

Sources of organic matter and microbial community structure in the sediments of the Visakhapatnam harbour, east coast of India

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ABSTRACT

Organic matter in the coastal sediments originates from various terrestrial and marine sources. Within the sampling sites, relative inputs from these sources may vary and can influence the microbial community structure. In order to identify the sources of organic matter, and its influence on microbial community structure in coastal environment, 19 surface sediment samples were collected from various stations in the Visakhapatnam harbour, east coast of India. These samples were analyzed for organic carbon (OC) content, bulk $\delta^{13}\text{C}$ signatures, and the concentration and composition of phospholipid fatty acids (PLFAs). Contents of OC, $\delta^{13}\text{C}_{\text{oc}}$ values, and PLFA concentrations varied spatially and ranged from 0.6 to 7.6 %, -29.32 to -23.75 ‰, and 0.30 to 33.30 $\mu\text{g g}^{-1}$ dw sediment, respectively. The bulk $\delta^{13}\text{C}_{\text{oc}}$ of sediments reflected mixed carbon sources from marine and terrestrial end members with dominance of the latter at most of the stations. The PLFA community was not influenced by concentration and source of OC. Saturated PLFAs were the most abundant followed by monounsaturated fatty acids (MUFAs), branched PLFAs, and polyunsaturated fatty acids (PUFAs). MUFAs indicate the abundance of Gram negative bacteria, cyanobacteria and microalgae. The Branched PLFAs (iso and anteiso) suggest the presence of Gram positive bacteria, Gram negative anaerobes and sulfate reducing bacteria. Similarly, PUFAs indicate the presence of eukaryotes. Moreover, the presence of trans-monounsaturated PLFAs in the harbour sediments imply that PLFA community was under stress due to contamination of the sampling sites by sewage and industrial waste, sulfur and petroleum products. Principal component analysis (PCA) based on concentrations of PLFAs established three factors that accounted for 81% of the total variance. The first factor contributed 57% of variance, and was mostly influenced by MUFAs and branched PLFAs. The second factor was controlled by PUFAs such as C20:3n6, C18:3n3, C20:4n6, C20:5n3 and C22:6n3, whereas the third factor was influenced by C20:3n3, C22:2 and C22:1n9. PLFA community in the Visakhapatnam harbour sediments was mostly dominated by bacteria along with some contribution from eukaryotes.

Keywords: OC, $\delta^{13}\text{C}_{\text{oc}}$, total lipids, PLFA, sediment, Visakhapatnam harbour, east coast of India

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1. Introduction

Organic matter (OM) in the near shore and coastal sediments is derived from various sources including primary production by phytoplankton or benthic microalgae, terrestrial plants, river runoff, municipal sewage and industrial discharge etc. (Hedges and Keil, 1995; Hedges et al., 1997). Stable carbon isotope ($\delta^{13}\text{C}_{\text{oc}}$) signatures of the various carbon sources are often different, and despite some overlap between different sources, they are powerful tracers of carbon inputs in various environments (Fry and Sherr, 1984; Prahl et al., 1994; Meyers, 1994; Schelske and Hodell, 1995). Geochemical biomarkers such as *n*-alkanes, alcohols, sterols, alkenones, and fatty acids also are often used to identify the carbon inputs from various organisms in marine environments (Volkman et al., 1992; Tolosa et al., 2003). However, these geochemical biomarkers and stable carbon isotopes do not differentiate between dead and live organisms.

In marine environment, living biomass consists of a complex mixture of prokaryotes and eukaryotes, whose combined activity determines nearly all of the elemental biogeochemical cycling (Boschker et al., 2001). The nature and sources of OM, nutrient status and pollution may influence the microbial community structure (Guckert et al., 1986; Bååth et al., 1995; Pennanen, 2001). Traditional techniques of isolation and culturing have not been adequate for characterization of microorganisms in environmental samples, especially in evaluating the natural microbial diversity (Fang et al., 2000; Delong and Pace, 2001). Thus to detect changes in bacterial biomass and natural microbial communities culture-independent techniques such as phospholipid fatty acid (PLFA) analysis (Pennanen et al., 1996; Pinkart et al., 2002), and genetic fingerprinting (Polymenakou et al., 2005), are routinely used.

PLFAs are the central component of the microbial cell membranes. They are present in reasonably constant amounts over a wide range of growth conditions and are rapidly hydrolyzed upon cell death (White et al., 1979; Russell and Nichols, 1999; Fang et al., 2000; Boschker and Middelburg, 2002). Furthermore, these compounds can be extracted from sediments easily and quantitatively (White et al., 1979; Balkwill et al., 1998). Hence, cellular membrane PLFAs represent a quantitative and sensitive tool to determine the living microbial biomass (White et al., 1979; Ringelberg et al., 1997; Fang et al., 2000), changes in microbial community composition (Volkman et al., 1980; Pinkart et al., 2002; Widenfalk et al., 2008), nutritional status of microbial community (Guckert et al., 1986; Kieft et al., 1994), general distribution of microorganisms with different metabolic activities (Kaneda, 1991), heavy metal-contaminated soils (Bååth et al., 1995; Pennanen, 2001), and environmental stress (Fang et al., 2004).

The Visakhapatnam harbour along the east coast of India is a natural harbour and is extensively used for various operations. It includes areas differentially impacted by anthropogenic activities (Subba Rao and Venkateswara, 1980; Sarma et al., 1996). It receives large inputs of OM from terrestrial, marine, and anthropogenic sources, and potentially sequesters a significant fraction of OC in its bed (Ganapati and Raman, 1973; Raman, 1995). Several reports are available on hydrography (Varadarajulu and Khadar, 1976) and various aspects of pollution of the harbour (Subba Rao and Venkateswara, 1980; Sarma et al., 1996). Crude oil, iron ore and sulfur dust spilled during loading and unloading operations further influence the physico-chemical conditions of the water in different channels of the harbour. The key factors controlling input, transfer and storage of this OM depend on tides, urban and industrial runoff, inadequate flushing and stagnant conditions (Sarma et al., 1982; Kadam and Bhangale, 1993). The effect of pollution stress is greatly seen in the biotic rather than the abiotic components of the harbour.

The aims of the present study were to 1) investigate the distribution of OM, 2) identify the sources of OM, 3) assess the live microbial biomass, and 4) evaluate the composition of microbial groups in the harbour sediments.

2. Materials and methods

2.1. Description of the study area

Visakhapatnam harbour is located at 17°41'825"N to 17°42'313"N and 83°16'881"E to 83°19'793"E, along the east coast of India (Fig. 1) at the mouth of river Meghadrigedda. The existence of this natural harbour has transformed the village once called as 'Vizag' into one of the fastest growing industrial cities of the world. It comprises of an inner harbour and outer harbour. There are 24 berths and 4 moorings to accommodate 28 ships at a time. Major industries like Visakhapatnam steel plant, HPCL oil refinery, fertilizer complex are setup near this port. Initially this port was used as a sea outlet for manganese ore. Subsequently, other bulk items such as iron ore, coking coal and oil products were handled by this natural harbour. During the year 2008, the harbour handled ~65 million tons of cargo.

2.2. Cleaning of glassware and purification of solvents

Before use all the glassware was washed with tap water, chromic acid, distilled water, ashed at 450 °C and rinsed with methanol and dichloromethane. All the solvents were of analytical grade and were double distilled before use. Sodium sulphate was heated at 450 °C. Silicic acid was washed with methanol, chloroform and dried prior to use.

2.3. Sample collection and analysis

Surficial sediment samples (top 10-15 cm) were collected from 19 stations (#) of the Visakhapatnam harbour using Van veen grab (Fig. 1 and Table 1). The sediment samples were frozen onboard and soon transferred to the laboratory and stored at -20 °C until lyophilization. The sediments were then freeze dried, homogenized using agate mortar and pestle to a fine powder and stored at -20 °C. The sediments were then used for the estimation of OC, $\delta^{13}\text{C}_{\text{oc}}$, total lipids and PLFAs.

2.4. Estimation of bulk geochemical parameters

Subsamples of sediments for OC and $\delta^{13}\text{C}_{\text{oc}}$ content were acidified with HCl (1N) to remove carbonates. Traces of HCl were removed by washing the sediments several times with distilled water. The sediments were then used for bulk OC and $\delta^{13}\text{C}_{\text{oc}}$ analysis. Concentrations of OC were determined by combusting preweighed samples in a CNS elemental analyzer (NCS 2500, CE instruments). Calibration was carried out using 2, 5-Bis-(5-tetra-butyl-benzoxazol-2-yl) thiopen (BBOT) as a standard. $\delta^{13}\text{C}_{\text{oc}}$ of OC was performed with the Thermo Finnigan Flash 1112 elemental analyzer, linked with a Thermo Finnigan Delta V plus Isotope Ratio MS. The overall analytical precision for replicate samples was within $\pm 1.6\%$ for OC and $\pm 0.2\text{‰}$ for $\delta^{13}\text{C}_{\text{oc}}$.

All isotopic compositions are reported as, per-mil (‰) relative to variation (δ) from the PDB standard.

$$\delta^{13}\text{C}_{\text{oc}} = \left\{ \left(\frac{^{13}\text{C}/^{12}\text{C}}{^{13}\text{C}/^{12}\text{C}} \right)_{\text{Sample}} / \left(\frac{^{13}\text{C}/^{12}\text{C}}{^{13}\text{C}/^{12}\text{C}} \right)_{\text{PDB}} - 1 \right\} \times 1000$$

2.5. Estimation of terrestrial carbon

To evaluate the relative proportions of terrestrial and marine carbon in sediments, a simple two end-member mixing model was employed (Schlunz et al., 1999; Hu et al., 2006). The following equation was used

$$\text{TC (\%)} = \frac{\delta^{13}\text{C}_{\text{marine}} - \delta^{13}\text{C}_{\text{sample}}}{\delta^{13}\text{C}_{\text{marine}} - \delta^{13}\text{C}_{\text{terrestrial}}} \times 100$$

Where, TC is the terrestrial carbon, $\delta^{13}\text{C}_{\text{sample}}$ is the measured $\delta^{13}\text{C}$ of the sample, $\delta^{13}\text{C}_{\text{marine}}$ is the marine end member (-20.5 ‰, average of $\delta^{13}\text{C}_{\text{oc}}$ -19 to -22 ‰ of marine organic matter, Fry and Sherr, 1984), $\delta^{13}\text{C}_{\text{terrestrial}}$ is the terrestrial end member (-29.32 ‰, most depleted $\delta^{13}\text{C}$ value from Table 1)

2.6. Extraction of lipids

Lipids from the lyophilized sediments were extracted following the modified method of Bligh and Dyer (1959). In brief sediments were shaken vigorously with chloroform: methanol: water (1:2:0.8, V/V/V) and kept standing for 24 h. Separation of the organic phase was achieved after adjusting the ratio of chloroform: methanol: water to 1:1:0.9 in a separating funnel. The total lipid extracts were pooled, filtered through Whatman No. 1 filter paper, and treated with anhydrous sodium sulphate to remove traces of water. The extract was rotary evaporated at 30 °C under vacuum and made to a known volume (1 ml). A known aliquot of the extract was used to determine total lipid content (Zaghden et al., 2005; Harji et al., 2008). The method showed a standard deviation of $\pm 5.3\%$, based on replicate analyses.

2.7. Separation of PLFAs

Organic phase containing the total lipids was reduced to a small volume and was transferred onto silicic acid columns (activated at 120 °C for 2 h). The column was eluted first with dichloromethane, followed by acetone and methanol. The methanol fraction (polar fraction) containing the PLFAs were reduced to a small volume using the rotary evaporator.

2.8. Preparation of Fatty acid methyl esters

Fatty acid methyl esters of the phospholipid fraction were prepared by mild alkaline methanolysis according to White et. al (1979). Phospholipid fraction was resuspended in methanol: toluene 1:1 (V/V) to which freshly prepared 0.2 M methanolic KOH was added. The mixture was incubated at 37 °C for 15 min, neutralized to pH 6 with 1 M acetic acid. The PLFAs were recovered using hexane and analyzed on gas chromatography (GC) and gas chromatography mass spectrometry (GC-MS).

2.9. Capillary GC and GC-MS analysis

GC analyses of the fatty acid methyl esters were done using Agilent GC 6890 equipped with a FID and SP-2560 fused silica capillary column (column length: 100m, film thickness: 0.20 μm , inner diameter: 0.25 mm). The temperature of injector and the detector was maintained at 260 °C. 1 μl of sample was injected in an on-column injector using nitrogen as a carrier gas at a flow rate of 20 cm sec^{-1} . The column oven temperature was programmed from 140 °C (5 min) to 240 °C (35 min) at a rate of 4 °C min^{-1} .

The quantification of PLFAs was done using the data handling system available on the instrument. The identification of individual PLFA was performed by comparing the retention times of PLFAs in the sample with those of authentic standards (FAME 37 mix, Supelco, USA). The peak areas were quantified relative to an internal standard (hexacosanoate methyl ester) added to the sample prior to GC- analysis.

Solvents were passed through the entire analytical procedure and run on GC and GC-MS as blanks to check the probable contamination. Sample values have been corrected for the blank levels. Recovery of the phospholipid standard (phosphatidyl choline) processed following the entire analytical procedure varied from 74 to 89 % (\bar{X} = 79.62 %, n=3)

Peak identification was further verified by analyzing the PLFAs on GC-MS (Shimadzu GC-MS-QP 2010) by comparing the EI spectra of sample PLFA to spectra from standards and published data. PLFA sample (1 μ l) was injected into a Shimadzu GC-2010 gas chromatograph interfaced with a Shimadzu GC-MS-QP 2010 mass selective detector. The gas chromatograph was equipped with the same 100-m polar capillary column (SP-2560) as above and the column was programmed as described above for the GC. The injector and source housing temperatures were maintained at 60 °C and 200 °C, respectively. The GC-MS system operated in full scan mode, scanning from m/z 50 to m/z 500. The prefixes "a" and "i" indicate anteiso- and iso-branching in PLFA.

2.10. Statistical analysis

A simple regression analysis was carried out to assess the relationship between different parameters using Microsoft Excel program installed in a personal computer (Sokal and Rohlf, 1981). Stations #2, #10 and #15 were excluded from statistical analysis because OC data were not available for these stations.

Principal component analysis (PCA) based on individual PLFA concentrations (Table 2) was carried out to distinguish differences in the PLFA community structure. Absence of PLFA compounds was replaced with zero. The variables were normalized in order to convert the data into a normal distribution with a mean of zero and variance of one, according to the following equation;

$$n_{ik} = (x_{ik} - \bar{x}) / s_i$$

where x is the concentration of PLFA (i) in sample (k), and s is the standard deviation (Reemtsma and Ittekkot, 1992). PCA was performed using Stat Soft, STATISTICA 6" software package and the principal components were calculated.

3. Results and discussion

3.1. Bulk parameters

Distribution of OC, $\delta^{13}\text{C}_{\text{oc}}$, total lipids and total PLFAs in the sediments of the Visakhapatnam harbour is shown in Table 1. OC varied from 0.6 % (station #4, Turning basin) to 7.6 % (station #5 H&Y Jetty-2/3). OC values are in good agreement with those reported earlier for other coastal areas of the world (Wang et al., 2006; Harji et al., 2008 and references within).

The sediments displayed a broad range of $\delta^{13}\text{C}_{\text{oc}}$ values (-29.32 to -23.75 ‰). Highly depleted value of $\delta^{13}\text{C}_{\text{oc}}$ (-29.32 ‰) was observed at the turning circle (station #17), and the relatively more enriched value (-23.75 ‰) was observed at the Fishery Jetty (station #19). The $\delta^{13}\text{C}_{\text{oc}}$ values varied from -22 to -19 ‰ and -28 to -26 ‰ for marine phytoplankton and terrestrial C3 plants, respectively (Fry and Sherr, 1984; Kendall et al., 2001). The $\delta^{13}\text{C}_{\text{oc}}$ values (-29.32 to -23.75 ‰) recorded for the surface sediments of the Visakhapatnam harbour (Table 1) were well within the range of values reported for marine (-22 to -19 ‰) and terrestrial C3 plants dominated OM (-28 to -26‰).

In order to assess the relative proportions of terrestrial and marine carbon, a two end-member mixing model was used (Schlunz et al., 1999; Hu et al., 2006). Terrestrial carbon accounted for 37 to 100 % of the total OC in sediments of the Visakhapatnam harbour (Table 1). Terrestrial carbon was relatively more abundant at most of the stations except at stations #7, #13 and #19. As compared to marine organic matter, terrestrial carbon is relatively more resistant to biodegradation (Meyers, 1994; McCallister et al., 2006). Therefore, preferential removal of marine organic matter may be responsible for the observed abundance of terrestrial organic matter in the sediments of Visakhapatnam harbor.

Total lipids varied from 300 $\mu\text{g g}^{-1}$ (station #14 and station #17) to 14 948 $\mu\text{g g}^{-1}$ (station #5) dw sediment (Table 1). Total lipid concentrations are in the range of values earlier recorded for the coastal sediments of other areas. For example, for the Marmugoa harbour sediments, the total lipids varied from 233 to 1448 $\mu\text{g g}^{-1}$ dw sediments (Harji et al., 2008). Similarly, for the sediments of Sfax coastal zone, Tunisia, the total lipids ranged between 1700 and 9100 $\mu\text{g g}^{-1}$ dw sediment (Zaghden et al., 2005). There was a fair relationship ($R^2 = 0.346$; $n=16$; $p= 0.01$) between total lipids and OC.

The concentrations of total PLFA varied between the sampling stations, and values ranged from 0.30 $\mu\text{g g}^{-1}$ (station #4) to 33.3 $\mu\text{g g}^{-1}$ (station #2) dw sediments. These values are in the range of values reported earlier from different environments. For example, total PLFA concentrations varied from 0.3 to 5.3 $\mu\text{g g}^{-1}$ dw sediments in the Thracian Sea, and from 4.5 to 10.7 $\mu\text{g g}^{-1}$ dw sediments in the

Augusta Bay of the Eastern Mediterranean Sea (Polymenakou et al., 2006). However, relatively higher values were observed in the sediments of Wadden Sea (36 to 71 $\mu\text{g g}^{-1}$; Langezaal et al., 2003), and a brackish lagoon in the German Baltic Sea ($>40 \mu\text{g ml}^{-1}$ of sediment; Boschker et al., 2001). Total PLFAs represent the current living community of organisms, since they are rapidly degraded following cell death (White et al., 1979; Bouillon et al., 2004; Alfaro et al., 2006; Bouillon and Boschker, 2006). Therefore, the observed variations in PLFAs indicate the spatial differences in living biomass in sediments of the harbour.

Total PLFAs accounted for a small fraction of the total lipids (0.02 to 1.04 %) and OC (0.001 to 0.076%). In order to assess the effect of OC, concentrations of PLFAs were normalized to OC. OC normalized total PLFAs varied from 15 $\mu\text{g g}^{-1}\text{OC}$ (station #6) to 761 $\mu\text{g g}^{-1}\text{OC}$, (station #16) (Table 1). There was no correspondence between concentrations of total PLFAs and OC. This was also supported by fairly significant but weak relationship ($R^2 = 0.22$; $n=16$; $p =0.02$) between these two parameters. Moreover, irrespective of the carbon sources, the PLFA community was mostly represented by MUFAs, branched PLFAs, and PUFAs (Fig. 5).

3.2. PLFA composition in the sediments

PLFAs are effective taxonomic markers to characterize the bulk composition of viable microbial communities (White et al., 1979). However, as there is an overlap in the PLFA composition between many species, it is not possible to assess the presence of individual species. In the sediments of the Visakhapatnam harbour, 38 PLFAs were identified. Concentrations of individual PLFAs varied from 1 ng g^{-1} (station #18) to 3153 ng g^{-1} (station #2) dw sediments (Table 2). At most of the stations, concentrations of individual PLFA varied, indicating the changes in the composition of PLFA community (Volkman et al., 1980; Elvert et al., 2003; Zhang et al., 2005).

Saturated PLFAs were generally the dominant PLFAs in most of the sediment samples (Fig. 2). PLFAs such as C14:0, C15:0, C16:0, C17:0, C18:0 and C20:0 were generally abundant among saturated group of PLFAs (Table 2). Among the saturated PLFAs, C16:0 was the most abundant accounting for 20 % (station #11) to 31 % (station #3) of the total identified PLFAs, except at station #18 (3 %) where the relative concentrations were relatively less (Fig. 3). These PLFAs are ubiquitous in organisms from a wide range of genera (White et al., 1979; Rajendran et al., 1992; Alfaro, et al., 2006). The high proportions of saturated PLFAs infer that a large fraction of viable microbial communities inhabiting the sediments can not be attributed to a certain functional group. Long chain saturated PLFAs (C20:0 – C24:0) were present but their concentrations were low in the sediments (Table 2). These PLFAs are

indicative of inputs from terrestrial plants and mangroves (Rajendran et al., 1992). However, these long chain fatty acids are also present at low concentrations in some diatoms (Volkman et al., 1980).

Monounsaturated PLFAs (MUFAs) are mainly produced by bacteria and microalgae (Wakeham, 1995; Rütters et al., 2002; Widenfalk et al., 2008). In the present study, the contribution of MUFAs varied from 15.6 to 39.1 % of the total identified PLFAs, and was the second most abundant class of the PLFA community (Fig. 2). MUFAs such as C14:1, C15:1, C16:1n9c, C17:1, C18:1 (C18:1n8, C18:1n9t, C18:1n9c), C20:1, C22:1n9 and C24:1 were present in most of the sediment samples (Table 2). Among these, C16:1n9c was the most abundant and accounted for 11 % (station #7) to 25.4 % (station #4) of the total identified PLFAs (Fig. 3). MUFAs C18:1n9t and C18:1n9c were also abundant and accounted for 1.8 to 12.2 % and 4.7 to 10.8 % of the total identified PLFAs, respectively. Both C16:1 and C18:1 fatty acids are characteristic indicator of methanotrophs or thio oxidizing bacteria (Boon et al. 1996; Boschker et al. 2005). C16:1n9c could be derived from bacteria and/or phytoplankton (Muri et al., 2004). PLFA such as C18:1n9c is present in bacteria, cyanobacteria, microalgae and fungi (Wakeham, 1995; Langezaal et al., 2003; Polymenakou et al., 2006). However, the contribution of ergosterol in the sediments was negligible (Harji et al., unpublished data) suggesting that the MUFAs were mostly derived from sources other than fungi in these harbour sediments.

Zooplankton grazing is an important link between lower and higher trophic levels. Generally, zooplankton feeding predominantly on phytoplankton contains elevated concentrations of long-chain MUFAs such as 20:1 and 22:1 (Parrish et al., 2000). Hence these PLFAs may be used as markers to determine the importance of zooplankton grazing. In our study, these zooplankton markers were observed at stations #2, #7, #12, #14, #15, #16 and #19 suggesting their role in the trophic food level.

Trans-monounsaturated PLFA (C18:1n9t) was one of the major PLFAs in the sediments of the harbour (Fig. 3). It appears that this PLFA is produced by bacteria from the corresponding cis- isomer in response to stress (Findlay et al., 1990; Kieft et al., 1994; Fang et al., 2004). Furthermore, the harbour is subjected to pollution due to municipal sewage, factory discharge, sulfur and petroleum products (Sarma et al., 1982; Kadam and Bhangale, 1993). Presence of these pollutants creates anoxic condition in the harbour which may put stress on benthic microbial communities including bacteria. Thus, the occurrence of trans-monounsaturated PLFAs in these sediments probably indicates the stress induced response of the local bacterial communities.

Polyunsaturated fatty acids (PUFAs) C18:2n6c, C18:3n3, C18:3n6, C20:3n3, C20:3n6, C20:4n6, C20:5n3 and C22:6n3 are synthesized mostly by eukaryotes (White et al., 1979; Volkman et al., 1998;

Brinis et al., 2004). Similarly, psychrophilic, halophilic, and barophilic marine genera of *γ-Proteobacteria* are able to produce these PUFAs since this group of lipid can play an important role in maintaining optimal membrane fluidity and hence making the organisms better adapted to the deep sea environment (Russell and Nichols, 1999; Nichols and McMeekin, 2002). *γ-Proteobacteria* is one of the most successful and widely distributed microbial groups in the marine environment (Giovannoni and Rappé, 2000). The contribution of PUFAs to the total identified PLFAs varied from 1.1 (station #2) to 16 % (station #11) in the harbour sediments (Fig. 2). The contribution from eukaryotes and *γ-Proteobacteria* to the total PLFA community was minor in the harbour sediments, was evident from the poor abundance of PUFAs.

The branched PLFAs i-15:0, a-15:0, i-16:0 and i-17:0 are well known biomarkers for Gram-positive bacteria, Gram negative anaerobes and sulfate reducing bacteria (O'Leary and Wilkinson, 1988; Wakeham, 1995; Rütters et al., 2002; Harvey et al., 2006; Widenfalk et al., 2008). However, these PLFAs are not detected in algae, phytoplankton, other microorganisms and invertebrates (Tunlid and White, 1992). In the Visakhapatnam harbour sediments, branched PLFAs accounted for 2.7 to 45.5 % of the total identified PLFAs. However, the contribution of these PLFAs to total identified PLFAs was higher (23.2 % to 45.5 %) at stations #2, #4, #7, #9, #11 and #13 (Fig. 2). Branched PLFAs, especially i-15:0 and a-15:0 were more abundant, together comprising 2.9 to 31.7% of the total identified PLFAs (Fig. 3). The presence of these PLFAs in the harbour sediments may be due to the anoxic conditions that prevail at most of the stations due to constant stagnation of the water (Sarma et al., 1982; Kadam and Bhangale, 1993). These branched PLFAs also have been reported from the anoxic environments of the Black Sea and the Arabian Sea (Wakeham, 1995; Wakeham et al., 2002).

In order to further define the PLFA community structure, PLFA data were processed using the principal component analysis (PCA). PCA produced three principal components (PC) which accounted for 81.2% of the total variability (Table 3). PC1 accounted for 57.0% of the variance with most negative loadings (-0.900 to -0.949) on C18:1n9c followed by C17:0, C24:0, C18:1n8, C16:0, C18:0, i-16:0, C14:0, C22:0, C15:0, i-17:0, C22:2 (Table 3). Of these, C18:1n9c, C17:0, C15:0, C18:1n8, i-16:0 and i-17:0 are produced by bacteria (Wakeham et al., 2002), while C18:1n9c is also derived from cyanobacteria and microalgae (Wakeham, 1995; Zhang et al., 2004, Alfaro et al., 2006). Saturated PLFAs C14:0, C16:0 and C18:0 are ubiquitous and hence are not indicators of any organisms (Volkman et al. 1998). PC1 was also influenced by C20:1 and C22:1 and C16:1n9c which indicates input from zooplankton, phytoplankton and bacteria (Parrish et al., 2000; Muri et al., 2004). Bacterial PLFAs i-15:0, a-15:0 and C18:1n9t and the algal PLFAs C20:3n6, C20:4n6, C20:5n3 and C22:6n3 also controlled the PC1.

PC2 accounted for 15.8% of the total variance, and was influenced by PUFAs C20:3n6, C20:4n6, C18:3n3 and C18:3n6 produced by eukaryotes such as green algae and red algae. This factor was also controlled by PLFAs such as C20:5n3 and C22:6n3 derived from diatoms and dinoflagellates. PC3 showed only 8.4% of the total variance, and was influenced by PLFAs C20:3n3 and C22:2 that are present in phytoplankton, algae and other eukaryotes, and C22:1n9 produced by zooplankton.

Principal component analysis of the PLFAs in the sediments was also used to group the stations. Sample scores for individual PLFAs showed three clusters. Stations #2, #7 and #13 were grouped in cluster 1, and stations #12, #15 and #16 in cluster 2. The remaining stations formed the third cluster. Stations belonging to cluster 1 were relatively enriched in iso and anteiso C15:0 and C23:0 PLFAs implying that these stations were relatively more influenced by the abundance of bacteria. Second cluster was mostly dominated by PUFAs indicating the importance of marine algae at these stations (Table 2, 3). In contrast, cluster 3 had those stations which contained relatively low concentrations of PLFAs (Table 2, 3). PCA analysis was useful to fairly classify the stations on the basis of PLFAs. However, in order to get a better picture of station groupings, a large number of samples need to be analyzed.

3.3. PLFA based community structure

Relative composition of the PLFA community was calculated using the most abundant groups of PLFAs present in the harbour sediments, except the saturated group which is ubiquitous in origin. Gram negative bacteria, cyanobacteria and algae are rich in MUFAs (C16:1n9c, C18:1n8, C18:1n9c). Branched (iso and anteiso) PLFAs are abundant in Gram positive bacteria, Gram negative anaerobes and sulfate reducing bacteria. PUFAs (C18:2n6c, C18:3n3, C18:3n6, C20:3n3, C20:3n6, C20:4n6, C20:5n3 and C22:6n3) are mostly present in eukaryotes. PLFA community observed in the Visakhapatnam sediments is presented in Fig.4. MUFAs, Branched PLFAs and PUFAs accounted for 19 (station #3) to 64 % (station #9), 14 % (station #18) to 61 % (station #4), and from 1 (station #2) to 37 % (station #11) of the total PLFA community. Shift in PLFA composition between the sediment stations indicates the changes in the structure of microbial communities (Frostegård et al., 1993).

4. Conclusions

Surficial sediments from Visakhapatnam harbour were collected and analyzed for OC, $\delta^{13}\text{C}_{\text{oc}}$, PLFA concentration and composition. $\delta^{13}\text{C}_{\text{oc}}$ signature and two end-members mixing model suggests that the OM was derived from mixed marine and terrestrial sources with relative abundance of latter at most of the stations. PLFA concentrations varied spatially indicating differences in the living biomass in the

harbour sediments. Furthermore, there was no correspondence between OC concentrations or sources and the concentrations or the composition of the total PLFAs. Saturated PLFAs were most abundant in the harbour sediments. These PLFAs are ubiquitous in nature, hence a major fraction of PLFAs in these sediments cannot be ascribed to any particular microbial group. The MUFAs and branched PLFAs were also the major PLFAs in the harbour sediments, suggesting major inputs from bacteria, cyanobacteria and algae. PUFAs were present in the sediments which imply that eukaryotes also contributed to the PLFA community. Presence of trans-monounsaturated PLFAs suggests that the PLFAs community was under stress due to pollution from various sources. Relative distribution of PLFA components strongly suggest that PLFA community was mostly dominated by bacteria in these sediments.

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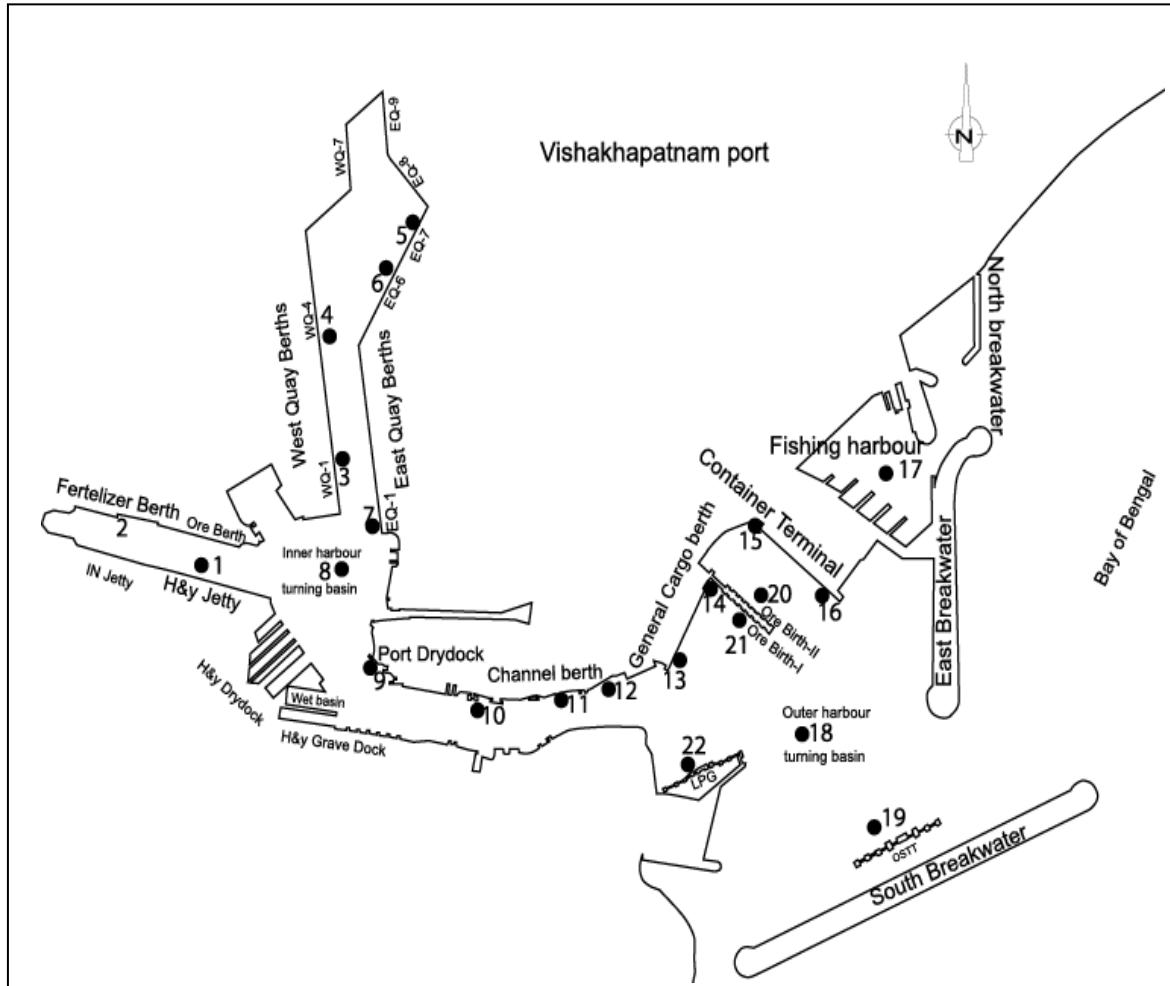


Fig.1. Map of the study area showing the sediment sampling stations in the Visakhapatnam harbour, east coast of India.

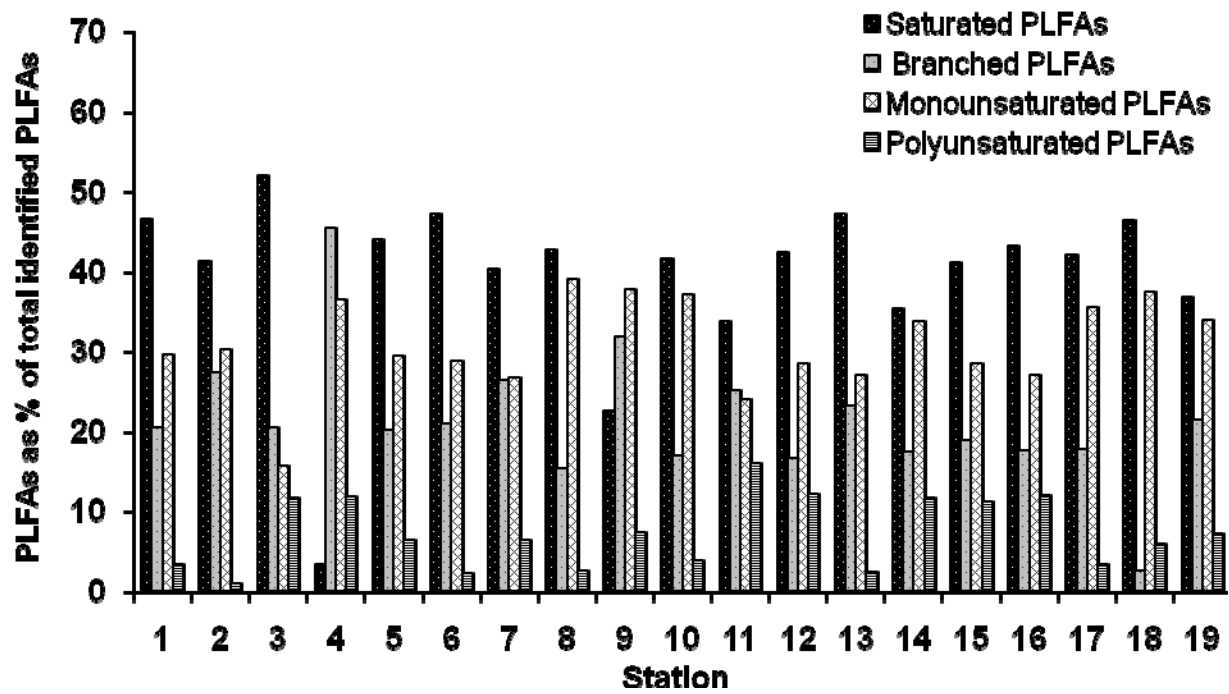


Fig.2: Distribution of saturated, branched, monounsaturated and polyunsaturated PLFAs as % of the total identified PLFAs in the sediments of the Visakhapatnam harbour, east coast of India.

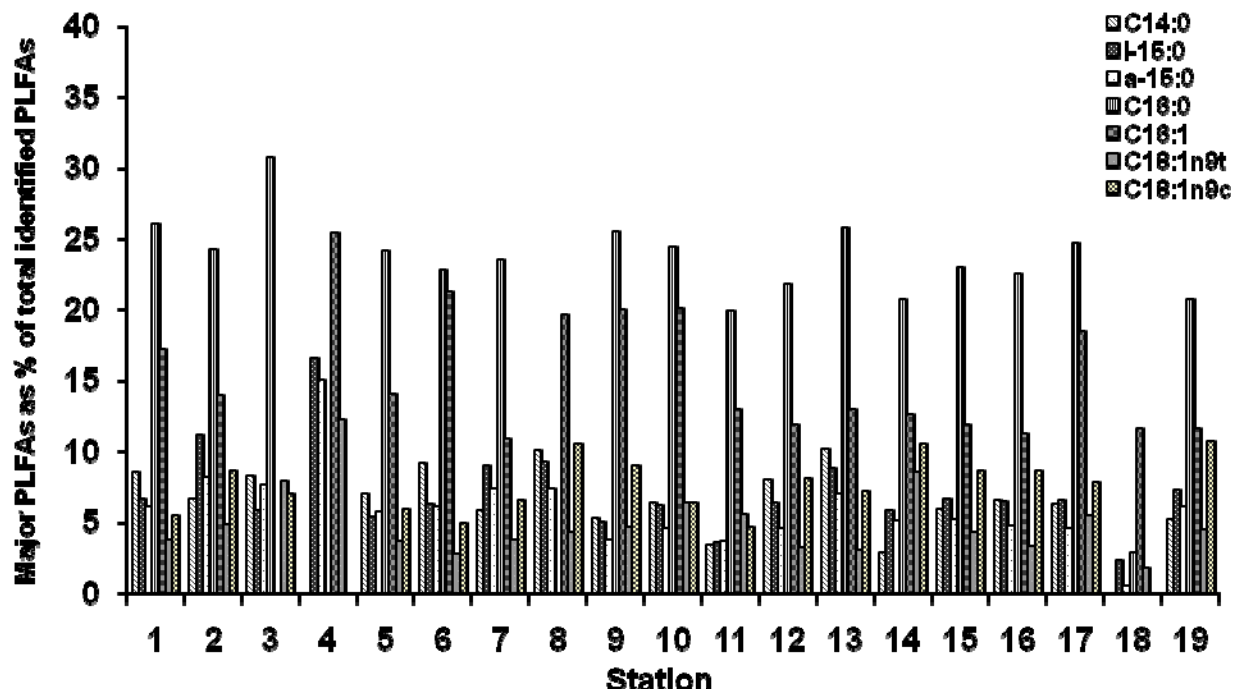


Fig 3: Distribution of major PLFAs as % of the total identified PLFAs in the sediments of the Visakhapatnam harbour, east coast of India.

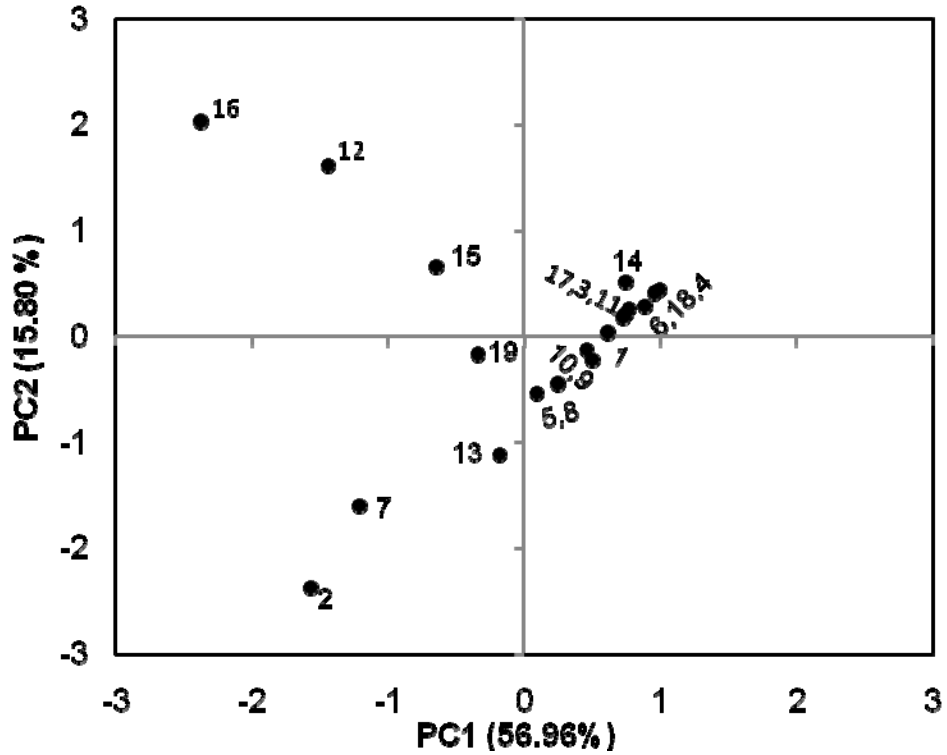


Fig 4: Distribution of PLFA community composition as % of the total identified PLFAs excluding saturated PLFAs in sediments of the Visakhapatnam harbour, east coast of India. Sum of PLFAs C16:1n9c, C18:1n8, and C18:1n9c was used to assess the abundance of Gram negative bacteria, cyanobacteria, and algae; sum of i- and a-15:0, i-16:0 and i-17:0 was used to evaluate the abundance of Gram +ve, anaerobic, and SO₄ reducing bacteria; and sum of PUFAs (C18:2n6c, C18:3n3, C18:3n6, C20:3n3, C20:3n6, C20:4n6, C20:5n3 and C22:6n3) was used to assess the abundance of eukaryotes

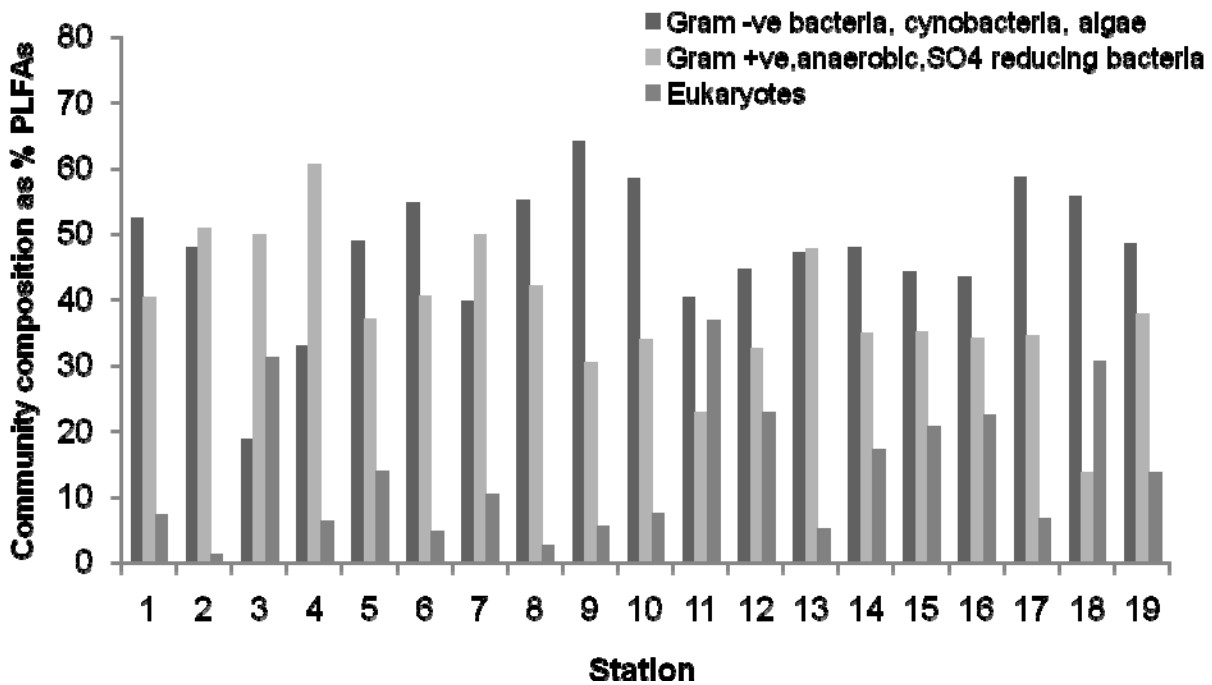


Fig. 5. (a) The scores of the first two principal components for the sampling sites for the PLFA compounds. Numbers indicate stations.

Table 1

OC, $\delta^{13}\text{C}_{\text{OC}}$, TC, MC, total lipids, total PLFAs and OC normalized total PLFA values in the sediments of the Visakhapatnam harbour, east coast of India.

Station	Sampling sites	Site code	OC (%)	$\delta^{13}\text{C}_{\text{OC}}$ (‰)	TC (%)	MC (%)	Total Lipids ($\mu\text{g g}^{-1}$) dw	Total PLFAs* ($\mu\text{g g}^{-1}$) dw	Total PLFAs* ($\mu\text{g gOC}^{-1}$)
1	West Quay Berth-1	VZPWQB-1	1	-25.35	55	45	4200	4.1	409
2	West Quay Berth-4	VZPWQB-4	-	-	-	-	4050	33.3	-
3	West Quay Berth-7	VZPSQB-7	6.7	-26.11	64	36	1528	1.9	28
4	Turning Basin	VZPTB	0.6	-26.05	63	37	1575	0.3	50
5	H&Y-Jetty 2/3	VZH&Y-2	7.6	-26.27	65	35	14 948	7.2	95
6	Fertilizer Berth	VZPFWRF	7.4	-29.18	98	2	6900	1.1	15
7	Jetty-8	VZPJT-8	4	-24.60	46	54	4083	15.9	397
8	Boat Basin Berth-3	VZpBBB-3	1.9	-27.37	78	22	5400	7.1	373
9	DC Jetty	VZPDCJT	2.4	-28.06	86	14	900	6.5	270
10	Dredger Berth	VZPDB	-	-	-	-	2724	5.2	-
11	General Cargo Berth S	VZPGCB-S	2.7	-26.76	71	29	600	1.6	58
12	General Cargo Berth N	VZPGCB-N	4.4	-27.04	74	26	1575	16.4	372
13	Ore Berth- 1	VZPORB-1	4.1	-24.35	44	56	2550	12.7	311
14	Ore Berth-2	VZPORB-2	1.3	-25.06	52	48	300	2.1	161
15	Container Berth-1	VZPCB-1	-	-26.88	72	28	2600	10.9	-
16	Container Berth-2	VZPCB-2	3	-25.77	60	40	2250	22.8	761
17	Turning Circle	VZPTRCL	0.7	-29.32	100	0	300	2.8	399
18	LPG Berth	VZPLPGB	1.2	-24.88	50	50	1718	0.5	39
19	Fishery Jetty	VZPFJT	1.3	-23.75	37	63	3825	8.4	643

- = No data; TC= terrestrial OC, MC= marine OC; * sum of identified and unidentified PLFAs; dw: dry weight sediment.

Table 2 PLFA concentrations (ng g⁻¹ dw sediment) in the sediments of the Visakhapatnam harbour, east coast of India.

PLFA (ng g ⁻¹)	Station																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
C12:0	-	74	-	-	-	-	-	-	-	-	-	148	-	-	71	298	-	-	-
C13:0	-	21	-	-	-	-	11	-	-	-	-	-	44	-	-	-	-	5	-
C14:0	195	870	136	0	315	74	483	217	205	196	35	626	637	26	360	743	100	0	267
i-15:0	152	1446	96	24	243	51	747	207	188	191	37	498	554	54	401	739	104	5	375
a-15:0	140	1060	126	22	259	49	617	155	149	140	37	358	439	46	314	545	73	1	316
C14:1	-	-	-	-	-	-	14	-	-	-	-	9	-	-	-	-	-	-	-
C15:0	66	308	37	-	134	27	221	122	100	84	34	216	218	17	117	245	41	-	118
i-16:0	59	341	39	11	149	28	307	82	63	63	18	186	200	27	152	275	42	-	145
C15:1	7	36	-	-	76	-	67	93	43	29	-	-	27	-	-	-	-	-	83
C16:0	593	3153	504	-	1085	185	1958	1045	-	751	204	1709	1613	189	1388	2564	391	6	1068
i-17:0	76	546	43	11	114	29	354	153	87	106	10	215	218	35	210	370	53	-	161
C16:1	391	1814	-	37	629	172	905	818	397	616	133	934	811	115	717	1280	292	26	601
C17:0	58	238	27	5	115	44	150	107	118	85	24	185	162	17	136	316	57	-	117
C17:1	24	50	-	-	69	-	82	63	-	61	11	115	84	7	62	118	23	60	107
C18:0	133	448	152	-	283	46	313	214	-	151	15	309	247	55	268	509	72	99	255
C18:1n8	39	252	-	-	112	-	167	67	29	44	-	152	130	11	118	208	45	-	126
C18:1n9t	85	632	130	18	163	22	313	191	87	197	57	256	190	78	260	381	86	4	232
C18:1n9c	125	1126	115	-	268	40	543	368	212	197	48	636	454	96	521	979	124	-	554
C18:2n6t	-	37	-	-	-	-	26	-	118	6	-	45	-	17	17	46	-	-	-
Unknown	37	157	32	-	138	14	172	34	162	25	157	48	43	-	67	80	14	-	111
C18:2n6c	77	10	191	-	287	18	194	108	31	111	3	210	152	27	224	367	53	-	151
C20:0	17	40	-	-	47	10	43	46	34	20	16	55	41	5	79	77	14	-	40
C18:3n6	-	-	-	7	-	-	63	-	-	-	129	45	-	3	160	301	-	14	39
C20:1	-	19	-	-	-	-	29	-	-	-	-	38	-	6	37	56	-	-	11
C18:3n3	-	30	-	-	-	-	23	-	-	-	-	141	-	8	65	171	-	-	10
C21:0	-	-	-	-	-	-	-	-	-	-	-	-	-	7	-	19	-	-	9
C20:2	-	21	-	11	-	-	23	-	-	-	-	35	-	-	25	46	-	-	17
C22:0	-	31	-	-	-	-	41	-	-	-	-	31	-	8	29	78	-	-	16
C20:3n6	-	-	-	-	-	-	-	-	-	-	-	22	-	4	-	23	-	-	-
C22:1n9	-	-	-	-	-	-	97	-	-	-	-	90	-	-	5	46	-	-	44
C20:3n3	-	-	-	-	-	-	35	-	-	-	-	-	-	4	-	-	-	-	21
C20:4n6	-	-	-	-	-	-	-	-	-	-	-	105	-	11	24	57	-	-	-
C23:0	-	137	-	-	-	-	94	-	-	-	23	-	-	5	-	-	-	-	-
C22:2	-	-	-	-	-	-	68	-	-	-	-	-	-	12	-	-	-	-	-
C24:0	-	49	-	-	-	-	49	-	-	-	-	58	-	-	47	76	-	-	17
C20:5n3	-	-	-	-	-	-	83	-	-	-	-	234	-	6	101	213	-	-	60
C24:1	-	-	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C22:6n3	-	39	-	-	-	-	24	-	-	-	34	123	-	16	60	135	-	-	80

- = not detected.

Table 3 Factor loading on the PLFA compounds using principal component analysis in the sediments of the Visakhapatnam harbour, east coast of India.

Variable	PC1	PC2	PC3
C18:1n9c	-0.949	-0.181	-0.200
C17:0	-0.944	-0.053	-0.213
C24:0	-0.943	0.172	0.108
C18:1n8	-0.943	-0.271	-0.102
C16:0	-0.930	-0.276	-0.161
C18:0	-0.924	-0.121	-0.143
i-16:0	-0.923	-0.362	0.019
C14:0	-0.917	-0.229	-0.223
C22:0	-0.911	0.233	0.112
C15:0	-0.909	-0.330	-0.115
i-17:0	-0.903	-0.386	-0.114
C20:2	-0.900	0.313	0.120
C20:1	-0.891	0.354	0.126
C16:1	-0.882	-0.334	-0.247
C18:1n9t	-0.866	-0.368	-0.199
i-15:0	-0.850	-0.455	-0.171
a-15:0	-0.846	-0.487	-0.132
C20:0	-0.821	0.082	-0.090
C18:3n3	-0.809	0.569	-0.032
C22:6n3	-0.796	0.496	0.044
C12:0	-0.794	0.523	-0.218
C20:5n3	-0.781	0.560	0.214
C17:1	-0.765	0.085	0.091
C22:1n9	-0.667	0.138	0.682
C20:3n6	-0.651	0.659	-0.007
C18:2n6c	-0.650	0.282	0.075
C20:4n6	-0.608	0.635	0.056
C18:3n6	-0.602	0.513	-0.013
C21:0	-0.496	0.459	-0.088
C23:0	-0.458	-0.701	0.113
Unknown	-0.449	-0.455	0.193
C14:1	-0.448	-0.107	0.793
C18:2n6t	-0.377	0.056	0.011
C20:3n3	-0.281	-0.352	0.806
C22:2	-0.259	-0.365	0.815
C13:0	-0.251	-0.580	-0.259
C15:1	-0.171	-0.564	0.203

