

Sources of hydrocarbons in sediments of the Mandovi estuary and the  
Marmugoa harbour, west coast of India

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## Abstract

Surface sediments were collected from various locations of the Mandovi estuary and the Marmugoa harbour. Sediments were analyzed for organic carbon (OC), total lipids, *n*-alkanes concentration and composition. Concentrations of OC, total lipids and *n*-alkanes varied spatially and ranged from 1 to 2.5 %, 176 to 1413 µg/g dry weight (dw) sediments, and 0.81 to 3.15 µg/g dw sediments of the Mandovi estuary, respectively; and from 0.6 to 2.9 %, 233 to 1448 µg/g dw sediments, and 1.6 to 10.7 µg/g dw sediments in the Marmugoa harbour, respectively. Long-chain, odd carbon *n*-alkanes (C<sub>23</sub>-C<sub>33</sub>) in the Mandovi estuary, whereas short-chain, even carbon *n*-alkanes (C<sub>11</sub>-C<sub>21</sub>) in the Marmugoa harbour sediments were more abundant. The total HC concentrations, *n*-alkane composition, CPI, UCM and other evaluation indices suggest the dominance of terrestrial hydrocarbons in the estuarine while petroleum derived hydrocarbons in the harbour sediments. This conclusion was further supported by the abundance of hopanes with C<sub>29</sub> to C<sub>34</sub> α, β compounds and steranes with C<sub>27</sub>, C<sub>28</sub> and C<sub>29</sub> compounds in the harbour sediments.

*Keywords:* Hydrocarbons; *n*-alkanes; source-determination; Mandovi estuary; Marmugoa harbour; west coast of India

## 1. Introduction

Organic matter (OM) in coastal sediments is derived from various sources such as terrigenous, marine, atmospheric and anthropogenic (Gogou et al., 2000; Wang et al., 2006). However, the fate of autochthonous and terrestrially derived OM in estuarine environments is not well understood (Hedges et al., 1997; Wu et al., 2004). The information about processes controlling the delivery of organic matter to coastal environments, and how the signatures of these inputs are reflected in newly deposited sediments is important to our understanding of global biogeochemical cycles.

Lipid molecules such as *n*-alkanes, fatty acids, alcohols and sterols are used to identify the sources of organic matter in marine and terrestrial samples (Volkman et al., 1992; Tolosa et al., 2003; Wu et al., 2004). Of these, the *n*-alkanes are commonly used to characterize organic matter of water, suspended matter and sediments from various environments (Ou et al., 2004; Gao et al., 2007). This is because *n*-alkanes are easy to analyse, and many are source specific. For example, bacteria normally show predominance of even carbon *n*-alkanes, mainly C<sub>18</sub> and C<sub>20</sub> (Han and Calvin, 1969). Elias et al. (2000) reported that even carbon *n*-alkanes in the C<sub>14</sub> - C<sub>22</sub> range originate from diatoms. On the other hand, planktonic organisms such as cyanobacteria and green, red and brown algae generally produce a simple mixture of odd carbon *n*-alkanes dominated by C<sub>15</sub>, C<sub>17</sub>, and C<sub>19</sub> with predominant compound being species dependent (Clark and Blumer, 1967; Gogou et al., 2000). Whereas, abundance

of straight chain odd carbon *n*-alkanes C<sub>25</sub>, C<sub>27</sub>, C<sub>29</sub> and C<sub>31</sub> has been used extensively as an indicator of terrestrial or land derived organic matter (Pearson and Eglinton 2000; Zhao et al., 2003).

A number of other indices such as concentrations of total hydrocarbons, major *n*-alkanes, the ratios of short chain/long chain *n*-alkanes, total *n*-alkanes/*n*-C<sub>16</sub>, *n*-C<sub>17</sub>/pristane and *n*-C<sub>18</sub>/phytane, carbon preference index (CPI) and the presence of an unresolved complex mixture (UCM) also have been used to identify the sources of *n*-alkanes in environmental samples (Bouloubassi et al., 2001; Ou et al., 2004; Gao et al., 2007). Similarly, because of their greater thermodynamic stability, hopanes with the 17 $\alpha$ , 21 $\beta$ -configuration, and steranes with 5 $\alpha$ , 14 $\alpha$  and 17 $\alpha$  configuration are useful to identify petroleum derived *n*-alkanes (Zaghdien et al., 2005; Gao et al., 2007). Furthermore,  $\delta^{13}\text{C}$  analysis is yet another useful technique to identify the sources of *n*-alkanes (Pearson and Eglinton 2000; Wu et al., 2004; Wang et al., 2006).

There are numerous studies on the physical, chemical and biological characterization of the waters of the Mandovi estuary and the Marmugoa harbour (Selvakumar et al., 1980; Qasim and Sen Gupta, 1981; Shirodkar and Sen Gupta 1985, Chanda et al., 1996). There also are studies on the total organic carbon (TOC) and the mineralogy of the surficial sediments of the Mandovi estuary, and total petroleum hydrocarbons in the coastal waters of Goa (Murty et al., 1976; Fondekar et al., 1980; Alagarsamy, 1991). In contrast, little is known about molecular level characterization of OM, especially *n*-alkane concentration and composition in the sediments of the Mandovi estuary and the Marmugoa harbour.

Therefore, the aims of the present study were, 1) to investigate the spatial distribution of total lipids, especially *n*-alkanes in sediments, 2) to characterize the composition of *n*-alkanes 3) to evaluate the sources of *n*-alkanes in the sediments and 4) to analyze sterane and hopane based biomarkers as indicators for petroleum inputs.

## **2. Materials and Methods**

### *2.1. Description of the Sampling areas*

Marmugoa harbour is located at 15.25 °N and 73.47 °E, whereas the Mandovi estuary is located at 15.35 °N and 73.47 °E along the west coast of India (Fig. 1a and 1b). Marmugoa harbour is one of the major harbours of India and is used to transport manganese and iron ores to various countries. It harbours a Ship building unit and a Dry dock for the construction, repair and painting of various vessels. Along the Mandovi estuary several small jetties, wharfs and boat repair workshops are located. It is used for navigation and also for the transport of iron and manganese ores. Jetties are used for berthing fishing vessels and barges. Mandovi estuary is banked on both the sides by mangrove vegetation.

### *2.2. Cleaning of glassware and purification of solvents*

Before use all the glassware was washed with tap water, chromic acid, distilled water, and rinsed with methanol, dichloromethane and *n*-hexane. All the solvents were of analytical grade and were double distilled before use. Sodium

sulphate was heated at 450 °C. Silica and alumina were washed with methanol, dichloromethane and *n*-hexane prior to use.

### *2.3. Sample collection and analysis*

Surficial sediment samples were collected using a Van Veen grab from 9 locations in the Mandovi estuary, and 10 locations in the Marmugoa harbour (Fig. 1a and 1b and Table 1). Sediments were lyophilized, powdered, sieved through 60- $\mu$ m filters and used for the estimation of organic carbon, total lipids and *n*-alkanes.

### *2.4. Estimation of organic carbon*

The total carbon (TC) in the sediments was estimated using the TOC analyzer (Shimadzu TOC – 5000 A, analyzer). In order to measure the inorganic carbon (IC), sediment sample was treated with orthophosphoric acid at 200 °C in presence of oxygen and the liberated carbon dioxide was analysed using TOC analyser. The total organic carbon was then obtained by subtracting the IC from the TC. The precision of the measurements was  $\pm 1.1$  % for TC based on replicate sediment analyses.

### *2.5. Extraction of lipids*

Total lipids from sediment samples were extracted using a modified method of Bligh and Dyer (1959). Briefly, freeze-dried sediment sample was ultrasonically extracted (15 x 3 min) with dichloromethane (DCM): methanol (MeOH) (2:1). This step was repeated two more times. The 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 40 °C under reduced pressure and made to a known volume (1 ml). A known aliquot was then applied onto the pre-weighed piece of the Whatman filter paper. The paper was dried in a vacuum desiccator and weighed. The weight of the empty Whatman paper was subtracted from the weight of Whatman paper plus solvent extract to get the weight of the total lipids (Zaghden et al., 2005). The method showed a standard deviation of  $\pm 5.8 \%$ , based on replicate analyses.

## 2.6. Separation of *n*-alkanes

The *n*-alkanes were separated from total lipids using silica-alumina column chromatography. Both silica and alumina were activated at 110 °C overnight and then deactivated with 5 % distilled water before use. The column (10 X 0.5 cm) was packed with silica (5 cm) followed by alumina (2 cm above silica) using *n*-hexane, and was topped by activated copper powder. The lipid was loaded onto the column and *n*-alkanes were eluted using 20 ml *n*-hexane. *n*-Hexane fraction was treated with anhydrous sodium sulphate to remove traces of water, reduced to near dryness under reduced pressure and methyl nonadecanoate was added as an internal standard. Subsequently, the sample was reduced to 100  $\mu$ l using dry nitrogen gas, and was adjusted to a known volume. A suitable aliquot was then analysed for *n*-alkanes using the capillary GC and/or GC-MS.

## 2.7. Capillary GC and GC-MS analysis

GC-analyses were performed using an Agilent GC 6890 equipped with a FID and SE-52 fused silica capillary column (30 m x 0.32 mm i. d. x 0.25  $\mu\text{m}$  film thickness). 1  $\mu\text{l}$  of sample was injected using an on-column injector when the temperature was maintained at 300  $^{\circ}\text{C}$ . The detector temperature was maintained at 325  $^{\circ}\text{C}$ . Nitrogen was used as a carrier gas at a flow rate of 1  $\text{ml min}^{-1}$  and the column oven temperature was 80  $^{\circ}\text{C}$  for 1 min, and then programmed from 80  $^{\circ}\text{C}$  to 150  $^{\circ}\text{C}$  at 6  $^{\circ}\text{C min}^{-1}$  and then from 150  $^{\circ}\text{C}$  to 280  $^{\circ}\text{C}$  at 4  $^{\circ}\text{C min}^{-1}$  and kept at 280  $^{\circ}\text{C}$  for 25 min.

The quantification of *n*-alkanes was done using the data handling system available on the instrument. Identification of the individual *n*-alkanes was performed by comparing the retention times of *n*-alkanes in samples with those of standards (Supelco, USA). The peak areas were quantified relative to an internal standard (methyl nonadecanoate) added to the sample prior to GC-analysis. Based on the peak area of the internal standard and the areas of all *n*-alkanes, the amount of UCM was calculated. Total hydrocarbons (THC) have been defined as the sum of concentrations of *n*-alkanes plus UCM. The Carbon Preference Index (CPI) for *n*-alkanes is defined as a summation of odd carbon number homologues over a range divided by the summation of even carbon number homologues over the same range (Elias et al., 2000; Gao et al., 2007). The standard deviation of the method was < 15 % based on replicate sediment analysis. Recovery of the standard *n*-alkanes ( $\text{C}_{14}\text{-C}_{30}$ ) processed following the entire analytical procedure varied from 78 to 98 % ( $X = 90 \pm 11.5$ ). Solvents were passed through the entire analytical procedure and were used as blank.



Hopanes and steranes were analysed using a GC-MS (Shimadzu -QP 2010) equipped with the RTX-5MS capillary column (30 m x 0.32 mm i. d. x 0.25  $\mu\text{m}$  film thickness). The injector and source housing temperatures were maintained at 60 °C and 250 °C, respectively. Helium was used as a carrier gas at a flow rate of 1.5 ml min<sup>-1</sup>. Sample (1  $\mu\text{l}$ ) was injected into an on-column injector. The GC oven temperature was programmed to rise from 80 to 120 °C at the ramp of 30 °C min<sup>-1</sup>, then from 120 to 320 °C at the ramp of 4 °C min<sup>-1</sup> and maintained at this temperature for 15 min. The GC-MS system operated in full scan mode, scanning from m/z = 50 to m/z = 500. Detection of steranes and hopanes were carried using ions at m/z = 191 and m/z = 217, respectively (Zaghden et al., 2005).

### **3. Results and discussion**

#### *3.1. Bulk parameters*

OC varied from 1.0 to 2.5 % and 0.6 to 2.9 %, whereas total lipids ranged between 176 and 1413  $\mu\text{g/g}$  dw sediments, and from 233 and 1448  $\mu\text{g/g}$  dw sediments of the Mandovi estuary and the Marmugoa harbour, respectively (Table 1). Total lipids were higher at stations # 5, # 6 and # 8 of the Mandovi estuary, and at the stations # 2 and # 3 of the Marmugoa harbour. Overall, total lipids tend to decrease from station # 2 to station # 6 in the harbour, while no particular trend was recorded for the estuarine sediments. The observed concentrations of OC and lipids are comparable to those reported for the sediments from other environments (Table 2).

### 3.2. Concentrations of Total Hydrocarbons (THC), total *n*-alkanes and UCM

Spatial variation in concentrations of THC, *n*-alkanes and UCM is shown in Table 1. Elevated concentrations of THC, *n*-alkanes and UCM were recorded at stations # 5 and # 6 of the Mandovi estuary, and at station # 4 of the Marmugoa harbour. Concentrations of THC, *n*-alkanes and UCM were generally higher by an order of magnitude in the sediments of the Marmugoa harbour. The levels of *n*-alkanes in the estuarine and the harbour sediments are comparable to those recorded for the surface sediments from other estuaries and coastal environments (Table 2). Overall, the observed concentration range generally typifies unpolluted to moderately polluted coastal sediments (Volkman et al., 1992; Tolosa et al., 1996).

UCM is a mixture of structurally complex isomers of homologous branched/cyclic hydrocarbons that cannot be resolved by capillary columns. Nevertheless, it is a useful indicator to assess petroleum contamination from anthropogenic sources (Volkman et al., 1992; Gogou et al., 2000; Ou et al., 2004). A weathering index, generally defined as the ratio of unresolved to resolved *n*-alkanes (UCM/ *n*-alkanes), is a useful tool to identify the presence of degraded petroleum hydrocarbons. Significant contamination by petroleum products exists when the value is >2.0 (Gogou et al., 2000; Ou et al., 2004). With the exception of stations # 5 and # 6, the UCM/ *n*-alkane ratio was low (< 2) (Fig. 2a). In contrast, the ratio was high (>2) for most of the stations in the harbour (Fig. 2b). The UCM concentrations and UCM/ *n*-alkane ratio suggest that harbour

sediments are more contaminated from petroleum inputs than the estuarine sediments. This was probably because the harbour is subjected to heavy shipping activities.

### 3.3. Composition of *n*-alkanes and the hydrocarbon indices

Estuary and the harbour sediments showed the presence of *n*-alkanes ranging between C<sub>11</sub> to C<sub>33</sub>. The *n*-alkanes in the C<sub>11</sub> – C<sub>22</sub> carbon range showed the dominance of even carbon *n*-C<sub>12</sub>, *n*-C<sub>14</sub>, *n*-C<sub>16</sub>, *n*-C<sub>18</sub> and *n*-C<sub>20</sub> at different stations, whereas for the C<sub>23</sub>–C<sub>33</sub> carbon range a odd carbon preference for *n*-C<sub>27</sub>, *n*-C<sub>29</sub>, *n*-C<sub>31</sub> and *n*-C<sub>33</sub> was recorded for both the estuarine and harbour sediments (Fig. 3a and 3b). In the estuarine sediments, the dominating *n*-alkanes were long chain odd carbon *n*-alkanes maximizing at *n*-C<sub>31</sub> and *n*-C<sub>29</sub> (Fig. 3a), whereas in the harbour sediments the dominating hydrocarbons were short chain even carbon *n*-alkanes (C<sub>12</sub>, C<sub>14</sub>, C<sub>16</sub>, C<sub>18</sub> and C<sub>20</sub>) followed by long chain odd numbered carbon (C<sub>29</sub>, C<sub>31</sub> and C<sub>33</sub>) (Fig. 3b). This was also evident from the low (< 1.0) and high (>1.0) ratios for short chain *n*-alkanes/long chain *n*-alkanes in the estuarine and harbour sediments, respectively (Table 3). The long chain, odd carbon *n*-alkanes are of terrestrial origin and can be found in waxes of higher plants (Brassell et al., 1978; Rieley et al., 1991), whereas high abundance of short chain even numbered *n*-alkanes indicates petroleum source (Stout et al., 2002; Wang and Fingas, 2005). Plankton generally produces a simple mixture of hydrocarbons dominated by short chain odd carbon *n*-C<sub>15</sub>, *n*-C<sub>17</sub> and *n*-C<sub>19</sub> (Goutx and Saliot, 1980; Gogou et al., 2000). Concentrations of these *n*-alkanes

(*n*-C<sub>15</sub>, *n*-C<sub>17</sub> and *n*-C<sub>19</sub>) were very low in both the sedimentary environments. This was probably because phytoplankton lipids are relatively more labile compared to terrestrial plant lipids (Meyers et al., 1984; Gagosian and Peltzer, 1986).

Short chain/long chain ratio for petroleum is 1.0, whereas marine animals, sedimentary bacteria and higher plants show relatively low values (<1.0) while algae and plankton produces >1.0 ratios (Clarck and Blumer, 1967; Gao et al., 2007). The ratio varied from 0.35 to 1.38 and 1.0 to 2.69 in the Mandovi estuary and the Marmugoa harbour sediments, respectively (Table 3). The ratio was < 1.0 for all the sediments (except station 1) sampled in the estuary. Low ratios were observed for the sediments collected from the riverine stations of the estuary probably due to the contribution from terrestrial plant *n*-alkanes (stations 5, 8 and 9) (Table 3). This is probably due to the proximity of these stations to the mangrove vegetation along the banks of the estuary, and/or due to the discharge of land-based effluents. At the station # 1 of the estuary, petroleum based sources appears to contribute more to organic matter than the terrestrial sources. For the harbour sediments the ratio was ≥1.0. This distribution of *n*-alkanes in harbour sediments indicates that they were derived from algal, bacterial and petroleum sources (Han and Calvin, 1969; Wang et al., 2006; Gao et al., 2007).

A CPI value of 1 or near 1 indicates the presence of petroleum derived *n*-alkanes, whereas CPI values of 4 to 10 have been recorded for the terrestrial plants (Pearson and Eglinton 2000; Wang et al., 2003). CPI<sup>a</sup> values varied from 1.0 to 3.2 and 0.3 to 1.1, whereas CPI<sup>b</sup> values varied from 3.4 to 5.7 and 1.6 to

3.7 in the estuary and the harbour sediments, respectively (Table 3). These values strongly support above conclusion that the *n*-alkanes in the estuarine sediments were mostly derived from terrestrial sources with some contribution from biogenic and/or petroleum sources. Conversely, it appears that the *n*-alkanes in the harbour sediments were mostly derived from petroleum sources with some contribution from terrestrial and biogenic sources (Volkman et al., 1992; Medeiros and Bicego, 2004; Gao et al., 2007).

In order to further differentiate the probable sources of organic matter in these sediments  $\sum n\text{-alkane}/n\text{-C}_{16}$ ,  $n\text{-C}_{17}/\text{Pristane}$  and  $n\text{-C}_{18}/\text{Phytane}$ , and  $(\text{Pristane} + \text{Phytane})/n\text{-C}_{17}$  ratios were used. The  $\sum n\text{-alkanes}/n\text{-C}_{16}$  ratio is usually <15.0 for petroleum contaminated samples (Gogou et al., 2000; Ou et al., 2004; Gao et al., 2007). The ratio varied from 14.56 to 148.93 and 6.84 to 38.95 in the estuarine and the harbour sediments, respectively (Table 3). The ratio is generally >15 for the estuarine sediments and <15 for harbour sediments. This again implies that the estuarine sediments were less contaminated from petroleum hydrocarbons than the harbour sediments.

Pristane (2,6,10,14 – tetramethylpentadecane) and phytane (2,6,10,14 – tetramethylhexadecane) are products of geological alteration of phytol and other isoprenoidyl natural products, and are not primary constituents of most terrestrial biota (Didyk et al., 1978; Li et al., 1995; Gao et al., 2007). However, pristane in the marine environment can be contributed by zooplankton and other higher marine animals while phytane is normal component of oil but also can be synthesized by the methanogenic and photosynthetic bacteria (Steinhauer and

Boehm, 1992; Sakata et al., 1997). In immature sediments, pristane and phytane are commonly produced from the phytol side chain of chlorophyll *a* and *b* (Ragan and Chapman, 1978) and therefore can indicate algal source in marine sediments.

The  $n\text{-C}_{17}$ /Pristane,  $n\text{-C}_{18}$ /Phytane and (Pristane + Phytane)/ $n\text{-C}_{17}$  ratios are useful to identify the presence of freshly derived or degraded petroleum hydrocarbons in sediments (Pavlova and Papazova, 2003; Gao et al., 2007) (Table 3). Low ( $<1.0$ )  $n\text{-C}_{17}$ /Pristane,  $n\text{-C}_{18}$ /Phytane ratios imply the presence of degraded petroleum hydrocarbons while higher ratios ( $>1.0$ ) suggest the presence of less degraded or relatively fresh hydrocarbons. The  $n\text{-C}_{17}$ /Pristane and  $n\text{-C}_{18}$ /Phytane ratio varied from 0.7 to 1.7, and 1.2 to 3.6 in the estuarine sediments, and from 0 to 54.1, and 1.0 to 11.5 in the harbour sediments (Table 3). Similarly,  $>1$  and  $<1.0$  (Pristane + Phytane)/ $n\text{-C}_{17}$  ratio indicates the presence of degraded and fresh petroleum hydrocarbons, respectively (UNEP 1995). This ratio varied from 1.3 to 3.1 and 0.2 to 11.3 in the estuary and the harbour sediments, respectively (Table 3). For most of the harbour sediments this ratio was  $<1$  indicating the presence of freshly derived petroleum hydrocarbons at these locations.

#### 3.4. Petroleum biomarkers: hopanes and steranes

In order to further substantiate the influence of petroleum at the sampling sites, qualitative analysis of hopanes and steranes was carried out. These compounds are resistant to environmental alteration. As a result they are often

used as proxies to detect petroleum contamination in recent sediments even after fairly extensive degradation and/or in the presence of biogenic compounds (Bouloubassi et al., 2001; Zaghden et al., 2005; Gao et al., 2007). Ion chromatograms ( $m/z=191$ ) indicate the presence of tricyclic terpanes, tetracyclic terpanes and pentacyclic triterpanes ( $C_{27}$  to  $C_{34}$  carbon atoms) in most of the samples (Table 4). However, the abundance of these compounds was greater in the Marmugoa harbour sediments (Table 4). Furthermore, the predominance of hopanes with configuration  $17\alpha$  (H),  $21\beta$  (H) strongly supports the petrogenic origin of the hydrocarbons in the sediments (Peters and Moldowan, 1993; Prince et al., 1994; Gogou et al., 2000). Similarly, ion chromatograms of steranes ( $m/z=217$ ) showed the presence of many stereoisomers such as steranes  $C_{27}$ -cholestane,  $C_{28}$ -cholestane and  $C_{29}$ -stigmastane with their 20S and 20R epimers in the harbour and the estuarine sediments (Table 4) (Philp, 1985; Zaghden et al., 2005; Gao et al., 2007). Nevertheless, these compounds were relatively more abundant in the harbour sediments confirming the conclusion that petroleum derived *n*-alkanes were more abundant in the harbour sediments.

### 3.5. Origin of hydrocarbons

The sum of *n*-alkanes of terrestrial ( $C_{23}$ ,  $C_{25}$ ,  $C_{27}$ ,  $C_{29}$ ,  $C_{31}$ ,  $C_{33}$ ), phytoplanktonic ( $C_{15}$ ,  $C_{17}$ ,  $C_{19}$  and  $C_{21}$ ), and petroleum origin ( $C_{12}$ ,  $C_{14}$ ,  $C_{16}$ ,  $C_{18}$ ,  $C_{20}$ ) was plotted in order to identify the dominant sources, and the order of their abundance (Fig. 4a and 4b). It was evident that the Mandovi estuary showed a dominance of terrestrial > petrogenic > planktonic *n*-alkanes, whereas the

Marmugoa harbour sediments exhibited an abundance of petrogenic > terrestrial > planktonic *n*-alkanes (Fig. 4a and 4b).

#### **4. Conclusions**

Sedimentary *n*-alkanes in the Mandovi estuary and the Marmugoa harbour were of natural as well as of anthropogenic origin. In the former, the *n*-alkanes were mostly derived from terrestrial higher plants followed by petroleum, phytoplankton, bacteria and algae. In contrast, *n*-alkanes in the harbour sediments were mostly of petroleum origin followed by some contribution from terrestrial higher plants, bacteria, algae and phytoplankton. The observed levels of *n*-alkanes will serve as useful baseline data for the future surveys.

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## Legends:

### Table 1

Spatial distribution of organic carbon (OC), total lipids, total HC, *n*-alkanes and UCM in the Mandovi estuary and the Marmugoa harbour sediments.

### Table 2

Distribution of organic carbon, total lipids and *n*-alkanes in the sediments of various environments.

### Table 3

The *n*-alkane indices in the sediments of the Mandovi estuary and the Marmugoa harbour.

### Table 4

Hopanes and steranes as petroleum biomarkers in the Mandovi estuary and the Marmugoa harbour sediments.

### Fig.1.

Map showing the sediment sampling stations in the Mandovi estuary (a) and the Marmugoa harbour (b).

### Fig.2.

Spatial variation in the UCM/*n*-alkane ratio in the sediments of the Mandovi estuary (a) and the Marmugoa harbour (b).

### Fig.3.

Relative distribution (%) of *n*-alkanes in the sediments of the Mandovi estuary (a) and the Marmugoa harbour (b).

### Fig.4.

Distribution of planktonic (PLK) (sum of C<sub>15</sub>, C<sub>17</sub>, C<sub>19</sub> and C<sub>21</sub>), terrigenous (TER) (sum of C<sub>23</sub>, C<sub>25</sub>, C<sub>27</sub>, C<sub>29</sub>, C<sub>31</sub>, C<sub>33</sub>) and petrogenic (PETRO) (sum of C<sub>12</sub>, C<sub>14</sub>, C<sub>16</sub>, C<sub>18</sub>, C<sub>20</sub>) *n*-alkanes in sediments of the Mandovi estuary (a) and the Marmugoa harbour (b).

Response to reviewers' comments:

Reviewer #1

1. Suggested comment has been included.
2. Suggested sentence has been included.
3. Capillary dimensions have been added.
4. Corrections have been made.
5. Corrections have been made.
6. The units for the table 2 have been included whereas table 3 consists of index values (ratios) which don't have units.

Reviewer #2: ENVINT-D-07-00616

Suggested change has been incorporated



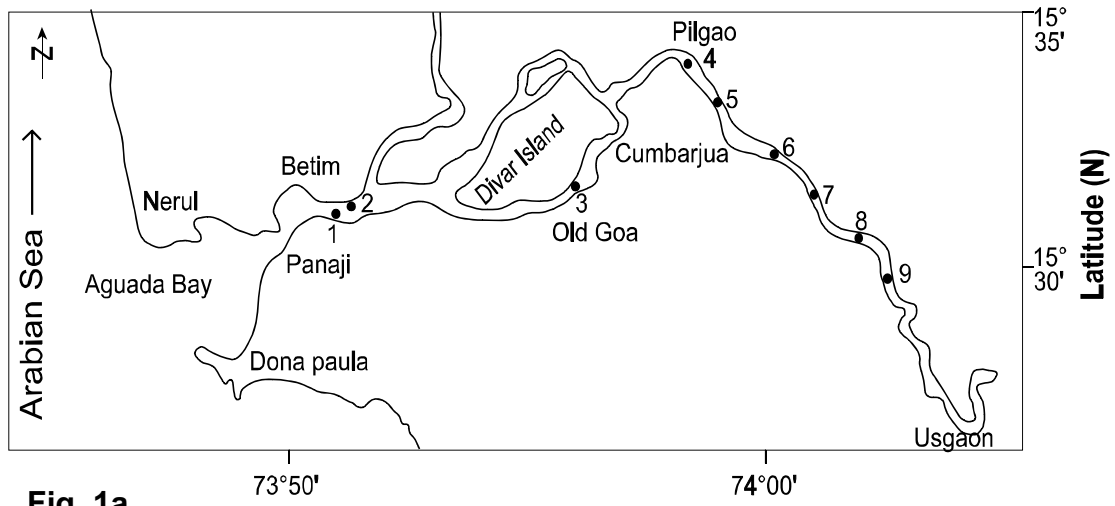


Fig. 1a

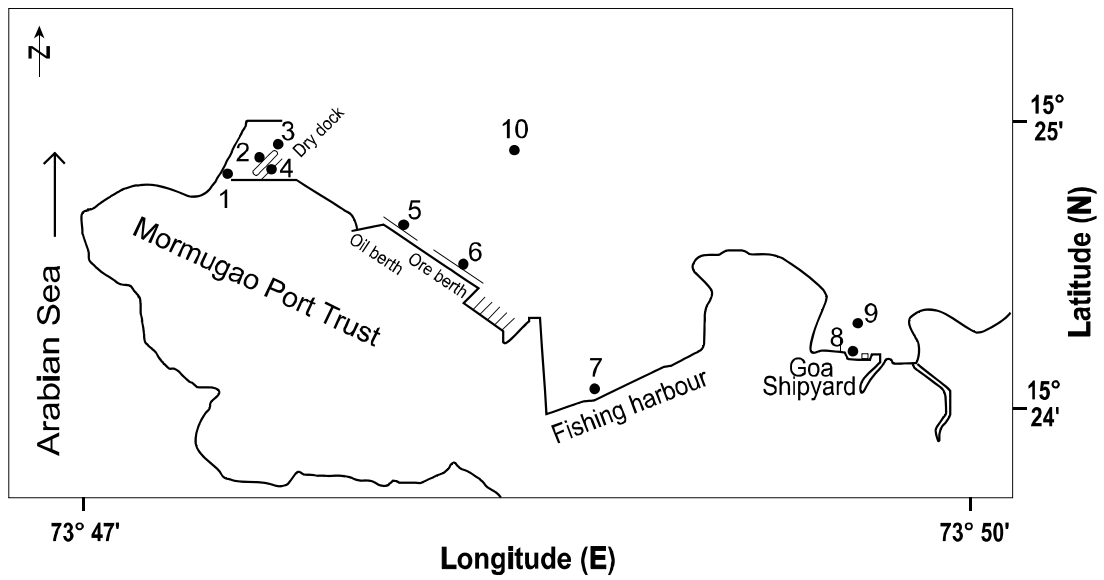


Fig. 1b

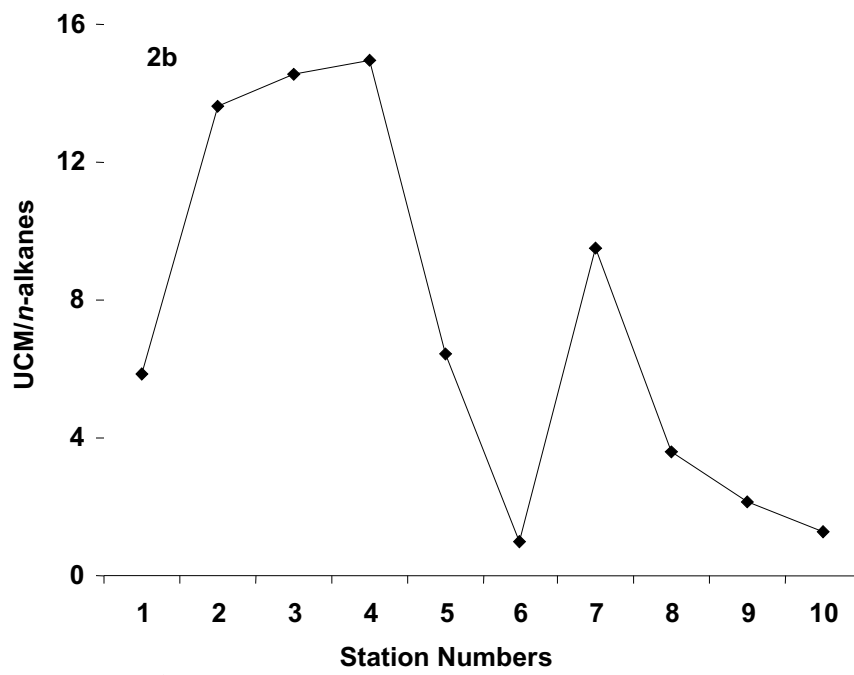
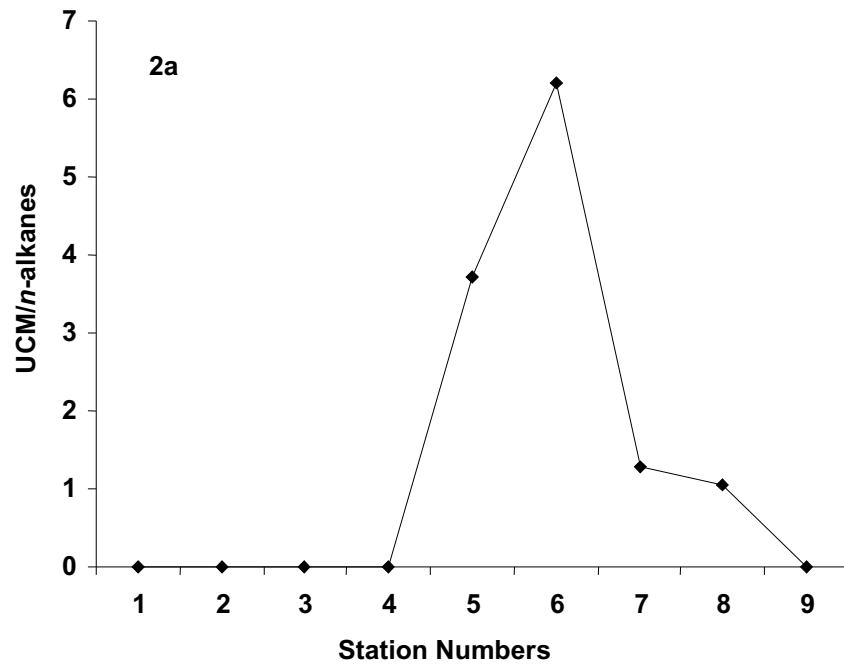
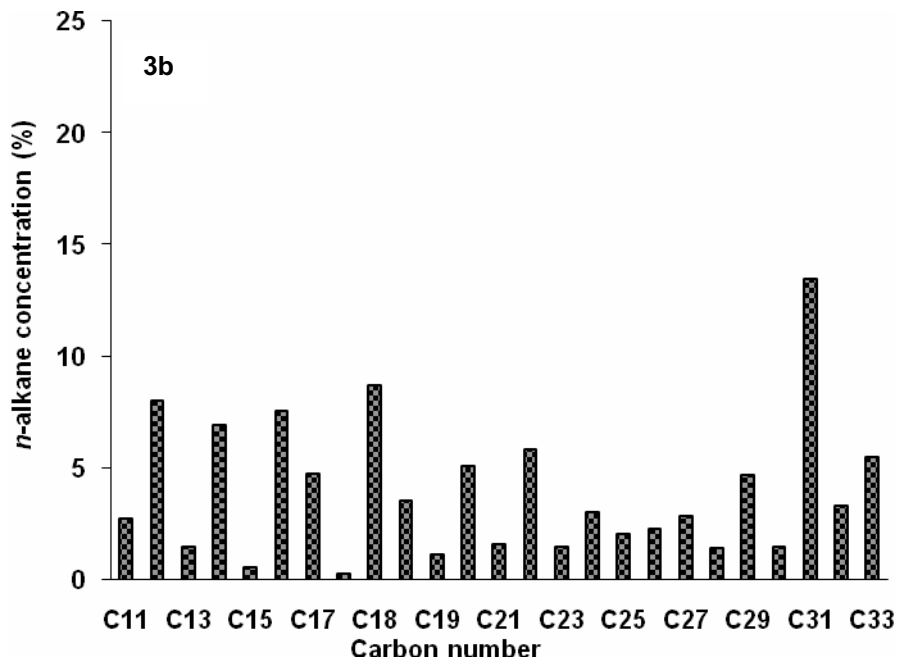
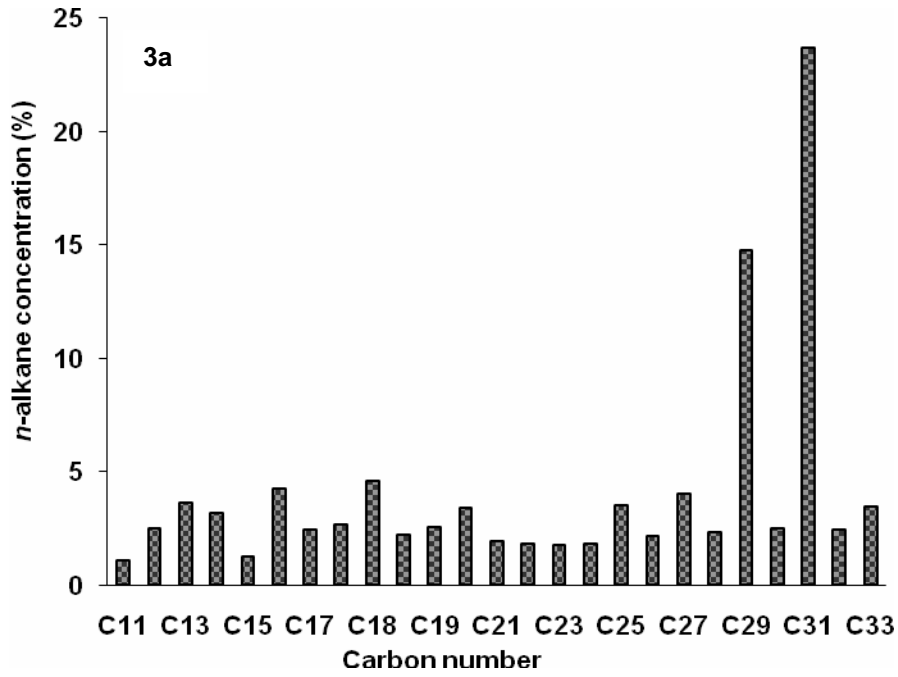
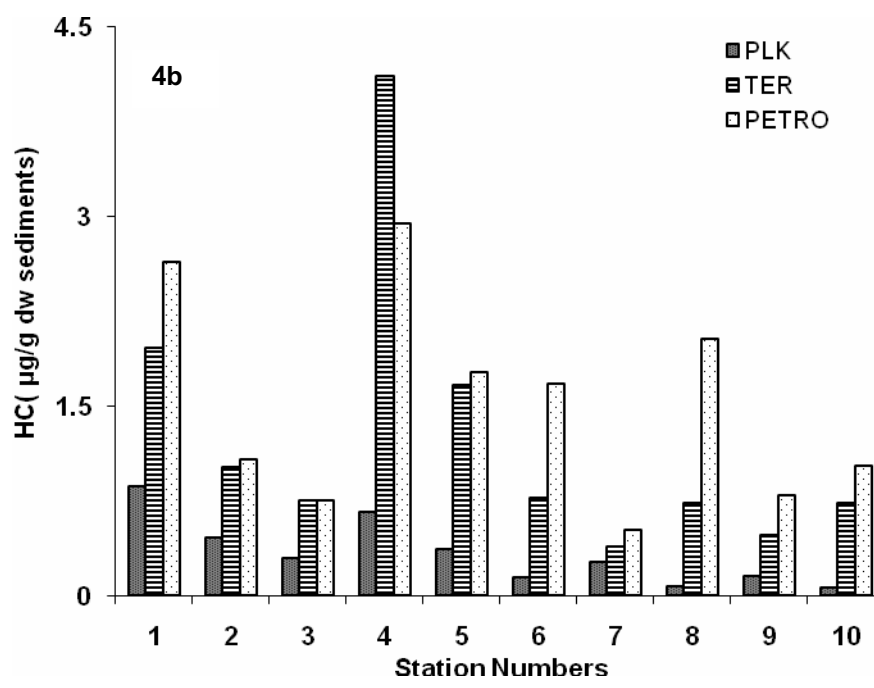
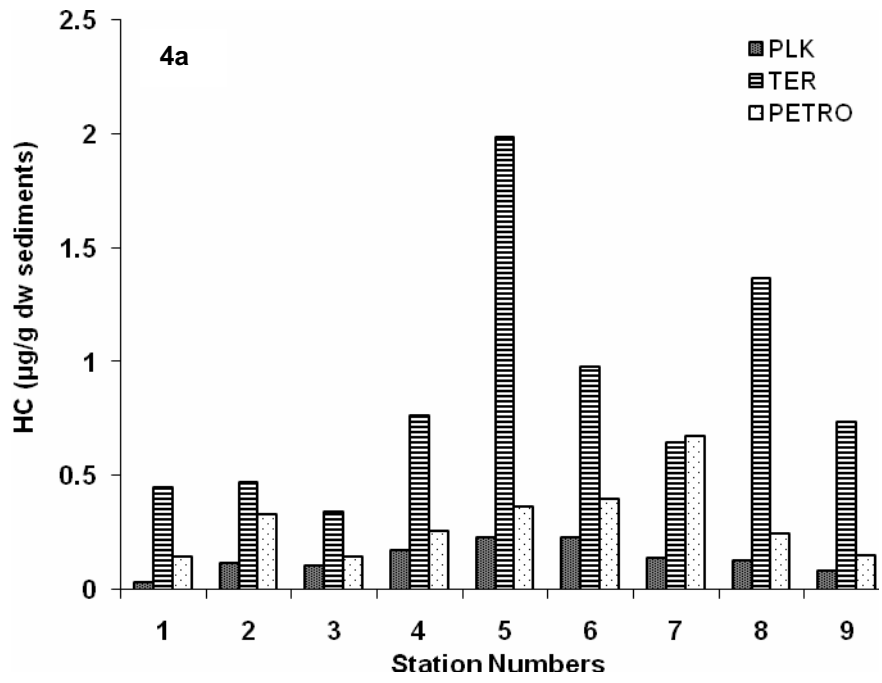


Fig. 2



**Fig. 3**



**Fig. 4**

## Potential Reviewers

Potential Reviewers:

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Table 1

Sampling sites		Sampling period	OC (%)	Total lipids	Total HC	<i>n</i> -alkanes	UCM
				(µg/g dw sediments)			
<b>Mandovi estuary</b>							
#1	Panjim harbour	16-May-01	1.0	597	1.1	1.1	nd
#2	Fish jetty Panaji	16-May-01	1.3	688	1.2	1.2	nd
#3	Old Goa	16-May-01	2.5	176	0.8	0.8	nd
#4	Pilgaon	16-May-01	1.1	736	1.5	1.5	nd
#5	Bicholim Sarmanas	16-May-01	1.7	1413	14.9	3.2	11.7
#6	Amona	16-May-01	2.0	981	14.8	2.1	12.7
#7	Mayana 1	16-May-01	1.6	538	4.4	1.9	2.5
#8	Mayana	16-May-01	2.3	970	4.5	2.2	2.3
#9	Pali	16-May-01	1.2	544	1.2	1.2	nd
<b>Marmugoa harbour</b>							
#1	Harbour Ferry Warf	6-Jun-01	2.7	953	46.8	6.8	40.0
#2	Dry dock lateral left	6-Jun-01	2.9	1448	48.8	3.3	45.4
#3	Dry dock posterior mouth	6-Jun-01	2.6	1232	37.4	2.4	35.0
#4	Dry dock lateral right	6-Jun-01	2.6	846	171.3	10.7	160.5
#5	Oil jetty	6-Jun-01	2.5	746	35.3	4.8	30.5
#6	Ore jetty	6-Jun-01	1.0	233	7.0	3.5	3.6
#7	Fish jetty	6-Jun-01	2.6	700	16.7	1.6	15.1
#8	Goa Shipyard inner	6-Jun-01	2.0	320	20.2	4.4	15.8
#9	Goa Shipyard outer	6-Jun-01	2.5	912	6.0	1.9	4.1
#10	Off harbour	6-Jun-01	0.6	620	6.0	2.6	3.4

nd: not detected

Table 2

Parameters	Concentrations	Locations	References
OC (%)	2.00 0.37 to 1.02 0.10 to 8.96 0.06 to 0.94 0.10 to 0.70 0.26 to 1.68 1.00 to 2.50 0.60 to 2.90	Eastern Lena Delta, New Siberian Islands Pearl River estuary, USA Jiaozhou Bay, China St Lawrence estuary, Quebec, Canada Yangtze Estuary, China Pearl River estuary, USA Mandovi estuary, India Marmugoa harbour, India	Fahl and Stein, 1997 Peng et al., 2005 Wang et al., 2006 Michaud and Pelletier, 2006 Zhou et al., 2007 Gao et al., 2007 Present work Present work
Lipids ( $\mu\text{g/g}$ dw sediments)	3500 40 to 1240 1700 to 9100 18.70 to 38.58 30 to 391 176 to 1413 233 to 1448	Coastal sediment, Egypt Gulf of Fos, France Sfax coastal zone, Tunisia Pearl River estuary, USA San Francisco Bay, USA Mandovi estuary, India Marmugoa harbour, India	Aboul-Kassim and Simoneit, 1996 Hostettler et al., 1999 Zaghden et al., 2005 Gao et al., 2007 Mille et al., 2007 Present work Present work
<i>n</i> -alkanes ( $\mu\text{g/g}$ dw sediments)	2.20 to 11.82 1.60 to 20.60 0.03 to 4.77 0.20 to 7.50 0.54 to 8.12 3.43 to 8.46 2.18 to 429.5 7.80 to 180 0.80 to 3.20 1.60 to 10.70	Changjiang estuary, China Fraser River Basin, Canada Sao Sebastiao, Brazil Patos lagoon estuary, Brazil Jiaozhou Bay, China Pearl River estuary, USA Sfax coastal zone, Tunisia Gulf of Fos, France Mandovi estuary, India Marmugoa harbour, India	Bouloubassi et al., 2001 Yunker et al., 2003 Medeiros and Bicego, 2004 Medeiros et al., 2005 Wang et al., 2006 Gao et al., 2007 Zaghden et al., 2007 Mille et al., 2007 Present work Present work

Table 3

Sampling sites	Short chain/long chain <i>n</i> -alkanes	CPI <sup>a</sup>	CPI <sup>b</sup>	$\sum n\text{-alk}/n\text{-C}_{16}$	<i>n</i> -C <sub>17</sub> /Pri	<i>n</i> -C <sub>18</sub> /Phy	(Pri+Phy)/ <i>n</i> -C <sub>17</sub>
<b>Mandovi estuary</b>							
#1	1.01	3.2	4.6	148.9	1.0	3.6	2.7
#2	0.97	1.4	4.1	33.5	1.3	2.5	1.9
#3	0.85	1.8	3.4	32.0	0.8	1.7	2.2
#4	0.59	2.0	4.1	24.8	1.7	2.3	1.7
#5	0.31	2.8	4.8	20.4	1.2	2.1	1.3
#6	0.69	1.8	4.3	14.6	0.7	1.2	2.2
#7	1.38	1.0	3.8	19.6	0.7	2.8	3.1
#8	0.35	3.1	5.7	26.3	0.8	1.4	1.9
#9	0.39	2.8	5.3	32.6	0.9	1.8	1.7
<b>Marmugoa harbour</b>							
#1	1.56	0.9	2.8	18.2	23.5	6.8	0.2
#2	1.15	0.9	1.9	14.7	54.1	11.5	0.2
#3	1.21	1.1	2.3	16.7	47.5	6.1	0.2
#4	1.00	1.1	3.3	38.9	0.4	1.5	0.8
#5	1.12	0.9	2.9	8.4	4.0	2.1	0.9
#6	2.12	0.4	2.2	8.8	0.1	2.5	1.8
#7	2.17	0.9	3.7	13.6	0.2	1.0	0.9
#8	2.69	0.3	1.6	7.3	0.8	1.8	11.3
#9	1.91	0.7	2.6	18.4	6.5	10.2	0.4
#10	1.31	0.6	1.9	6.8	0.03	1.4	9.1

Short chain: *n*-alkanes < C<sub>22</sub> carbon number; long chain: *n*-alkanes > C<sub>23</sub> carbon number;

CPI<sup>a</sup>: carbon preference index in the range C<sub>11</sub>-C<sub>33</sub>; CPI<sup>b</sup>: carbon preference index in the range C<sub>23</sub>-C<sub>33</sub>

*n*-alk/*n*-C<sub>16</sub>: ratio of sum of *n*-alkanes (C<sub>11</sub> - C<sub>33</sub>) and *n*-C<sub>16</sub>

*n*-C<sub>17</sub>/Pri: ratio of *n*-C<sub>17</sub> and pristane; *n*-C<sub>18</sub>/Phy: ratio of *n*-C<sub>18</sub> and phytane; (Pri+Phy)/*n*-C<sub>17</sub>: ratio of Pristane+Phytane and *n*-C<sub>17</sub>



Table 4

Hopanes/Steranes	MF	MW	Marmugoa harbour sampling sites						Mandovi estuary sampling sites			
			# 1 %	# 4 %	#6 %	#7 %	#8 %	#9 %	# 4 %	# 5 %	#7 %	#9 %
Tricyclic terpanes												
C <sub>23</sub> -Tricyclic terpane	C <sub>23</sub> H <sub>42</sub>	318	-	-	-	-	+	-	-	-	-	-
Tetracyclic terpanes												
17,21-seco-hopane	C <sub>24</sub> H <sub>42</sub>	330	-	-	-	-	+	-	-	-	-	-
Pentacyclic triterpanes or hopanes												
18α(H)-22,29,30-Trisnorneohopane	C <sub>27</sub> H <sub>46</sub>	370	-	-	-	-	-	-	-	-	-	-
17β(H),18α(H),21α(H)-28,30-Bisnormoretane	C <sub>28</sub> H <sub>48</sub>	384	-	-	-	-	-	-	-	+	-	-
17α(H),21β(H)-30-Norhopane	C <sub>29</sub> H <sub>50</sub>	398	+	+	+	+	-	+	+	+	-	-
17α(H),21β(H)- Hopane	C <sub>30</sub> H <sub>52</sub>	412	+	-	-	+	+	+	+	+	-	-
17α(H),21β(H)- 30-Homohopane(22S)	C <sub>31</sub> H <sub>54</sub>	426	+	-	-	+	+	-	+	+	+	+
17α(H),21β(H)- 30-Homohopane(22R)	C <sub>31</sub> H <sub>54</sub>	426	+	-	-	+	+	-	+	-	+	-
17α(H),21β(H)- 30,31-Bishomohopane(22S)	C <sub>32</sub> H <sub>56</sub>	440	+	-	-	+	+	+	+	-	+	+
17α(H),21β(H)- 30,31-Bishomohopane(22R)	C <sub>32</sub> H <sub>56</sub>	440	+	-	-	+	+	+	+	-	+	-
17α(H),21β(H)- 30,31,32-Trishomohopane(22S)	C <sub>33</sub> H <sub>58</sub>	454	+	-	-	+	+	+	+	-	-	-
17α(H),21β(H)- 30,31,32-Trishomohopane(22R)	C <sub>33</sub> H <sub>58</sub>	454	+	-	-	+	+	-	+	-	-	-
17α(H),21β(H)- 30,31,32,33-Tetrakishomohopane(22S)	C <sub>34</sub> H <sub>60</sub>	468	-	-	-	+	-	-	-	-	-	-
17α(H),21β(H)- 30,31,32,33-Tetrakishomohopane(22R)	C <sub>34</sub> H <sub>60</sub>	468	+	-	-	+	+	+	-	-	-	-
Regular steranes												
5α(H),8α(H),14β(H)-Cholestane (20S)	C <sub>27</sub> H <sub>48</sub>	372	+	-	-	+	+	-	+	-	-	-
5α(H),8α(H),14β(H)-Cholestane (20R)	C <sub>27</sub> H <sub>48</sub>	372	+	-	-	+	+	-	+	-	-	-
24-Methyl-5α(H),14β(H),17β(H)-Cholestane (20S)	C <sub>28</sub> H <sub>50</sub>	386	+	-	-	-	+	-	+	-	-	-
24-Methyl-5α(H),14β(H),17β(H)-Cholestane (20R)	C <sub>28</sub> H <sub>50</sub>	386	+	-	-	+	+	-	+	-	-	-
5α(H),14α(H),17α(H)-Stigmastane(20S)	C <sub>29</sub> H <sub>52</sub>	400	+	-	-	+	+	-	+	-	-	-
5α(H),14α(H),17α(H)-Stigmastane(20R)	C <sub>29</sub> H <sub>52</sub>	400	+	-	-	-	+	-	+	-	-	-

MF: molecular formula; MW: molecular weight; +: present; -: absent