

n-Alkanes in surficial sediments of Visakhapatnam harbour, east coast of India

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Surface sediments collected from 19 stations along Visakhapatnam harbour were analysed for organic carbon (OC), $\delta^{13}\text{C}_{\text{oc}}$, total lipids (TL), total hydrocarbon (THC), *n*-alkane concentration and composition. OC, $\delta^{13}\text{C}_{\text{oc}}$, TL and THC ranged from 0.6% to 7.6%, -29.3 to -23.8‰ , 300 to 14,948 $\mu\text{g g}^{-1}$ dw, and 0.2 to 2,277 $\mu\text{g g}^{-1}$ dw, respectively. Predominance of even carbon numbers *n*-alkanes $\text{C}_{12}\text{--}\text{C}_{21}$ with carbon preference index (CPI) of <1 suggests major microbial influence. Fair abundance of odd carbon number *n*-alkanes in the range of $\text{C}_{15}\text{--}\text{C}_{22}$ and $\text{C}_{23}\text{--}\text{C}_{33}$ indicates some input from phytoplankton and terrestrial sources, respectively. Petrogenic input was evident from the presence of hopanes and steranes. The data suggest that organic matter (OM) sources varied spatially and were mostly derived from mixed source.

1. Introduction

In coastal sediments, organic matter (OM) is derived from various sources including autochthonous and allochthonous sources. Transport and storage of OM in coastal areas assume much significance as these areas play an important role in global carbon cycle, and also serve as a source of OM to the open ocean (Gogou *et al.* 2000; Bouloubassi *et al.* 2001). The role played by coastal areas in both natural and disturbed carbon cycle is yet to be fully understood in terms of global carbon change (Wu *et al.* 2001). In order to better understand the process of carbon cycling, it is essential to characterize the nature and sources of OM.

A number of compounds including carbohydrates, lignin phenols and lipids are often used to evaluate the nature and sources of OM. Lipids

account for a small fraction of OM but when characterised at molecular level, they provide valuable information on the sources of OM (Meyers 2003; Volkman 2006). For example, lipid compounds such as *n*-alkanes, sterols, alcohols and fatty acids are used to assess sources of OM in marine and terrestrial sediments (Volkman *et al.* 1992; Tolosa *et al.* 2009). Of these, *n*-alkanes are produced by a broad range of organisms. Moreover, *n*-alkanes are easy to analyse and hence, often used to identify the sources of OM in water, suspended matter and sediments from different environments (Harji *et al.* 2008; Kameyama *et al.* 2009; Maioli *et al.* 2011). This is because the assemblage of *n*-alkanes found in marine biota is different from that found in terrestrial biota. For example, marine planktons such as algae and cyanobacteria exhibit predominance of short chain, odd carbon *n*-alkanes mainly at C_{15} , C_{17} and C_{19}

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while terrestrial plants exhibit predominance of long chain, odd carbon *n*-alkanes at C₂₃, C₂₅, C₂₇, C₃₁ and C₃₃ (Clark and Blumer 1967; Cranwell 1973; Brassel *et al.* 1978; Sonibare and Sojnu 2009; Ahad *et al.* 2011). Similarly, the predominance of short chain, even carbon number *n*-alkanes in the range of C₁₂–C₂₂ is attributed to micro-organisms including diatoms and petroleum (Grimalt and Albaiges 1987; Garg and Bhosle 2004). Furthermore, indices such as unresolved complex mixture (UCM), carbon preference index (CPI), pristane/phytane, *n*-C₁₇/pristane and *n*-C₁₈/phytane ratios are useful to identify *n*-alkane sources (Volkman *et al.* 1992; Commendatore and Esteves 2004; Zaghden *et al.* 2005; Hu *et al.* 2009).

Visakhapatnam harbour, a semi-enclosed water body is one of the largest and busiest ports of India along the east coast of India. Since the beginning of 1950s, many large scale basic and other major industries were set up by the government and the private sector in its vicinity. The harbour is land-locked and this has resulted in limited natural flushing processes enhancing pollution stress on the harbour (Raman 1995). Studies on biological, chemical and geophysical parameters have indicated the influence of anthropogenic activities on the harbour and also their subsequent effects on the biological community and the ecosystem as a whole (Subbarao and Subbarao 1994; Raman 1995; Sultana and Rao 1998; Harji *et al.* 2010). Presently, we are studying distribution as well as molecular level characterization of OM in coastal and deep sea sediments. Such studies provide useful information on the nature, sources and cycling of OM. In the present study, we report on the distribution and molecular level characterization of OM. The objectives of this study were formulated to

- assess the distribution of organic carbon (OC), stable carbon isotopes, total lipid (TL) and *n*-alkanes in coastal sediments,
- identify the sources of OM using a combined approach of stable carbon isotopes, and *n*-alkanes,
- get an insight into the contribution of OC from different sources, and
- to assess if the harbour sediments were influenced by petroleum products.

2. Materials and methods

2.1 Description of the sampling area

Visakhapatnam harbour is located at 17°41'825''–17°42'313''N and 83°16'881''–83°19'793''E, along

the east coast of India (figure 1) at the mouth of a surface drain known as Meghadrigedda. It consists of an inner harbour and an outer harbour. There are 24 berths and 4 moorings to accommodate 28 ships at a time. The effluents of an oil refinery, a steel plant, a fertilizer plant and a lead and zinc smelter in the vicinity are discharged into this harbour. The harbour handles items such as manganese and iron ore, coal and oil products. Added to this, it receives most of the urban run-off and waste water discharge.

2.2 Cleaning of glassware and solvent purification

All the glassware was cleansed by washing with tap water, chromic acid and distilled water are heated to 450°C for 6 hours. After this, it was rinsed with methanol and dichloromethane. The solvents were of analytical grade and were double distilled before use. Sodium sulphate was ashed at 450°C and silicic acid, silica and alumina were washed with methanol and chloroform and dried prior to use.

2.3 Sample collection and analysis

Surficial sediment samples (the top 10–15 cm) were collected from various stations along the Visakhapatnam harbour (figure 1) using a Van-Veen grab. The samples were frozen onboard and soon transported to the laboratory in an ice box and stored at –20°C. The sediment samples were then lyophilized, powdered using agate mortar and pestle and stored at –20°C until analysed. The sediments were then used for the estimation of OC, δ¹³C, TL and *n*-alkane concentration and composition.

2.4 Estimation of bulk geochemical parameters

Samples for OC and δ¹³C_{oc} analysis were treated with 1N HCl, and subsequently washed with distilled water to remove the HCl traces. The finely decarbonised sediments were packed and loaded onto CNS elemental analyzer (NCS 2500, CE instruments) for OC analysis. 2, 5-Bis-(5-tetrabutyl-benzoxazol-2-yl) thiopen (BBOT) was used as a standard for calibration. For δ¹³C_{oc} analysis, Thermo Finnigan Flash 1112 elemental analyzer, linked with a Thermo Finnigan Delta V plus Isotope Ratio MS was used. The δ¹³C values are reported in the delta notation relative to Pee Dee Belemnite (PDB) standard. The analytical precision was within ±1.6% for OC and ±0.2‰ for δ¹³C_{oc}. All the OC isotopic compositions are reported in parts per million (‰) relative to

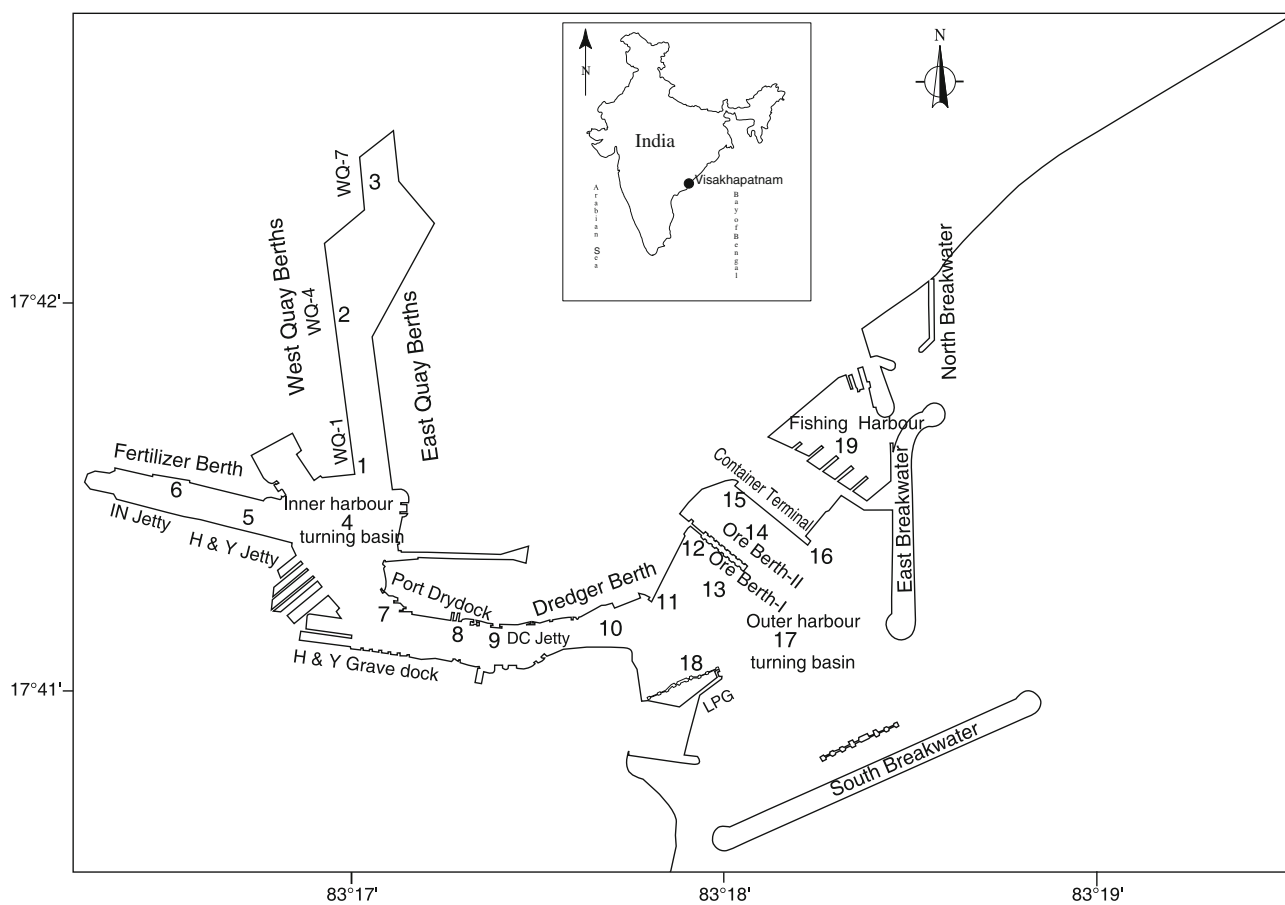


Figure 1. Map of the study area showing sampling stations in the Visakhapatnam harbour, east coast of India.

variation (δ) from the PDB standard and calculated as follows:

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

2.5 Extraction of lipids

Total lipids (TL) were extracted from lyophilized sediments following the method of Bligh and Dyer (1959) and estimated following Harji *et al.* (2008). In brief, sediments were shaken vigorously with chloroform:methanol:phosphate buffer (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 extracts were combined, treated with anhydrous sodium sulphate and filtered through Whatman No.1 filter paper. The filtrate was concentrated using a rotary evaporator under reduced pressure and made to a known volume (1 ml). A known aliquot was then put on pre-weighed Whatman filter paper. The paper was dried in a vacuum desiccator and weighed again. The weight of TL was obtained after subtracting

the weight of the empty filter paper from the filter paper containing dried lipid extract (Zaghden *et al.* 2005; Harji *et al.* 2008). The method showed a standard deviation of $\pm 5.3\%$ based on replicate analyses.

2.6 Separation of n-alkanes

Separation of *n*-alkanes from TL was done by silicic acid and silica-alumina column chromatography. Before use, silicic acid was activated for two hours at 120°C and packed onto glass column (10 × 0.5 cm). TL was loaded onto the column and eluted with dichloromethane. The neutral lipid fraction thus, obtained, was concentrated at 30°C using a rotary evaporator under reduced pressure.

The neutral lipid fraction eluted with dichloromethane was further subjected to silica-alumina column chromatography. Both silica and alumina were activated at 110°C overnight and then deactivated with 5% distilled water before use. The glass column (10 × 0.5 cm) was filled with silica and alumina and topped with activated copper powder using hexane. Neutral lipid fraction was loaded onto the column, and *n*-alkanes

were eluted using *n*-hexane. The *n*-hexane fraction was treated with anhydrous sodium sulphate and reduced to near dryness using a rotary evaporator under reduced pressure. The final volume of the *n*-alkanes was adjusted (100 μ L) after adding methyl nonadecanoate as the internal standard. A suitable aliquot of the fraction was analysed using the GC and GC-MS.

2.7 GC and GC-MS analysis

The *n*-alkanes were analysed using GC (Agilent GC model 6890 Plus) equipped with FID and SE-52 fused silica capillary column (30 m \times 0.32 mm i.d \times 0.25 μ m film thickness). A sample of 1 μ l was injected using on-column injector when the injector temperature was maintained at 300°C. The temperature of the detector was maintained at 325°C. The carrier gas was nitrogen and the flow rate was 1 ml min⁻¹. Column oven temperature was programmed at 80°C for 1 min, and then from 80° to 150°C at 6°C min⁻¹, and then from 150° to 280°C at 4°C min⁻¹ and kept at 280°C for 25 min.

The quantification of the *n*-alkanes was done using the data handling system available on the instrument. The *n*-alkanes were identified using authentic standards (*n*C₁₁–*n*C₃₃) procured from Supelco, USA. The peak areas were quantified with respect to the internal standard (methyl nonadecanoate). Based on the area of the internal standard and the areas of all *n*-alkanes in the sample, the concentration of UCM was calculated. The sum of the concentrations of the *n*-alkanes and UCM has been defined as total hydrocarbon (THC). The CPI for *n*-alkanes is defined as the ratio of the sum of odd carbon number homologues in a specified range to the sum of even carbon number homologues in the given range (Cranwell 1973; Elias et al. 2000). Recovery of 10 standard *n*-alkanes (C₂₀–C₂₉) varied from 75 to 92% (X = 81% \pm 7.9). Standard deviation for the method was within 15% based on replicate analysis. Solvents were passed through the entire analytical procedure and were used as blank.

Verification of the *n*-alkane peaks as well as determination of hopanes and steranes were done on GC-MS Shimadzu QP 2010. Capillary column RTX-5MS (30 m \times 0.32 mm i.d \times 0.25 μ m film thickness) was used. Injector temperature was 60°C and source housing temperature was maintained at 250°C. Carrier gas used was helium and flow rate was maintained at 1.5 ml min⁻¹. Sample of 1 μ l was injected using an on-column injector. Oven temperature was programmed to rise from 80° to 120°C at a ramped rate of 30°C min⁻¹, then from 120° to 320°C at a ramped rate of 4°C min⁻¹ and kept at this temperature for 15 min. GC-MS operation was in full scan mode, scanning

from *m/z* 50 to *m/z* 500. Detection of hopanes and steranes was done using ions at *m/z* 191 and *m/z* 217, respectively (Zaghden et al. 2005; Harji et al. 2008).

2.8 Statistical analysis

A simple correlation was used to assess relationship between different parameters using Microsoft Excel program.

3. Results and discussion

3.1 Bulk parameters

The distribution of OC, $\delta^{13}\text{C}_{\text{oc}}$ and TL are presented in table 1. OC ranged from 0.6% (station 4) to 7.6% (station 5). OC values are comparable to those which have been reported from other coastal regions (Harji et al. 2008 and references therein). The $\delta^{13}\text{C}_{\text{oc}}$ values in the Visakhapatnam harbour varied from -29.3‰ to -23.8‰ . Highly depleted value (-29.3‰) was observed at station 17 while relatively enriched value (-23.8‰) was observed at station 19. The $\delta^{13}\text{C}_{\text{oc}}$ values for marine OM have been reported to be in the range of -21 to -19‰ while for terrestrial plants with C3 pathway, it is in the range of -33 to -22‰ (Fry and Sherr 1984; Pancost and Boot 2004). Our $\delta^{13}\text{C}_{\text{oc}}$ values

Table 1. Geochemical data for surficial sediments from Visakhapatnam harbour, east coast of India.

Station no.	OC %	$\delta^{13}\text{C}_{\text{oc}}$ ‰	TL	T Alk	THC	UCM
			(μg g ⁻¹ dw)			
#1	1	-25.4	4200	11	344	332
#2	–	–	4050	23	550	519
#3	6.7	-26.1	1528	19	847	822
#4	0.6	-26.1	1575	8	166	157
#5	7.6	-26.3	14,948	31	327	295
#6	7.4	-29.2	6900	18	2277	2244
#7	4	-24.6	4083	16	860	843
#8	1.9	-27.4	5400	7	457	446
#9	2.4	-28.1	900	9	386	372
#10	–	–	2724	23	318	291
#11	2.7	-26.8	600	13	13	nd
#12	4.4	-27	1575	8	195	185
#13	4.1	-24.4	2550	6	258	252
#14	1.3	-25.1	300	2	2	nd
#15	–	-26.9	2600	10	11	nd
#16	3	-25.8	2250	5	5	nd
#17	0.7	-29.3	300	0.2	0.2	nd
#18	1.2	-24.9	1718	0.4	1	nd
#19	1.3	-23.8	3825	11	948	933

OC: Organic carbon, TL: total lipid, T Alk: total alkane, THC: total hydrocarbon, UCM: unresolved complex mixture.

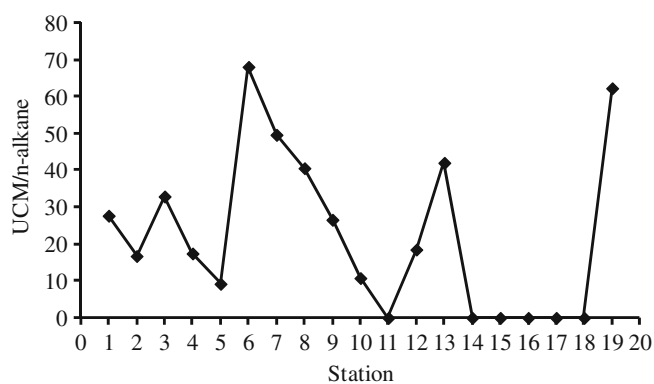


Figure 2. Spatial trend of UCM/*n*-alkane along the Visakhapatnam harbour, east coast of India.

for the harbour sediments indicate that OM was derived from both marine and terrestrial C3 plant sources. TL ranged from 300 (stations 14 and 17) to 14,948 $\mu\text{g g}^{-1}\text{dw}$ (station 5). These TL values are in the range of values reported earlier for coastal sediments (Harji *et al.* 2008). Significant correlation was observed between OC and TL ($R = 0.710$, $n = 14$, $p < 0.01$), suggesting common origin for these compounds.

3.2 Concentrations of THC, total *n*-alkanes and UCM

The distribution of THC, total *n*-alkanes and UCM is shown in table 1. Concentrations of THC ranged from 0.2 (station 17) to 2277 $\mu\text{g g}^{-1}\text{dw}$ (station 6). The concentrations of THC observed for the Visakhapatnam harbour sediments are comparable to those reported from other coastal areas (Mille *et al.* 2007; Harji *et al.* 2008). THC showed a

significant positive correlation with OC ($R = 0.762$, $n = 14$, $p < 0.001$), suggesting that THC was an important contributor to the OC. Sediments are considered as unpolluted, if the THC concentration is below 10 $\mu\text{g g}^{-1}\text{dw}$ (Farrington and Tripp 1977; Volkman *et al.* 1992; Readman *et al.* 2002). In our study, out of the 19 stations, 14 stations (stations 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14 and 19) showed values $>10 \mu\text{g g}^{-1}\text{dw}$, indicating that these stations were contaminated with petroleum input.

The total *n*-alkanes ranged from 0.2 (station 17) to 31 $\mu\text{g g}^{-1}\text{dw}$ (station 5) (table 1). Concentrations of *n*-alkanes were relatively higher at stations 2, 3, 5, 6 and 10 than those observed at other stations. The concentrations of total *n*-alkanes recorded in the present study are comparable to those reported earlier for other coastal regions. For example, Harji *et al.* (2008) reported total *n*-alkanes in the range of 1.6 to 10.7 $\mu\text{g g}^{-1}\text{dw}$ from the sediments of Marmugoa harbour while Wagener *et al.* (2012) reported in the range of 7.66 to 57.22 $\mu\text{g g}^{-1}\text{dw}$ from the sediments of Guanabara Bay.

The concentrations of the UCM ranged from 157 (station 4) to 2244 $\mu\text{g g}^{-1}\text{dw}$ (station 6). UCM is a mixture of structurally complex isomers of homologous branched/cyclic hydrocarbons that cannot be resolved by capillary GC columns (Bouloubassi and Saliot 1993; Readman *et al.* 2002; Gao *et al.* 2007). In general, the presence of UCM is indicative of degraded/weathered petroleum contamination although, bacterial reworking of OM cannot be ruled out (Farrington and Tripp 1977; Volkman *et al.* 1992; Hu *et al.* 2009). UCM was observed at stations 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13 and 19. A weathering index expressed as the ratio of

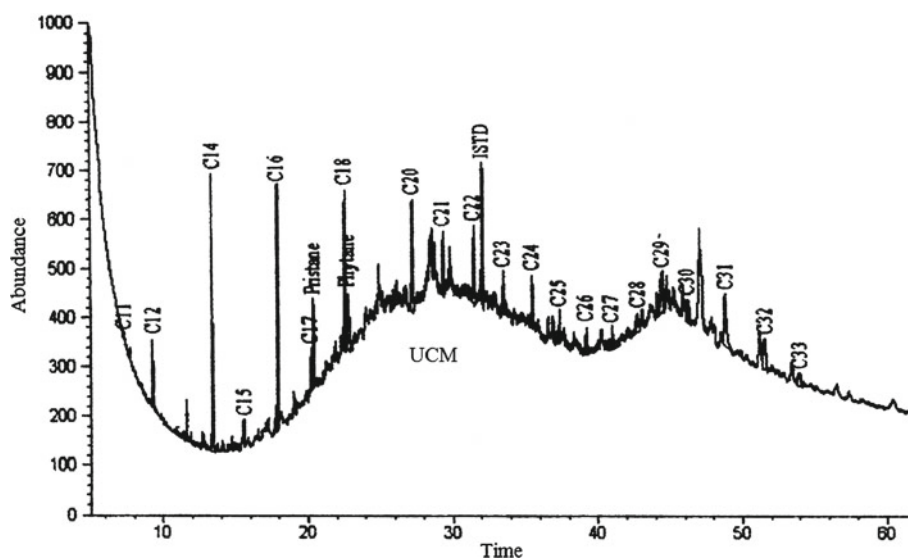


Figure 3. A representative gas chromatogram profile of *n*-alkanes at station 3 in the Visakhapatnam harbour, east coast of India.

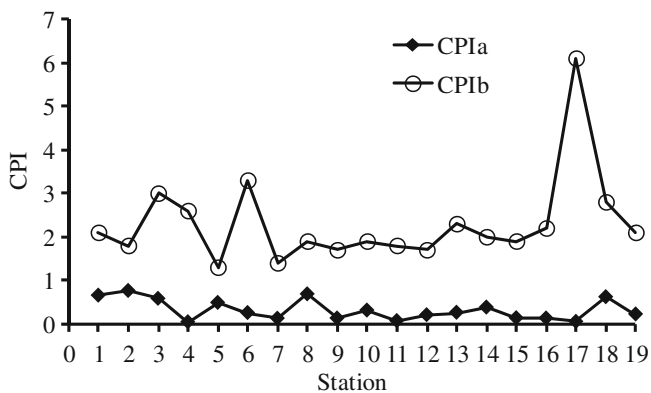


Figure 4. Spatial variation of CPI (carbon preference index) in surface sediments of Visakhapatnam harbour, east coast of India. $CPI^a = (n-C_{11} + n-C_{13} + n-C_{15} + n-C_{17} + n-C_{19} + n-C_{21}) / (n-C_{12} + n-C_{14} + n-C_{16} + n-C_{18} + n-C_{20})$; $CPI^b = (n-C_{23} + n-C_{25} + n-C_{27} + n-C_{29} + n-C_{31}) / (n-C_{22} + n-C_{24} + n-C_{26} + n-C_{28} + n-C_{30} + n-C_{32})$.

UCM to resolved *n*-alkanes (U/R) is used as a diagnostic criterion for petroleum pollution. Values >4 occur when there is widespread petroleum contamination (Mazurek and Simoneit 1984; Readman *et al.* 2002). In our study, the U/R ratio was high (>4) for all the UCM contaminated stations (figure 2). This suggests a strong influence of petroleum inputs on the sediments of the harbour.

3.3 Composition of *n*-alkanes

The *n*-alkanes ranging from C_{11} to C_{33} were observed in the surface sediments of the Visakhapatnam harbour. The distribution of short chain *n*-alkanes (C_{11} to C_{22}) demonstrated the predominance of even carbon *n*-alkanes C_{12} , C_{14} , C_{16} , C_{18} and C_{20} (figure 3). The predominance of these short chain even *n*-alkanes indicates the contribution of marine microorganisms and petroleum products to the OM of surface sediments of the harbour (Grimalt and Albaiges 1987; Garg and Bhosle 2004; Harji *et al.* 2008). Similarly, at some stations abundance of short chain odd *n*-alkanes such as

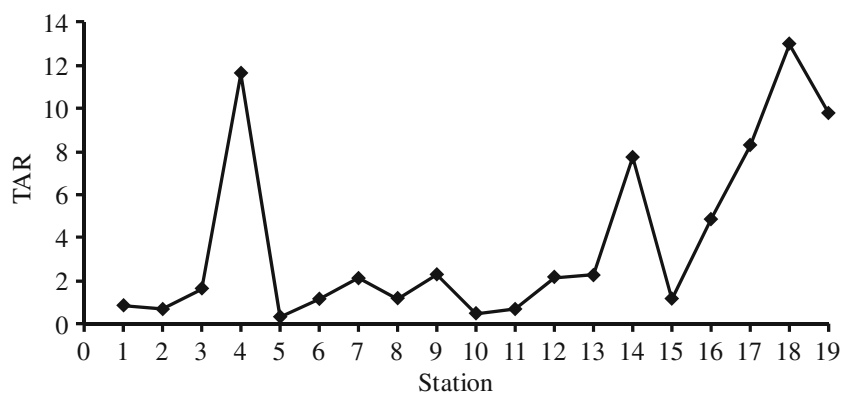


Figure 5. Spatial trend of TAR (terrigenous aquatic ratio) in the surface sediments of Visakhapatnam harbour, east coast of India.

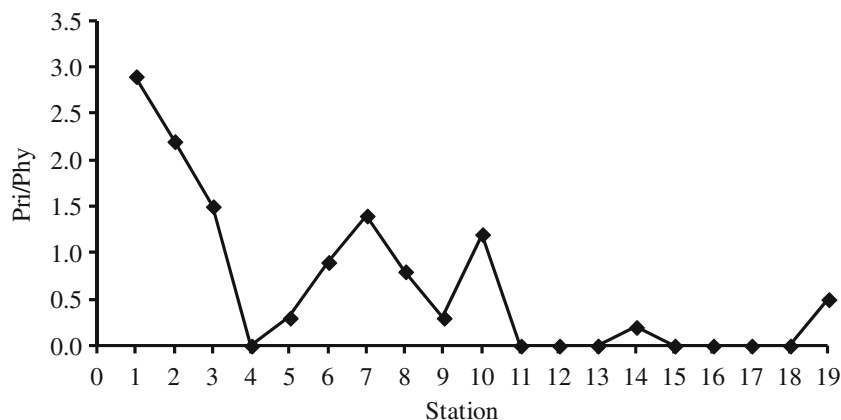


Figure 6. Spatial trend of Pri/Phy (pristane/phytane) in the surface sediments of Visakhapatnam harbour, east coast of India.

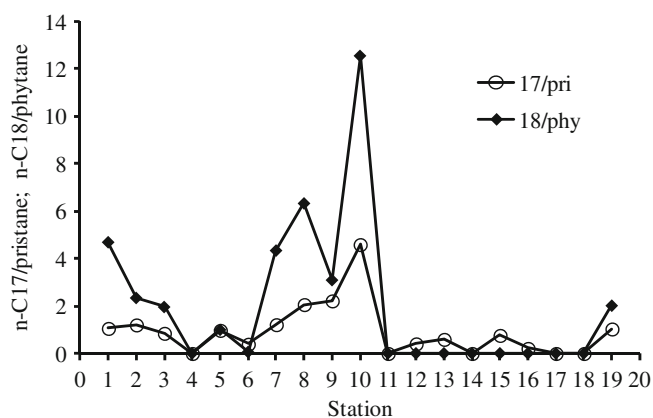


Figure 7. Spatial variation of *n*-C₁₇/pristane and *n*-C₁₈/phytane in the Visakhapatnam harbour, east coast of India.

C₁₅, C₁₇ and C₁₉ was recorded. Short chain odd *n*-alkanes C₁₅, C₁₇ and C₁₉ are produced by phytoplankton (Clark and Blumer 1967; Cranwell 1973; Pearson and Eglington 2000; Sonibare and Sojinu 2009). Therefore, the presence of these short chain odd carbon *n*-alkanes indicates the contribution from marine phytoplankton. The long chain odd carbon *n*-alkanes such as C₂₃, C₂₅, C₂₇, C₂₉, C₃₁ and C₃₃ were also present at some stations. These long chain odd carbon *n*-alkanes are derived from terrestrial plants (Clark and Blumer 1967; Cranwell 1973; Pearson and Eglington 2000; Sonibare and Sojinu 2009).

To further assess the possible sources of *n*-alkanes in the sediments of the Visakhapatnam harbour, CPI was calculated. CPI is a measure of homologous odd over even *n*-alkanes in a specified range of carbon numbers and is routinely used as a source indicator in marine sediments. The CPI (i.e., C₁₁+C₁₃+C₁₅+C₁₇+C₁₉+C₂₁/C₁₂+C₁₄+C₁₆+C₁₈+C₂₀+C₂₂) for the short chain *n*-alkanes is designated as CPI^a. The CPI^a values of 1 or near 1

indicate inputs from petroleum products, whereas <1 indicates inputs from microorganisms including bacteria and diatoms (Clark and Blumer 1967; Garg and Bhosle 2004; Ahad *et al.* 2011). Stations 1, 2, 3, 8 and 18 exhibited values close to 1 indicating petrogenic inputs. Lower CPI^a values (0.1–0.5) were observed at stations 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 19, suggesting algal and bacterial sources at these stations (figure 4). The CPI (i.e., C₂₃+C₂₅+C₂₇+C₂₉+C₃₁+C₃₃/C₂₄+C₂₆+C₂₈+C₃₀+C₃₂) for the long chain *n*-alkanes (C₂₃–C₃₃) is designated as CPI^b. The CPI^b values varied between 3 and 20 for higher vascular plants (Brassel *et al.* 1978; Wang and Fingas 2003). Our CPI^b values varied from 1.3 to 6.1. At stations 3, 4, 6, 17 and 18, the CPI^b values indicate the influence of terrestrial OM (figure 4).

In order to evaluate the relative distribution of terrestrial OM and aquatic algae at the sampled stations, the terrigenous-aquatic ratio (TAR) was calculated (Bourbonniere and Meyers 1996; Peters *et al.* 2005; Mille *et al.* 2007). TAR was calculated as follows:

$$\text{TAR} = (C_{27} + C_{29} + C_{31}) / (C_{15} + C_{17} + C_{19}).$$

The ratio is >1 for terrestrial input and <1 for algal input (Bourbonniere and Meyers 1996). Our values ranged from 0.3 to 13 (figure 5). The values were >1 at stations 3, 4, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18 and 19 indicating higher terrestrial input over marine algae at these stations. However, the dominance of long chain *n*-alkanes could be overestimated considering the preferential utilization of labile, short chain *n*-alkanes (Peters *et al.* 2005; Hu *et al.* 2009).

Contamination by petroleum-related products can be further assessed with the help of pristane and phytane. Pristane and phytane are isoprenoid hydrocarbons originating from geologic alteration

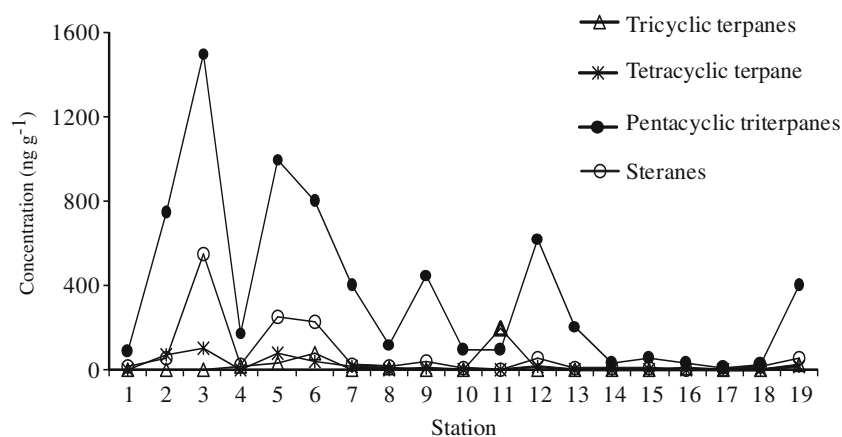


Figure 8. Distribution of tricyclic terpanes, tetracyclic terpanes, pentacyclic triterpanes and steranes in the Visakhapatnam harbour, east coast of India.

of phytol and other isoprenoidyl products and are not primary constituents of most terrestrial biota (Peters and Moldowan 1993; Gao et al. 2007). They are usually present in most petroleum as major constituents and are considered as good indicators of petroleum contamination (Volkman and Maxwell 1986; Readman et al. 2002; Zaghden et al.

2005). However, in marine environments, pristane can also be derived from zooplanktons and other marine animals while phytane can be contributed by methanogenic and photosynthetic bacteria (Volkman et al. 1992; Le Dreau et al. 1997). In the present study, pristane/phytane ratio varied from 0 to 2.9 (figure 6). For uncontaminated site,

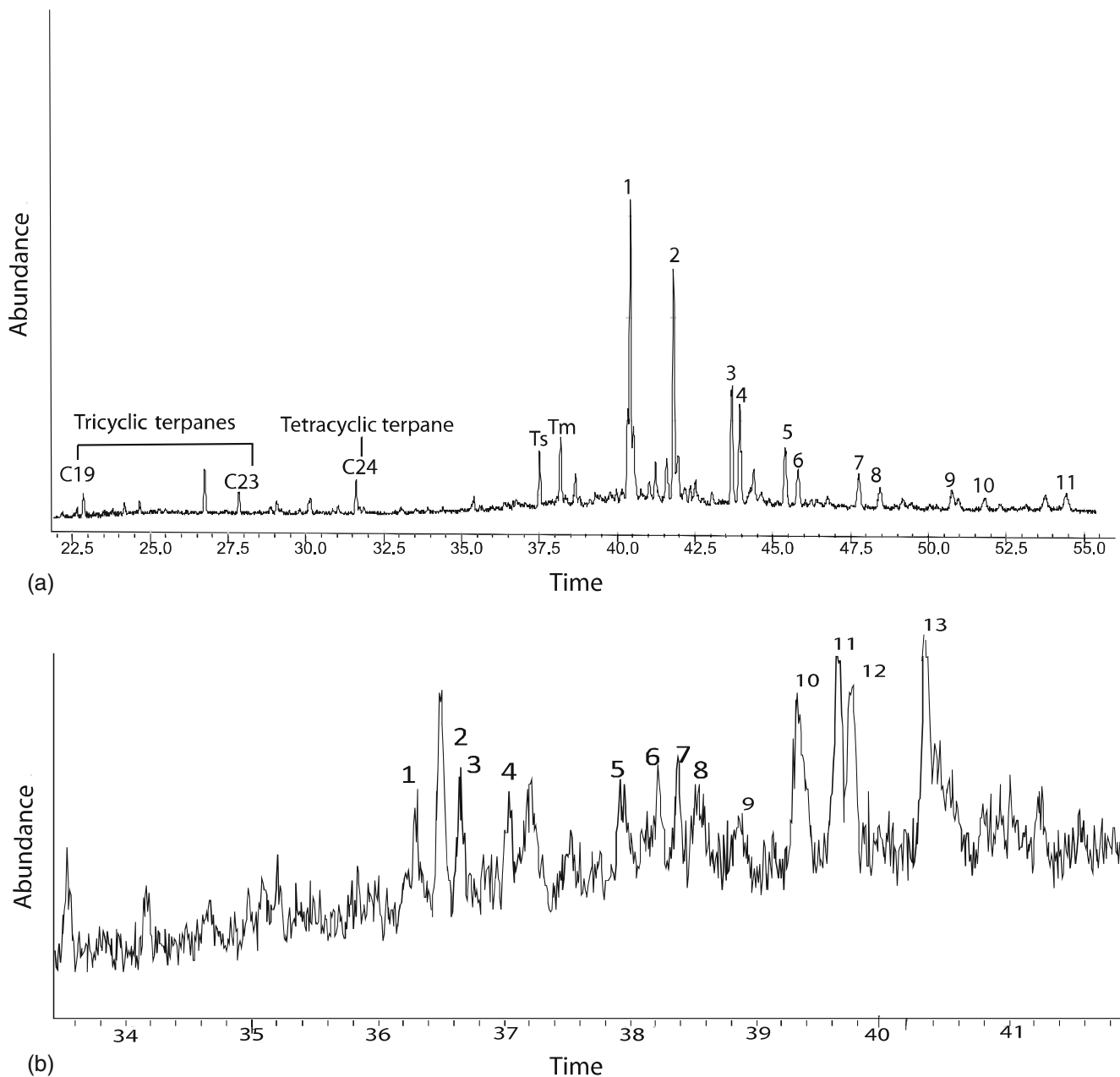


Figure 9. (a) Mass fragmentograms of m/z 191 (hopanes) at station 3 in the surface sediments of Visakhapatnam harbour, east coast of India. Ts: $18\alpha(\text{H})$ -22,29,30-trisnorneohopane; Tm: $17\alpha(\text{H})$ -22,29,30-trisnorhopane; 1: $17\alpha(\text{H})$, $21\beta(\text{H})$ -30-Norhopane; 2: $17\alpha(\text{H})$, $21\beta(\text{H})$ -Hopane; 3: $17\alpha(\text{H})$, $21\beta(\text{H})$ -30-homohopane(22S); 4: $17\alpha(\text{H})$, $21\beta(\text{H})$ -30-homohopane(22R); 5: $17\alpha(\text{H})$, $21\beta(\text{H})$ -30,31-bishomohopane(22S); 6: $17\alpha(\text{H})$, $21\beta(\text{H})$ -30,31-bishomohopane(22R); 7: $17\alpha(\text{H})$, $21\beta(\text{H})$ -30,31,32-trishomohopane(22S); 8: $17\alpha(\text{H})$, $21\beta(\text{H})$ -30,31,32-trishomohopane(22R); 9: $17\alpha(\text{H})$, $21\beta(\text{H})$ -30,31,32,33-tetrakishomohopane (22S); 10: $8-17\alpha(\text{H})$, $21\beta(\text{H})$ -30,31,32,33-tetrakishomohopane; 11: $17\alpha(\text{H})$, $21\beta(\text{H})$ -pentakishomohopane (22S). (b). Mass fragmentograms of m/z 217(Steranes) in the surface sediments of Visakhapatnam harbour, east coast of India. 1: $27\alpha\alpha\alpha$ (S); 2: $27\alpha\beta\beta$ (R); 3: $27\alpha\beta\beta$ (S); 4: $27\alpha\alpha\alpha$ (R); 5: $28\alpha\alpha\alpha$ (S); 6: $28\alpha\beta\beta$ (R); 7: $28\alpha\beta\beta$ (S); 8: $28\alpha\alpha\alpha$ (R); 9: $29\alpha\alpha\alpha$ (S); 10: $29\alpha\beta\beta$ (R); 11: $29\alpha\beta\beta$ (S); 12: $29\alpha\alpha\alpha$ (R); ($\alpha\alpha\alpha = 5\alpha(\text{H})$, $14\alpha(\text{H})$, $17\alpha(\text{H})$ -steranes; $\alpha\beta\beta = 5\alpha(\text{H})$, $14\beta(\text{H})$, $17\beta(\text{H})$ -steranes. R and S = R and S at C-20 configuration).

pristane/phytane ratio varies from 3–5 whereas for petroleum contaminated sites the ratio is ≤ 1 (Zaghden *et al.* 2005; Gao *et al.* 2007). The values were ≤ 1 at stations 5, 6, 7, 8, 9, 10, 14 and 19 indicating petroleum input at these sites.

Further, $n\text{-C}_{17}$ /pristane and $n\text{-C}_{18}$ /phytane ratios are used to evaluate the presence of freshly derived and degraded petroleum hydrocarbons in the sediments (Commendatore and Esteves 2004; Harji *et al.* 2008). The value is < 1 for degraded petroleum and > 1 for fresh petroleum input (UNEP 1995; Harji *et al.* 2008). In our study, the $n\text{-C}_{17}$ /pristane values ranged from 0.2 to 4.6 (figure 7). The ratios indicated that fresh petroleum input was present at stations 1, 2, 3, 7, 8, 9 and 10 whereas, degraded petroleum was observed at stations 6, 12, 13, 14, 15, 16 and 18. This was further supported by the $n\text{-C}_{18}$ /phytane ratios which ranged from 0.1 to 12.5 (figure 7).

3.4 Hopanes and steranes

The presence of petroleum compounds was substantiated by the presence of hopanes and steranes. Hopanes and steranes are resistant to environmental alteration and are routinely used to fingerprint petroleum contamination in sediments (Volkman 1986; Peters and Moldowan 1993; Zaghden *et al.* 2005; Harji *et al.* 2008; Hu *et al.* 2009). Hopanes (8–1602 ng g⁻¹ in stations 17 and 3, respectively) and steranes (0–554 ng g⁻¹ in stations 17 and 3, respectively) were detected at the study site (figure 8). Ion chromatograms for hopanes (m/z 191) showed the presence of tricyclic and tetracyclic terpanes and pentacyclic triterpanes (figure 9a). Of the total hopanes present, pentacyclic triterpanes accounted for 96% and tetracyclic and tricyclic terpanes together constituted 4%. Of this, the presence of 17 α (H), 21 β (H) configuration (47%) and 18(H)-22,29,30-trisnorneohopane (5%) strongly supports the presence of petroleum compounds (Shi *et al.* 2008). High concentrations of these compounds were observed at stations 2, 3, 5, 6, 7, 9, 12, 13 and 19. Tetracyclic and tricyclic terpanes are found in crude oil and fossil fuel, respectively (Moldowan and Seifert 1983; Aboul-Kassim and Simoneit 1996; Sanchez and Permanyer 2006). These were detected at stations 3, 5, 6, 12 and 19. The mass fragmentograms of steranes (m/z 217) reveal the presence of C₂₇ and C₂₈ cholestane and C₂₉ stigmastane with their 20S and 20R epimers (figure 9b). Steranes are strong indicators of petroleum input as they are absent in unpolluted recent surface sediments (Mackenzie *et al.* 1982). In this study, steranes were detected in varying concentrations in all the stations except for station 17. Elevated concentration of steranes was observed at stations 2, 3, 5, 6, 7, 9, 12, and 19.

Major steranes detected in the study site were C₂₉ stigmastane (54%) followed by C₂₇ (26%) and C₂₈ steranes (20%) with their 20S and 20R epimers. The composition of C₂₇–C₂₉ steranes indicates a mixed source of origin of the constituent oils. Generally, C₂₉ steranes indicate petroleum input derived from terrestrial sources while C₂₇ steranes are derived from marine sources (Volkman 1986; Peters and Moldowan 1993; Gao *et al.* 2007). The dominance of C₂₉ steranes in the present study indicates terrestrial source of origin of petroleum. Earlier studies had attributed the dominance of C₂₉ steranes in sediments to be typical of non-marine crude oil (Hu *et al.* 2009; Bouloubassi *et al.* 2001). However, it is also known that higher abundance of C₂₉ steranes could possibly indicate microbial activity in the host environment as C₂₇ and C₂₈ steranes are more susceptible to microbial degradation (Ollivier and Magot 2005). Hence, the abundance of C₂₈ steranes in the study site due to microbial activity cannot be ruled out.

4. Conclusion

In summary, the geochemical parameters and n -alkane distribution in Visakhapatnam harbour sediments indicate that the harbour sediments were influenced by a mixed contribution from terrestrial and marine sources. The n -alkane distribution indicates that the OM in the harbour is mainly contributed by microbial activity and petroleum products followed by terrestrial sources of input. The contribution of marine phytoplankton, though minimal, was observed. This was further supported by the carbon preference index (CPI) and terrigenous-aquatic ratio (TAR) and unresolved complex mixture (UCM)/ n -alkane ratio. The presence of high UCM is also a positive indicator of weathered oil residues. This was further substantiated by $n\text{-C}_{17}$ /pristane and $n\text{-C}_{18}$ /phytane ratios. The compositional pattern of hopanes and steranes strongly indicate that the harbour is contaminated by petroleum related compounds. This study may be used as baseline information in future source studies of OM from n -alkanes and may help in mitigating incongruities between source allocations and the magnitudes of prospective sources.

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