7.1 Introduction

Several neutral lipid classes are used as biomarkers (alcohols, sterols, terpenoids etc) because of their relatively high specificity in source assignments and better resistance to bacterial degradation than other classes of organic compounds (Volkman, 2006). Fatty alcohols can be efficient biomarkers for distinguishing marine and terrestrial organic matter. Short-chain (<C_{20}) n-alcohols are produced by marine and freshwater organisms, while long-chain (≥C_{22}) n-alcohols with a strong even-over-odd carbon number preference are derived from waxes of terrestrial higher plants (Jeng et al., 2003; Treignier et al., 2006; Xu and Jaffe, 2007; Costa et al., 2011). Phytol (3,7,11,15-tetramethyl-2-hexadecen-1-ol), a diterpenoid alcohol present as an esterified side-chain of the chlorophyll-a molecule, is a general marker for primary producers and a suitable indicator of “fresh” organic matter derived from autotrophs due to its high reactivity (Jeng and Huh, 2004; Rontani and Volkman, 2003; Volkman et al., 2007; Costa et al., 2011).

Sterols are ubiquitous components of cellular membranes in eukaryotic organisms including phytoplankton, zooplankton and higher
plants in which they are known to improve the mechanical properties of phospholipids bi-layers, while prokaryotic organism do not generally biosynthesise these compounds (Volkman, 1986). They represent a class of molecular biomarkers that can be used to differentiate between allochthonous, autochthonous and anthropogenic lipid carbon sources in estuarine environment. The unique structural features such as position of double bond, alkylation in the ring system and side chain, and stereochemistry makes them suitable tracers of organic matter sources. The source specificity, broad diversity and their distinguishable diagenetic products makes them excellent indicators to delineate provenance as well as the diagenetic pathway of sedimentary organic matter (Volkman, 2006; Rontani et al., 2009).

Prevalence of 4-methyl sterols such as 4α, 23, 24-trimethyl-5α-cholest-22(E)-en-3β-ol (dinosterol) indicate a significant diatom flagellate contribution, although certain diatoms have been found to synthesise these sterols (Volkman et al., 1993; Hudson et al., 2001). Even though certain algae produce C29 sterols, proportionately high abundance of C29 sterols are indicative of terrestrial inputs (Laureillard and Saliot, 1993; Li et al., 1995; Hudson et al., 2001). Terrestrial plants have been shown to have a high abundance of 24-ethylcholest-5-en-3β-ol (sitosterol), 24-methylcholest-5-en-3β-ol (campesterol) and stigmasterol (Volkman, 1986; Jaffè et al., 1995; Bianchi, 2007). The major sterol biomarkers used in the study are furnished in Table 7.1.
Table 7.1 Name and structure of major sterol biomarkers used in the study

<table>
<thead>
<tr>
<th>Sl No</th>
<th>IUPAC Name</th>
<th>Common Name</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cholest-5-en-3β-ol</td>
<td>Cholesterol</td>
<td><img src="image1.png" alt="Structure" /></td>
</tr>
<tr>
<td>2</td>
<td>5α-Cholestan-3β-ol</td>
<td>Cholestanol</td>
<td><img src="image2.png" alt="Structure" /></td>
</tr>
<tr>
<td>3</td>
<td>24-Methylcholesta-5,22E-dien-3β-ol</td>
<td>Brassicasterol</td>
<td><img src="image3.png" alt="Structure" /></td>
</tr>
<tr>
<td>4</td>
<td>24-Methylcholesta-5-en-3β-ol</td>
<td>Campesterol</td>
<td><img src="image4.png" alt="Structure" /></td>
</tr>
<tr>
<td>5</td>
<td>24-Ethylcholesta-5,22E-dien-3β-ol</td>
<td>Stigmasterol</td>
<td><img src="image5.png" alt="Structure" /></td>
</tr>
<tr>
<td>6</td>
<td>24-Ethylcholesta-5-en-3β-ol</td>
<td>Sitosterol</td>
<td><img src="image6.png" alt="Structure" /></td>
</tr>
<tr>
<td>7</td>
<td>24-Ethyl-5α-cholestan-3β-ol</td>
<td>Stigmastanol</td>
<td><img src="image7.png" alt="Structure" /></td>
</tr>
<tr>
<td>8</td>
<td>4α, 23,24-Trimethyl-5α-cholesta-22-en-3β-ol</td>
<td>Dinosterol</td>
<td><img src="image8.png" alt="Structure" /></td>
</tr>
</tbody>
</table>
Cholesterol is the most abundant and ubiquitous sterol in the environment due to its multiple sources (Pratt et al., 2008). Even though cholesterol is mainly animal sterol, it is also produced by other organisms including diatoms, microbial communities, macrophytes, algae, phytoplankton and zooplankton (Logan et al., 2001; Reeves and Patton, 2001; Azevedo, 2003). Thus, the use of cholesterol on its own as a biomarker for organic matter is limited and often been used in the ratio form with other sterols. The sterol coprostanol, which is produced by bacterial reduction of cholesterol in the digestive systems of higher animals (McCalley et al., 198; Brown and Wade 1984), is largely used to characterise sewage inputs to aquatic ecosystems (Isobe et al., 2004; Cordeiro et al., 2008). Pentacyclic triterpenoids have been used to characterise the contribution of vascular plant sources in sedimentary environment (Koch et al., 2003; Boot et al., 2006). Pentacyclic triterpenoids with oleanane, ursane, taraxerane, lupane and friedoleanane skeleton is identified in mangroves and terrestrial plants but not in marine organisms. The identification of fatty alcohols, sterols and pentacyclic terpenoids can give important source information and signal of organic matter remineralisation pathway. The major pentacyclic triterpenoids employed in the study are presented in Table 7.2
Table 7.2  Major pentacyclic triterpenoid biomarkers used in the present study.

<table>
<thead>
<tr>
<th>Sl No</th>
<th>Common Name</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Germanicol</td>
<td><img src="image" alt="Germanicol structure" /></td>
</tr>
<tr>
<td>2</td>
<td>β-Amyrin</td>
<td><img src="image" alt="β-Amyrin structure" /></td>
</tr>
<tr>
<td>3</td>
<td>Friedelin</td>
<td><img src="image" alt="Friedelin structure" /></td>
</tr>
<tr>
<td>4</td>
<td>Betulin</td>
<td><img src="image" alt="Betulin structure" /></td>
</tr>
<tr>
<td>5</td>
<td>Taraxerol</td>
<td><img src="image" alt="Taraxerol structure" /></td>
</tr>
<tr>
<td>6</td>
<td>Lupeol</td>
<td><img src="image" alt="Lupeol structure" /></td>
</tr>
</tbody>
</table>
7.2 Results

7.2.1 Fatty alcohols in the surface sediments

A total of 18 fatty alcohols (C_{12}-C_{28}), including short chain, long chain and branched were identified from surface sediments of the Cochin estuary and the relative abundance of each fatty alcohol is furnished in Table 7.3. Six branched chain fatty alcohols were identified including 6, 10, 14-trimethylpenta-decan-2-ol, 3,7,11,15-tetramethyl-hexadecanol and phytol. Barmouth region is characterised by high abundance of phytol, short chain fatty alcohols including hexadecanol, tetradecanol and octadecanol. The long chain fatty alcohols were not identified in the barmouth region. Bolghatty region exhibited high abundance of hexadecanol, octacosanol, 13-methyl pentadecanol and tetracosanol. Unlike bar mouth region, this station showed presence of long chain fatty alcohols such as octacosanol and docosanol.

The inner part of the estuary exhibited the predominance of long chain fatty alcohols. This area is characterised by higher abundance of docosanol, hexacosanol and octacosanol. The abundance of short chain fatty alcohols decreased towards the inner part of the estuary. In all samples, there was an even over odd predominance in the n-alcohol chain length. However, in Fisheries harbour region, pentadecanol displayed high abundance. The distribution of n-alcohols is bimodal and characterised by even carbon predominance. In all the samples, n-C_{16} was the most dominant compound among short chain n-alcohols, while C_{22} and C_{28} was the most dominant in long chain alcohols.
Table 7.3 Percentage distribution of major fatty alcohols identified from the surface sediments of the Cochin estuary

<table>
<thead>
<tr>
<th>Fatty Alcohol</th>
<th>S1</th>
<th>S2</th>
<th>S4</th>
<th>S5</th>
<th>S6</th>
<th>S7</th>
<th>S9</th>
<th>S10</th>
<th>S14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dodecanol (C₁₂)</td>
<td>2.61</td>
<td>3.75</td>
<td>0.43</td>
<td>3.54</td>
<td>1.78</td>
<td>0.90</td>
<td>0.68</td>
<td>-</td>
<td>0.54</td>
</tr>
<tr>
<td>Tetradecanol (C₁₄)</td>
<td>5.91</td>
<td>7.63</td>
<td>0.44</td>
<td>2.49</td>
<td>3.33</td>
<td>2.56</td>
<td>3.03</td>
<td>-</td>
<td>1.75</td>
</tr>
<tr>
<td>Methyl Tetradecanol (Me-C₁₅₀)</td>
<td>4.12</td>
<td>11.57</td>
<td>1.10</td>
<td>4.52</td>
<td>3.67</td>
<td>2.86</td>
<td>2.09</td>
<td>-</td>
<td>3.15</td>
</tr>
<tr>
<td>Pentadecanol (C₁₅)</td>
<td>2.02</td>
<td>5.02</td>
<td>0.00</td>
<td>25.23</td>
<td>1.11</td>
<td>0.79</td>
<td>0.73</td>
<td>-</td>
<td>0.70</td>
</tr>
<tr>
<td>6,10,14-trimethylpentadecan-2-ol</td>
<td>2.09</td>
<td>3.47</td>
<td>0.48</td>
<td>1.38</td>
<td>1.49</td>
<td>1.01</td>
<td>0.74</td>
<td>-</td>
<td>0.89</td>
</tr>
<tr>
<td>Hexadecanol (C₁₆)</td>
<td>13.76</td>
<td>23.18</td>
<td>4.42</td>
<td>8.58</td>
<td>13.85</td>
<td>10.82</td>
<td>6.09</td>
<td>2.84</td>
<td>15.52</td>
</tr>
<tr>
<td>Heptadecanol (C₁₇)</td>
<td>2.55</td>
<td>4.15</td>
<td>1.35</td>
<td>1.65</td>
<td>2.72</td>
<td>2.87</td>
<td>1.13</td>
<td>0.96</td>
<td>3.30</td>
</tr>
<tr>
<td>3,7,11,15-tetramethyl hexadecanol</td>
<td>-</td>
<td>-</td>
<td>1.89</td>
<td>-</td>
<td>1.70</td>
<td>1.93</td>
<td>0.99</td>
<td>1.29</td>
<td>2.35</td>
</tr>
<tr>
<td>Octadecanol (C₁₈)</td>
<td>11.28</td>
<td>9.91</td>
<td>3.57</td>
<td>4.91</td>
<td>10.93</td>
<td>10.10</td>
<td>4.78</td>
<td>3.15</td>
<td>6.33</td>
</tr>
<tr>
<td>Phytol</td>
<td>38.23</td>
<td>-</td>
<td>6.30</td>
<td>1.15</td>
<td>8.93</td>
<td>15.18</td>
<td>3.15</td>
<td>27.30</td>
<td>2.59</td>
</tr>
<tr>
<td>18-methyl nonadecanol</td>
<td>5.57</td>
<td>3.57</td>
<td>4.35</td>
<td>3.47</td>
<td>6.53</td>
<td>5.60</td>
<td>3.75</td>
<td>2.84</td>
<td>2.24</td>
</tr>
<tr>
<td>Docosanol (C₂₂)</td>
<td>8.86</td>
<td>5.50</td>
<td>21.28</td>
<td>9.48</td>
<td>23.01</td>
<td>15.36</td>
<td>22.85</td>
<td>9.77</td>
<td>13.85</td>
</tr>
<tr>
<td>Tricosanol (C₂₃)</td>
<td>0.85</td>
<td>-</td>
<td>2.17</td>
<td>-</td>
<td>1.24</td>
<td>1.67</td>
<td>1.90</td>
<td>0.21</td>
<td>1.08</td>
</tr>
<tr>
<td>22-methyltricosanol</td>
<td>2.16</td>
<td>3.03</td>
<td>14.53</td>
<td>5.31</td>
<td>6.60</td>
<td>9.54</td>
<td>18.17</td>
<td>15.35</td>
<td>22.84</td>
</tr>
<tr>
<td>Pentacosanol (C₂₅)</td>
<td>-</td>
<td>-</td>
<td>2.64</td>
<td>-</td>
<td>-</td>
<td>0.87</td>
<td>2.21</td>
<td>2.46</td>
<td>4.30</td>
</tr>
<tr>
<td>Hexacosanol (C₂₆)</td>
<td>-</td>
<td>1.20</td>
<td>13.03</td>
<td>1.75</td>
<td>4.32</td>
<td>7.29</td>
<td>9.54</td>
<td>16.88</td>
<td>10.12</td>
</tr>
<tr>
<td>Heptacosanol (C₂₇)</td>
<td>-</td>
<td>-</td>
<td>2.29</td>
<td>0.00</td>
<td>-</td>
<td>0.56</td>
<td>1.95</td>
<td>-</td>
<td>2.67</td>
</tr>
<tr>
<td>Octacosanol (C₂₈)</td>
<td>-</td>
<td>18.03</td>
<td>19.71</td>
<td>26.52</td>
<td>8.79</td>
<td>10.11</td>
<td>16.23</td>
<td>16.94</td>
<td>5.76</td>
</tr>
</tbody>
</table>
7.2.2 Sterols and pentacyclic triterpenoids in the surface sediments

The identification of individual sterols is confirmed by co-injection with authentic standards and by the comparison of individual mass spectra with published MS data (Volkman, 1986; Duan, 2000). The presence and abundance of various sterols showed large variations in the study region. The major sterols identified in the surface sediments of Cochin estuary are furnished in Table 7.4.

<table>
<thead>
<tr>
<th>Name</th>
<th>S1</th>
<th>S2</th>
<th>S4</th>
<th>S5</th>
<th>S6</th>
<th>S7</th>
<th>S9</th>
<th>S10</th>
<th>S14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cholestanol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Brassicasterol</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Stigmastanol</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Stigmasterol</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Sitosterol</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Dinosterol</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ detected, - not detected

Cholesterol, cholestanol, brassicasterol, campesterol, stigmasterol, β-sitosterol, stigmastanol and dinosterol were identified in the bar mouth region. The dominance of C_{27} sterols is indicative of a zooplankton source, even though they are common in algae (Volkman, 1986; Lu and Zhai, 2006). The cholesterol contributed 29% of total sterols detected in the barmouth region. Brassicasterol, campesterol and dinosterol were the significant sterols in the barmouth region. Brassicasterol, dinostanol, cholestanol contributed 13%, 8% and 8% of total abundance respectively, while sitosterol contributed 13% of total abundance.

All estuarine stations except S4 and S10 showed the presence of cholesterol and cholestanol. Cholesterol/cholestanol ratio ranged from 0
Sterols as Biomarkers

Brassicasterol was detected in stations S1, S5, S6, S7 and S14. The major penatacyclic triterpenoids identified include: germamicol, amyrin, betulin and friedelin. The entire estuarine area showed the presence of germamicol. The relative abundance of germamicol displayed an increasing pattern from barmouth region to the inner parts of the estuary. The chromatogram of pentacyclic triterpenoids are given in Appendix.

7.3 Discussion

Many previous studies pointed out the predominance of even number carbon chain between C_{12} and C_{28} as a characteristic feature of fatty alcohols in marine sediments (Sever and Parker, 1969; Volkman et al., 1981; Mudge and Norris, 1997). Short chain fatty alcohols predominantly originate from marine organisms including plankton and microalgae, but it is also reported that these compounds are derived from unspecified terrigenous sources (Mudge and Seguel, 1999; Seguel et al., 2001). Short chain fatty alcohols are also produced by freshwater organisms (Triegnier et al., 2006) and Volkman et al., 1999 identified zooplankton as major source of fatty alcohols in marine sediments.

Comparatively high abundance of short chain fatty alcohols in the barmouth region is attributed to the increased input of autogenous organic matter to the surface sediments. C_{12}, C_{14}, C_{16} and C_{18} fatty alcohols contributed 33.56% of total fatty alcohols in this region, while the long chain fatty alcohols remained below the detection level. The long chain fatty alcohols showed an increasing pattern from barmouth region to the inner part of the estuary and short chain fatty alcohols exhibited a decreasing pattern. Either C_{22} or C_{28} n-alcohols predominated in the
estuarine sediments. The abundance of C_{22} and C_{24} n-alcohols may be attributed to aquatic macrophytes (Ficken et al., 1998). The predominance of long chain fatty alcohols indicates the input of terrestrial higher plants (Fukushima and Ishiwatari, 1984; Mudge and Norris, 1997; Gao et al., 2008). The relatively high abundance of allochthonous material in the inner part of the estuary is not only due to the input of terrestrial organic matter but also due to the difference in reactivity of marine derived and terrestrial derived organic materials.

Odd chain and branched chain alcohols were detected in the surface sediments. Branched fatty alcohols are usually resulting from bacterial metabolism (Parkes, 1987; Mudge and Norris, 1997) of even chain length precursors. Therefore, the degree of bacterial metabolism in sediment samples can be assessed by using the ratio between the even chain precursor and odd carbon numbered methyl derivative of fatty alcohols. The presence of branched and odd chain fatty alcohols are signals of bacterial degradation processes acting in the surface sediments.

Phytol (3, 7, 11, 15-tetramethyl-2-hexadeca-1-ol) was identified in all the stations except at Bolgatty region. The relative abundance ranged from 0 to 38.23% in total fatty alcohols. The barrowth region (S1) and Poothotta region (S10) of the estuary showed high abundance of phytol. It derives from the side chain of Chl-\(\alpha\) and is a typical marker of phytoplankton (Shi et al., 2001; Pearson et al., 2007). It can also arise from the hydrolysis of bacterio-chlorophyll \(\alpha\) of purple sulphur bacteria (Marchand and Rontani, 2003). Previous studies reported that sedimentary bound phytol is derived from both terrestrial plants and phytoplankton (Chikaraishi and Naraoka, 2005), while free phytol in sediments is predominantly from phytoplankton inputs (Pearson et al., 2007).
3, 5, 17, 15 – hexadeca-1-ol (dihydroxyphytol) was also identified in surface sediments. Dihydroxyphytol is previously reported in faecal pellets of copipods (Prahl et al., 1984a,b) and relative percentage of dihydroxyphytol to phytol can be used to estimate the input of faecal zooplanktonic material in aquatic systems (Christodoulou et al., 2009). The dihydroxyphytol was identified only in the inner part of the estuary suggests the zooplankton grazing in the inner estuary. The isoprenoid 6, 10, 14 – trimethyl pentadecan-2-one (phytene) is an important component in many sites of the estuary and showed maximum abundance in the Bolgatty region. Phytone is the early oxidation product of phytol and the distribution of phytone can be used as an indicator of bacterial activity (Brooks and Maxwell, 1974; Rontani and Acquaviva, 1993; Marchand and Rontani, 2003; Pearson et al., 2007). Isoprenoid ketones may be produced in several processes including bacterial degradation and photosensitised oxidation of free phytol, photodegradation of chlorophyll phytol side chains and photosensitised oxidation of some isoprenoid hydrocarbons such as pristine and phytane (Rontani et al., 1992).

Cholesterol was identified in most of the estuarine region during the present study. Cholesterol has multiple sources in aquatic systems. Even though cholesterol is the main animal sterol (Puglisi et al., 2003) it is also produced by diatoms, microbial community, macrophytes, phytoplankton and zooplankton (Reeves and Patton, 2001; Azevedo, 2003). The barmouth region showed maximum cholesterol concentration. C27 sterols, cholesterol and dehydrocholesterol have been used to indicate inputs of zooplankton grazing (Gagosian et al., 1981) and they have been also reported as algal/phytoplankton marker (Volkman et al., 1998). The relative high abundance of cholesterol in the barmouth region suggests an important
contribution of zooplankton or other marine fauna which are well known to contain high abundances of this compound, although phytoplankton contribution cannot be excluded. The C\textsubscript{28} sterol, 24-methyl cholesta-5,2-dien-3\beta-ol (brassicasterol) is considered as diatom marker (Brassell et al, 1982). Even though brassicasterol was detected in the inner part of the estuary (Fisheries harbour region and Shipyard region) the maximum abundance was observed in the barmouth region which indicated significant input of diatom derived organic matter in the barmouth region of the estuary. This observation is consistent with the reported result of chemotaxonomic analysis of sediments using pigment markers which identified the abundance of fucoxanthin in the barmouth region (Aneeshkumar and Sujatha, 2012).

Another C\textsubscript{28} sterol detected in sediments was campesterol, which is used as a terrestrial biomarker, but they are also biosynthesised by diatoms (Xu et al., 2006). Campesterol has already been reported in mangrove sediments of Cochin (Narayanan, 2006; Kumar, 2011) and northern part of Cochin estuary (Kumar, 2011). Campesterol was detected around the Cochin harbour region and remained below detectable limit towards the Thaneermukkom bund. Previous studies showed that mangroves contain significant amount of campesterol. Maximum campesterol content was reported in mangrove species \textit{Acanthus ilicifolius} (15.5%), while minimum concentration in \textit{Avicennia marina} (0.9%) (Sunilkumar and Antony, 1994; Subramanian, 2000; Kumar, 2011). The abundance of campesterol in the harbour area is attributed to the close proximity of two mangrove ecosystems Murikumpadam and Mangalavanam with this area. One of the dominant mangrove species in these ecosystems includes \textit{Acanthis ilicifolius}. Hence, the abundance of campesterol may
Sterols as Biomarkers

Sterols represent a signal of mangrove derived organic matter in surface sediments of Cochin estuary.

Terrestrial organic matter can be recognised by the presence of C29 sterols, which are the predominant sterols produced by vascular land plants. The presence of stigmasterol detected in most of the sites. Sitosterol was detected in the barmouth region as well as in the inner part of the estuary. Sitosterol has been used as a marker of allochthonous materials in estuarine environment (Laureillard and Saliot, 1993; Mudge and Norris, 1997) due to its high abundance in land plants. In the present study, β-sitosterol did not follow the expected trend to trace the terrestrial materials with decreasing relative abundance from Thaneermukkam bund to the barmouth region. Sitosterol was found to be more concentrated in the bund region as well as in the barmouth region. A comparison of sitosterol with other lipid biomarker compounds such as n-alkanes and fatty acids, strongly suggests that the sitosterol might be a mixture of terrestrial as well as marine origin (Sangiorgi et al., 2005).

The predominance of sitosterol is reported in many microalgae including some diatom species (Volkman, 1986; Barrett et al., 1995). Hence, the high abundance of sitosterol in the barmouth region indicated the autochthonous and allochthonous additions of sitosterol into the sediment, making complexities while using it as a molecular biomarker.

As compared to the above mentioned sterols, dinosterol is considered more taxonomically specific molecular biomarker because of its predominance in dinoflagellates (Volkman et al., 1993; Mansour et al, 1999; Leblond and Chapman, 2004). The wide spread abundance of dinosterol in surface sediments of the Cochin estuary, underlines the
importance of dinoflagellates of the organic matter pool in the surface sediments.

The dinosterol has been reported as an abundant sterol component in dinoflagellate species *Prorocentrum micans*, *Lingneodinium polydrum*, *Gymnodinium* and *Alexandrium tamarense* (Sangiorgi et al., 2005). The abundance of *Prorocentrum* and *Gymnodinium* was previously reported in Cochin estuary by many authors in Cochin estuary (Gopinathan et al., 1974; Martin et al., 2012). Even though dinoflagellates have been reported as the major source of dinosterol, this sterol has also been identified in certain diatom species (Budge and Parrish, 1998; Hudson et al., 2001). A recent study identified 43 species of diatoms and 40 species of dinoflagellates in the Cochin estuary (Aneeshkumar and Sujatha, 2012). Analysis of chlorophyll pigment markers identified the presence of peridinin which indicated the abundance of dinoflagellates in the Cochin estuary (Aneeshkumar and Sujatha, 2012).

The pentacyclic triterpenoids is dominated by germanicol and this biomarker compound reported in organic matter derived from mangrove *Rizophora mangle* (Koch et al., 2003). The presence of organic matter derived from angiosperm to the total organic matter was identified by the presence of lupeol. Betulin was detected in most of the stations which indicated the presence of mangrove derived organic matter in the sediments of the study area. The triterpenoid biomarker friedoleanane was identified in estuarine sediments and this compound was reported in fluvial and lacustrine sediments whose origin is attributed to the bark and leaves of numerous angiosperms (Jaffe et al., 1996; Otto and Simoneit, 2001; Kumar, 2011).
7.4 Biomarker proxies for the Cochin Estuary

Every estuary is unique in terms of their morphological features, climatic settings, tidal incursion and chemical processes. The development of a set of biomarker proxies which suits better for a particular estuary is important in order to acquire better knowledge regarding the organic matter cycling in that estuary.

Several biomolecules are frequently used as biomarkers which are indicators of the origin of organic matter in sediments in order to derive information about the carbon cycling in estuaries. The underlying goal driving quantitative biomarker analysis is the determination of the quantity of a given compound originating from one source compared to another, with the aim of deriving information on past or present environmental conditions and/or processes (Panetta and Gelin, 2009). When using molecular biomarkers in complex estuaries like Cochin estuary, the different transport processes, which are dominated by river discharge, vertical and lateral transport through the water column, the transport from adjacent mangrove ecosystems, urban and industrial inputs have to be considered. The study focuses on three major inputs which include: algal, terrestrial and bacterial derived organic matter.

The bulk parameter approach revealed the dominance of terrestrial derived organic matter in surface sediments. The fraction of terrestrial organic matter in the total organic matter pool ranges from 13 to 74% in the surface sediments as estimated by $\delta^{13}$C based two end member mixing model. The $\delta^{15}$N exhibited complex spatial and seasonal distributions in the study area. It was found that the dynamic cycling of nitrogen through various biogeochemical and organic matter degradation processes modifies...
the OC/TN ratios and δ¹⁵N to a considerable degree. The relative contribution of terrestrial derived organic matter in surface sediments displayed a gradual increase from inner part of the estuary to the seaward side which suggest an increase in contribution of marine autogenous organic matter towards the seaward side. Biomarker analysis provides an added dimension to the understanding of the carbon cycle in estuarine systems, in many cases highlighting important details not revealed by bulk analyses alone.

The short chain homologous of n-alkanes, n-alcohols and alkanoic acids are associated with the aquatic flora. These compounds can be used to infer dynamics of algal derived organic matter in estuarine systems. It was observed that the results of n-alkanes and n-alcohols in the present study are consistent with the aforementioned general feature. Conversely, the C₁₆ n-alkanoic acid showed very high concentrations than higher chain n-alkanoic acids which may be due to the influx of sewage derived organic matter in estuarine sediments. Hence the use of short chain fatty acids is inadequate to track the algal derived organic matter in this estuary. The very low abundance of PUFA makes more complexities in the source characterisation process by alkanoic acid biomarker tool. The distribution of short chain n-alkanes and the use of terrestrial to aquatic ratio is found to be the better biomarker proxy in this estuary. The distribution of phytol also can be a relevant tool for the same.

PUFAs are less resistant to diagenetic degradation than monounsaturated and saturated fatty acids (Farrington et al., 1988; Wakeham and Canuel, 1990). PUFAs were present in very low concentrations in the samples analysed which was indicative of a strongly degraded material in surface sediments which in turn provide primary indication about the bacterial mineralisation
process in sediments. Further for delineating the distributional dynamics of bacterial derived organic matter, branched and odd carbon numbered fatty acids were found to be a reliable proxy in the Cochin estuary. Comparatively high concentrations of branched and odd chain concentration in sediments can be used as an effective tool for the assessment of bacterial derived organic matter. The inference about bacterial input, which can be extracted from short chain n-alkanes, is more or less masked by the overlapping of source input through petroleum spillage.

Homologues series of long-chain n-alkanes, n-alkanols and fatty acids are typical terrigenous lipids found in marine sediments (Poynter et al., 1989; Bird et al., 1995; Kuypers et al., 1999; Huang et al., 2000). These compounds are abundant constituents of terrestrial higher plant epicuticular waxes (Eglinton and Hamilton, 1963), occurring as protective coating on leaves and stems. Plant waxes can be washed off the leaf surface by rain and transported into the ocean by freshwater runoff (Bird et al., 1995). From the result of the present study, it was found that all the aforementioned biomarker compounds can provide effective source information in the study area. The triterpenoid biomarker germanicol displayed widespread abundance in the study region and this biomarker can be employed to get an account of the terrestrial derived organic matter in the estuarine sediments.

7.5 Conclusions

The significant abundance of the straight chain and branched chain fatty alcohols emphasises their potential applicability as molecular markers. The sets of sterols identified in the present study include cholesterol, cholestanol, brassicasterol, campesterol, stigmasterol, stigmastanol,
sitosterol and dinosetrol. The abundance of terrestrial derived sterols underlines the prevailing importance of terrestrial end member in the sediment organic pool and their preferential preservation in the estuarine salinity gradient. The relatively high abundance of dinosterol suggested that this compound can be effectively employed to assess the dianflagellate derived organic matter in surface sediments.

References


