.

Figure 3: Percentages of fatty alcohols

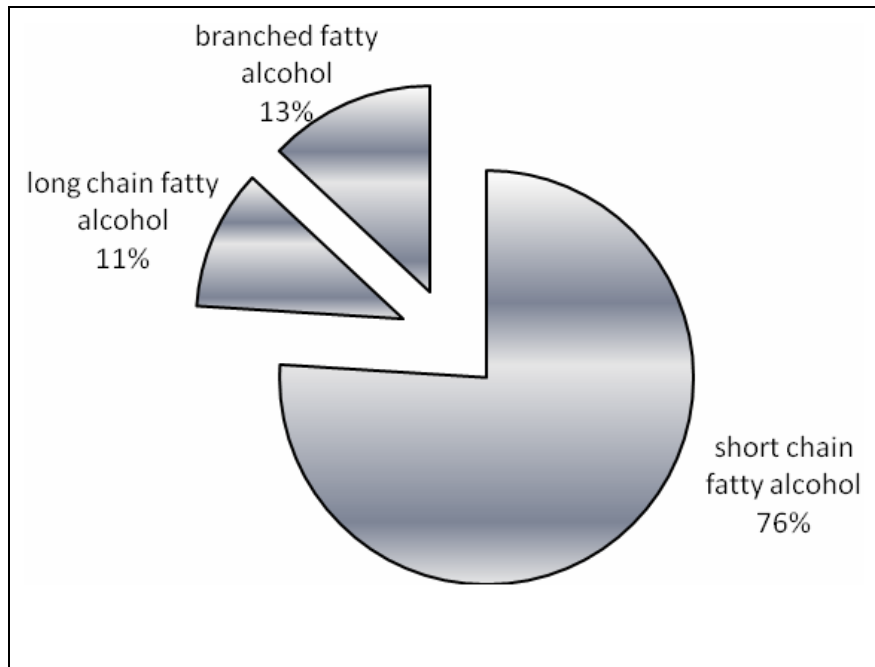


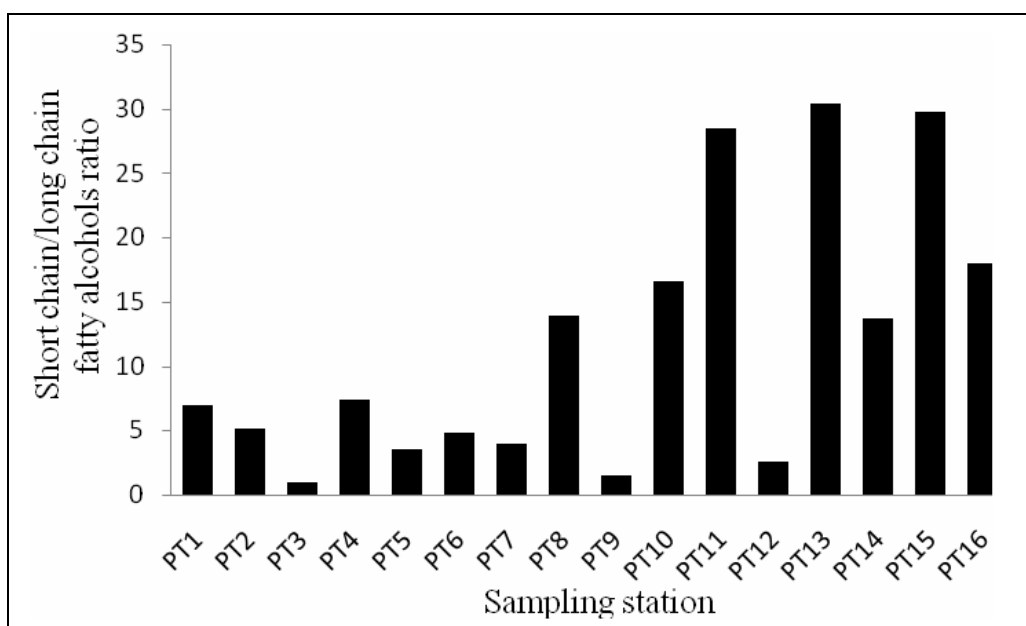
Table 2: Fatty alcohols concentration for each sampling station (ng g⁻¹ dry weight)

	PT1	PT2	PT3	PT4	PT5	PT6	PT7	PT8	PT9	PT10	PT11	PT12	PT13	PT14	PT15	PT16
C ₁₂	1079.25	981.14	794.57	696.56	602.5	839.61	704.9	1188.4	1137.61	946.02	1221.58	690.7	1008.23	1990.79	776.47	788.35
bC ₁₃	50.66	45.09	16.09	45.97	55.17	30.5	104.82	88.72	14.44	58.7	10.75	85.34	9.36	87.87	7.2	98.52
C ₁₃	604.93	492.26	392.83	296.22	222.6	355.86	309.17	597.05	503.74	445.3	604.84	246.88	494.26	1011.79	385.65	367.67
C ₁₄	20.25	0	5.85	0	0	11.18	9.62	0	0	11.17	0	30.39	0	11.14	0	3.54
iC ₁₅	217.72	177.27	204.64	0	88.71	129.33	120.94	179.52	245.71	164.09	0	183.1	144.85	313	0	198.51
aC ₁₅	7.5	0	0	0	0	9.86	22.23	5.93	0	5.78	0	17.89	0	18.09	0	17.27
bC ₁₅	29.47	0	0	0	0	10.38	0	0	0	0	0	66.06	0	0	0	75.57
C ₁₅	15.25	0	0	5.27	4.73	6.57	9.65	4.13	0	4.83	0	25.05	0	13.31	0	29.71
C ₁₆	130.02	21.49	181.8	25.6	10.26	54.52	78.89	8.96	8.95	49.07	0	152.76	0	69.22	0	1463.3
iC ₁₇	8.5	0	0	44.81	39.18	19.26	44.08	61.29	248.57	64.91	0	14.25	0	107.49	0	27.72
aC ₁₇	15.19	0	0	62.79	5.04	7.73	9.95	11.25	5.79	4.59	0	19.45	0	9.01	0	17.21
C ₁₇	12.23	0	4.51	5.23	23.86	6.37	14.56	10.57	7.08	13.9	0	12.9	0	5.21	0	108.97
C ₁₈	61.76	14.34	11.43	20.55	10.3	38.98	51.19	37.22	298.27	15.75	0	66.42	27.84	17.27	21.75	806
C ₁₉	51.8	81.47	452.79	42.06	35.61	55.57	28.65	35.45	351.95	55.1	3.03	70.89	24.04	62.54	19.53	67.31
C ₂₀	43.83	0	0	21.21	8.22	26.92	33.69	0	0	6.52	0	14.37	0	8.31	0	54.53
C ₂₁	72.46	104.77	650.3	41.18	42.85	44.07	32	37.7	453.24	70.38	30.19	111.46	23.21	69.6	19.79	40.55
C ₂₂	112.91	8.27	29.15	4.59	61.3	109.59	96.88	15.99	13.66	23.03	0	178.55	0	53	0	68.29
C ₂₃	95.33	194.35	1222.9	97.44	107.2	95.04	77.81	52.72	1078.26	0	34.14	199.46	27.98	96.98	20.69	84.74
C ₂₄	9.41	0	0	8.27	45.19	42.22	106.42	29.04	0	0	0	10.39	0	13.4	0	11.32

b-branched compound; i-iso; a-anteiso

The role of fatty alcohols as a biomarker has limitations due to indefinable specific species which make it impossible/almost impossible to identify a single source of each compound. However, many studies have established that the major input of organic matter into the aquatic system can be still assessed based on the compound categories according to their carbon number range. The application of ratios could also provide a strong indication of the major sources of organic matter in the study area in addition to the individual concentration data of fatty alcohols. The most applied fatty alcohol ratios is the ratio of short chain to long chain compounds, which has been used widely to assess the predominance of marine or terrestrial contributions (Mudge & Norris, 1997; Treignier et al., 2006). The ratio of 1 indicates an equal amount of short chain and long chain fatty alcohols, values >1 indicates a greater concentration on short chain, while a value <1 indicates that long chain compounds are dominant but usually familiar in the freshwater systems. The ratio $[\Sigma(C_{12}-C_{20})/\Sigma(C_{21}-C_{24})]$ was plotted in Figure 4; the values >1 calculated in most of the sampling stations reaching a maximum of 30 at station PT13 and PT15, with the lowest value of 1 calculated at station PT3 and PT9.

Figure 4: Short chain/long chain fatty alcohol ratio for each sampling station



Both distribution patterns of fatty alcohols and the ratio support the high input of the short chain compound ($C_{12}-C_{20}$). Short chain compounds predominantly originate from marine organisms but it is also reported that these compounds are derived from unspecified terrigenous sources (Mudge & Seguel, 1999; Seguel et al., 2001). However, $\leq C_{20}$ compounds are assumed to predominantly have marine sources such as phytoplankton, zooplankton and bacteria (Mudge & Norris, 1997; Treignier et al., 2006). According to Volkman et al. (1999), zooplankton has been identified as a major contributor of fatty alcohols in marine sediments. Moreover, short chain fatty alcohols are believed to derive from freshwater organisms (Treignier et al., 2006). In this study though, it is not possible that these are the major input of short chain compounds, due to the distance between the study area and terrestrial and riverine inputs. As reported earlier, C_{12} and C_{13} are the most abundant compounds in the study area, which is in conjunction with the study area's location at sea.

The next most abundant fatty alcohols, which constitute 13% of the total fatty alcohols, are branched compounds. These compounds mainly originate from microbial inputs but are also reported to have zooplankton and microalgae as sources (Mudge & Norris, 1997; Volkman et al., 1999; Mudge & Duce, 2005). Individually, the C_{13} compound is the major fatty alcohol found in most samples, with a concentration which ranged from 222.60 to 1011.79 ng g⁻¹ d.w and a mean value of 1015.99 ng g⁻¹ d.w (Table 2). As reported by Mudge & Norris (1997) based on the literature of Parkes (1987),

branched fatty alcohols are usually produced from bacterial metabolism, for example the *iso*-C₁₃ compound is formed from a straight chain C₁₂ compound. Therefore, a high concentration of branched compounds also indicates a high population of bacteria communities.

The least quantified compound is long chain fatty alcohol (C₂₁-C₂₄) which is indicative of terrigenous organic matter mainly derived from higher vascular plants (Mudge & Norris, 1997; Mudge & Seguel, 1999; Seguel et al., 2001; Treignier et al., 2006). This was also apparent in the marine sediments even though far from terrestrial input, which can probably be attributed to the physical processes, particularly by transportation via coastal currents and the subsequent deposition of the compounds. There is a small quantity of literature that documents other sources of long chain compounds, such as microalgae (especially cyanobacteria, freshwater euglenophytes and aquatic macrophytes) (Volkman et al., 1998; Ficken et al., 2000). However, these sources are not the main origin of the compounds. Somehow, this explains the occurrence of long chain compounds in marine surface sediments, especially at station PT3 and PT9.

4. Summary and Concluding Remarks

The results presented here illustrate a mixture of short chain, long chain and branched compounds of fatty alcohol which originate from various sources. Short chain fatty alcohol comprised a major fraction of total fatty alcohol and accounted for 76% of the total fatty alcohol, which indicates a high input from marine organisms. Ratios of short chain to long chain fatty alcohol confirmed that the major input in the study area are marine organisms. Moreover, whilst a long chain compound is synonymous to higher plant terrestrial input, in this study the compound is likely to have been derived from aquatic macrophytes due to the fact that the distance between the sampling stations and terrestrial input is rather too far to have received terrestrial organic matter. Branched compounds were also identified in high concentrations and constituted 13% of the total fatty alcohol. This is a strong indicator that there is a high population of bacteria in the sampling area, since branched compounds are formed as a result of bacterial activities. Overall, the results obtained synchronize with the study area and its' location in the middle of open sea.

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