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Identification of sources of tar balls deposited along the Goa coast, India, using fingerprinting techniques

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Abstract

Deposition of tar balls along the coast of Goa, India is a common phenomenon during the southwest monsoon. Representative tar ball samples collected from various beaches of Goa and one Bombay High (BH) crude oil sample were subjected to fingerprint analysis based on diagnostic ratios of n-alkane, biomarkers of pentacyclic tri-terpanes and compound specific stable carbon isotope ($\delta^{13}\text{C}$) analysis to confirm the source. The results were compared with the published data of Middle East Crude Oil (MECO) and South East Asian Crude Oil (SEACO). The results revealed that the tar balls were from tanker-wash derived spills. The study also confirmed that the source is not the BH, but SEACO. The present study suggests that the biomarkers of alkanes and hopanes coupled with stable carbon isotope analysis act as a powerful tool for tracing the source of tar balls, particularly when the source specific biomarkers fail to distinguish the source.

Key words: Tar balls, fingerprint, biomarkers, alkanes, hopanes, $\delta^{13}\text{C}$

1. Introduction

Goa, situated along the central west coast of India, is one of the major global tourist destinations because of its attractive beaches with essential recreational activities. The economy of Goa primarily depends on tourism. However, the Goa coast is often under the menace of tar balls, which has become a common phenomenon during the southwest monsoon season (**Dhargalkar et al., 1997**). During August 2010, most of the Goa beaches were affected by tar ball deposition, whereas, in May 2011, the tar ball deposit was found only along the Mandrem beach, north Goa. This deposition attracts a huge public concern as the on-going recreational activities on beaches get seriously affected, which in turn affects the tourism industry, and the Government of India is concerned about this environmental problem. Moreover, the tar ball being a carcinogenic, it affects the marine life and tar ball pollution degrades health of the coastal waters off Goa. Therefore, tracing of the sources of these tar balls is highly important and essential. In this context, as a part of the scientific project, 'Finger-printing of oil spills and tar balls along the Indian coast', the present study has been carried out.

1.1. Recent techniques in source identification of tar balls

Several studies have been conducted worldwide for tracing the source of tar balls deposited along the beaches since 1970s. However, there is no single method that can fully fingerprint the oil spills. Fingerprint analysis mainly depends on the type of oil spilled, ambient prevailing weather and oceanographic conditions and weathering processes. Spreading and evaporation are the initial processes for weathering to start, followed by dissolution, biodegradation, photooxidation, emulsification and sedimentation, and therefore, properties of the spilled crude oil alter significantly. Pristane ($C_{19}H_{40}$) and Phytane ($C_{20}H_{42}$) are the most abundant isoprenoid compounds in oil and have been widely used for the estimation of degree of oil bio-degradation in the environment (**Wang and Stout, 2007**). The quantitative comparisons of the following ratios: heptadecane/pristane (C_{17}/Pr), octadecane/phytane (C_{18}/Ph), pristane/phytane (Pr/Ph) and heptadecane/octadecane (C_{17}/C_{18}) were found to be useful in identification of unknown source of tar balls as these ratios are less affected by the weathering of oil (**Zafirou et al., 1972**). These ratios were used to identify the sources of tar balls deposited along the Saudi coast of Arabian Gulf after the oil spill at Nowruz oil field (**Fayad, 1986**). Biomarkers were established in identification of petroleum-derived contaminants in the marine environments (**Bence et al., 1996; Boehm et al., 1997; Kvenvolden et al., 1995; Volkman et al., 1997; Wang et al., 1994a, 1994b, 1999; Zakaria et al., 2000, 2001**). These biomarkers were hydrocarbon groups normally present in petroleum and its products, and are used for chemical fingerprinting because they preserve most of the carbon skeleton of the original product. The terpane and sterane biomarkers are less degradable and undergo less weathering as compared to n-alkanes and isoprenoid hydrocarbons (**Volkman, 1984**). The fingerprinting of terpane and steranes is a powerful tool for tracking the source of long-term weathered oil. Christensen et al (2007) reviewed the practical aspects of chemometrics for oil spill fingerprinting and provided a basis for the use of chemometric

methods in tiered oil spill fingerprinting. Biomarker compounds such as isoprenoid alkanes, hopanes and polycyclic aromatic hydrocarbons (PAHs) have been proposed as molecular markers to identify the sources of oil pollution. The diagnostic ratios of alkanes, hopanes and polycyclic aromatic hydrocarbons were used to categorize the sources and weathering of tarballs deposited along the Malaysian beaches. Low molecular weight/high molecular weight ratios (L/H) of both alkanes and PAHs together are useful in categorizing the weathering effects of tar balls (**Chandru et al., 2008**). However, in cases such as the diagnostic ratios of biomarkers are unable to distinguish the source, or when the samples are formed due to mixing of different crude oils, the GC and GC/MS data fingerprint techniques could be, therefore in such conditions, the compound specific isotope analysis can be a useful tool to identify or to differentiate the different samples particularly for the sample that have undergone extensive weathering, sample having the absence of biomarker compounds (steranes and terpanes); and the products have the similar chromatograms (Mansuy et al., 1997, Yim et. al., 2012). When diagnostic ratios of biomarkers fail to distinguish the source or when different crude oils are mixed with, the normal fingerprint techniques may be ambiguous and inconclusive. In such situations, the compound specific isotope analysis will be a useful tool to identify or differentiate different samples, particularly the samples that have undergone extensive weathering, samples in which biomarker compounds such as steranes and terpanes are absent and the products having similar chromatograms (Yim et. al., 2012). The $\delta^{13}\text{C}$ values of individual n-alkanes are unaffected by the weathering processes and can be used as a potential tool to identify the source of an oil spill, especially in the case of spills that have low concentration of biomarkers (Li et al., 2009). Mazeas et al (2002) studied the oil residues, oil bird feathers and tar balls that are deposited along the Atlantic coast of France after the Erika oil spill. He used alkane, PAH quantification in addition to the bulk and molecular $^{13}\text{C}/^{12}\text{C}$ ratio measurements. Bulk stable carbon isotopic composition confirmed the source of north Atlantic coast samples as Erika oil spill, whereas these values are higher for the samples collected along the southern part of the coast. If carbon isotopic profiles of different samples have similar appearance it shows that all the samples have come from a single source, whereas, if each sample has individual isotopic profile, that shows that they are from independent sources. Based on the carbon isotopic profiles, Mazeas et al., 2002 confirmed that oil residues deposited along the Pereire beach have the same isotopic profile as tar balls that were collected along the Salie beach. He also found that sample of Crohot beach and small tar ball sample collected from the Salie beach shows individual isotopic profiles, indicating that they have originated from independent sources. Mazeas et al., 2001 described the new analytical procedure to measure the stable carbon isotopic composition of polycyclic aromatic hydrocarbons (PAHs) in petroleum and sediments. He applied this procedure to sediment samples that are contaminated by the Erika tanker spill and confirmed that its contamination is due to the Erika oil spill. Betti et al., (2011) also provided a review of uses of isotope analysis: a tool to track the source and levels of chemical and biological contamination and tracking the organic pollutants.

The present study primarily aims at applying the fingerprint techniques, such as diagnostic ratios of alkanes and triterpanes coupled with compound specific isotope analysis, on the tar balls that are deposited along the Goa coast, in an attempt to identify the source of tar balls. The results are compared with the available MECO and SEACO database and the results of BH sample analysis. This is the first study on fingerprinting of tar balls in the coastal waters of India.

2. Methodology

2.1. Sample collection

Altogether six tar ball samples, representing five locations on Goa beaches and one BH Crude oil – assuming it as the possible source, were used for analysis in the present study. Four tar ball samples were collected from Candolim and Calangute beaches in the north Goa and Colva and Benaulim beaches in the south Goa, whereas, two samples were collected from the Mandrem beach in the north Goa. **Fig. 1a** shows the international tanker route and the location of BH. **Fig.1b** shows the location of tar balls sampling along the Goa coast during 30-31 August 2010 and on 20 May 2011, and the closest tanker route across the Arabian Sea. Tar ball samples were collected by hand using plastic hand gloves, packed with aluminium foil and kept in labelled zip lock bags, and transferred to the laboratory for further analysis.

2.2. Chemicals

Authentic standards for alkanes and PAH were purchased from Sigma Aldrich, USA, and hopane standard 17 β (H)21 α (H)-hopane (C₃₀17 β) was purchased from Chiron, Norway. A 100-200 mesh silica gel was baked at 400°C for 4 hours, cooled, then activated at 200°C overnight and deactivated with 5% of distilled water. Organic solvents were distilled in glass before use. All glassware were washed with detergents and tap water and then rinsed with methanol, acetone and hexane.

2.3. Extraction and Fractionation

BH crude oil and the collected tar ball samples were precisely weighed as 22.5mg and 35mg, respectively and dissolved in excess n-hexane and transferred to 5% H₂O deactivated silica gel column (1cm i.d x 9cm). Hydrocarbons ranging from alkanes to PAH were eluted with 20 mL of DCM/hexane (1:3 v/v); 5 gm of activated copper was added to the collected elute and allowed to stand overnight to react with sulphur. Then the copper was removed and the sample was subjected to roto-evaporation to decrease the volume to 2 mL. The sample was then transferred to the fully activated silica gel column (0.47cm i.d x 18 cm). Two fractions were eluted. The first fraction was eluted with 4 mL of hexane containing the aliphatic and alicyclic hydrocarbons (Aliphatic hydrocarbon Fraction). The second fraction was eluted with 16 mL of hexane/DCM (3:1, v/v) containing the higher molecular weight PAHs (Aromatic Fraction). These two fractions were evaporated to dryness under the

nitrogen blow down and rinsed with DCM HPLC grade and transferred to 1.5mL ampule, and added with 200 μ L isoootane for further GC-MS analysis.

2.4. Gas Chromatography-Mass spectroscopy (GC/MS) analysis

Analyses for *n*-alkane, PAHc and hopanes were performed on a Shimadzu QP5050A gas chromatograph and mass spectrometer with a Shimadzu AOC-20i autosampler. A 30m \times 0.25mm id (0.25 μ m film thickness) fused silica capillary column was used. The carrier gas used was helium (1mL/min). The injector and detector temperatures were set to 230°C and 300°C, respectively for alkane and PAH analysis, whereas, they were 300°C and 230°C for hopane analysis. The oven temperature program for alkane and PAHs was: 2 min hold at 70°C; ramp to 150°C at 30°C/min; and ramp to 310°C at 4°C /min and finally a 15 min hold at 310°C. The oven temperature program for hopane analysis was 2 min hold at 5°C; ramp to 300°C at 6°C/min; and finally a 15 min hold at 300°C.

The *n*-alkanes, pristane and phytane were identified by comparing first the retention time with authentic standards within a range of +/- 0.1 min, and then, the mass spectra with authentic standards and National Institute of Standards and Technology (NIST) spectral reference library, and finally, the particular ratios of several relatively abundant ions. The mode of analysis was electron ionization (EI) Scan mode with 70 eV. The absolute quantification method with external standard has been employed for quantification of individual compounds. The pentacyclic tri-terpenoids present in the aliphatic hydrocarbon fraction were determined with electron ionization (EI) SIM mode with 70 eV by monitoring *m/z* 191. The 17 β (H)21 α (H)-hopane (C₃₀17 β) was identified by comparing retention time with a authentic standard. Other hopanes were confirmed by comparison of their retention time with the authentic hopane standard 17 β (H)21 α (H)-hopane and mass spectra from published data. The quantification of pentacyclic tri-terpenoids was relative to the response of *m/z* 191 of 17 β (H)21 α (H)-hopane. The response factor of individual hopanes relative to the standard was assumed to be 1.0.

Selected PAHs were analyzed with electron ionization (EI)-SIM mode with 70 eV from aromatic fraction. PAHs were monitored for Dibenzothiophene(*m/z*-146), Phenanthrene(*m/z* -178), 3-Methyl-Phenanthrene(*m/z*-192), 2-Methyl-Phenanthrene(*m/z*-912), 9-Methyl-Phenanthrene(*m/z*-192), 1-Methyl-Phenanthrene(*m/z*-192), 2-Methyl Anthracene(*m/z*-192), Fluoranthene(*m/z*-202), Pyrene, (*m/z*-202), 1-MethylPyrene(*m/z*-216), Chrysene (*m/z*-228), Benzo(k)fluoranthene (*m/z*-252), Benzo(e)Pyrene (*m/z*-252) and Benzo(a)Pyrene (*m/z*-252). The absolute quantification method with external standard (EPA 16 PAH) was employed for quantification of individual compounds. Reproducibility of the whole analytical procedure was checked through triplicate analysis of BH. Relative standard deviation of the individual alkanes and hopane standard 17 β (H)21 α (H) concentration were less than 10% and 15%, respectively.

2.5. Gas Chromatography-Isotope Ratio Mass Spectroscopy (GC/IRMS)

Carbon isotopic ratios of individual n-alkanes have been determined on Thermo Delta V-plus GC-C-IRMS coupled with Trace GC interfaced with oxidation-reduction reactor (980-650°C). The GC was equipped with a DB-5 fused silica column (30m X 0.25mm inner diameter with 0.25 µm film thickness). The oven temperature program was: 1 min hold at 70°C; ramp to 140°C at 10°C/min; again ramp to 300°C at 6°C /min and finally a 30 min hold at 310°C with Helium as the carrier gas. Results were reported in the standard delta notation as per mil (‰) deviations from the VPDB (Vienna pee Dee belemnite). Reported isotope ratios represent average values of duplicate analysis. Instrumental standard deviation for the analysis is <0.3‰ based on repeated analysis of calibrated lab tank with respect to IAEA standards (cellulose).

3. Results and Discussion

3.1. General

Several methods have been developed in the recent past to investigate the oil composition, weathering processes and the source (**Zakaria et al., 2000, 2001; Wang et al., 2003, 2006; Chandru et al., 2007**). Fingerprinting of unknown oil spills and their products using GC/MS is the best method available today to identify the source. In the present study, we have taken 6 representative tar ball samples and one crude oil sample for specific target analyses, including individual n-alkanes (C₁₂ to C₃₆), isoprenoid hydrocarbons (pristane and phytane) and PAHs to characterize the samples. In addition, the biomarkers such as pentacyclic terpanes and the compound specific stable carbon isotope ratios were also used to fingerprint the source of the tar ball. The data for MECO and SEACO were taken from **Zakaria et al (2000)**.

Tar balls form whenever incidents such as tanker accidents, oil well blow-outs, natural oil seepage and discharge of ballast water and tanker-wash occur. When the spilled oil mixes with sand or any other material by wave action, the tar ball forms and acquires in situ property of the ambient area. The composition of tar ball, therefore, gives primary information on its origin. The Carbon Preference Index (CPI) defined by Bray and Evans (1961) is one such method, which gives information on composition of tar balls. The CPI value is approximately 1.0 for floating tar balls (Zsolnay, 1978) and petroleum oil (Wang et al., 1998). The CPI values of the present samples also range from 1.09 to 1.11 (Table 1), which indicates that the present tar ball samples contain petroleum, and they have floating tar ball characteristics.

3.2. Diagnostic ratios of n-alkanes

It is necessary to find out the weathering effect of tar balls to correlate tar ball properties with the kind of crude oil. For this purpose, Alkanes and PAHs compounds were studied. The ratio L/H (Low Molecular Weight/High Molecular Weight) indicates the relative depletion of Lower Molecular Weight (LMW) compounds relative to the

Higher Molecular Weight (HMW) compounds. If L/H ratio of tar balls is less than that of crude oil, then the higher molecular weight compounds are dominant over the LMW compounds. The L/H-alkane ratios for present tar ball samples are in the range 0.69-0.93 (Table 1), except for Mandrem-1 sample, whereas, for crude oil such as MECO and SEACO (Zakaria et. al, 2000) and BH (one sample, Table 1), L/H ratios range between 3.35 and 4.60, 1.62 and 4.52 and 2.25, respectively. The tar ball samples show very low L/H ratios compared to all crude oils because of depletion of LMW components due to their weathering (evaporation and dissolution). Mandrem-1 has the highest ratio of 3.92, indicating that the residence time of this sample is very less, as it is not affected by weathering. Therefore, Mandrem-1 sample might be of recent origin compared to other samples. If all the tar ball samples have an identical chromatogram, and the diagnostic ratios C_{17} /pristane, C_{18} /phytane and pristane/phytane are approximately the same, it indicates that all the samples have come from the unique source (**Wang et al., 1998**). In the present analysis it was found that the ratios of C_{17} /pristane, C_{18} /phytane, pristane/phytane and C_{17}/C_{18} are 0.98-1.58, 0.93-1.38, 0.73 - 0.81 and 0.76 -0.87, respectively. Thus, we find that not only the diagnostic ratios of all the samples are nearly the same, the respective chromatograms also found to be nearly the same (Fig. 2).

The occurrence of UCM (Unresolved Complex Mixture) in the sample indicates active microbial degradation in the samples (**Teal et al, 1992; Barakat et al., 2001; Dutta and Harayama, 2000; Atlas, 1995; Peters and Moldwan, 1993**). UCM is an envelope/hump that appears in the GC traces between the solvent baseline and baseline of resolved peaks. It is an indicator of the biodegradation or heavily weathering petroleum (Volkman et al., 1997). Booth et al (2007) studied the identification of individual petroleum derived hydrocarbons in unresolved complex mixtures (UCMs) accumulated by mussels. They resolved very narrow peaks using the two dimensional GC coupled with time-of-flight-MS. ToF-MS detects narrow chromatographic peaks very fast upto 1000 full scan spectra s^{-1} . Low L/H ratios for alkanes suggest that samples lost the lower molecular weight components. The absence of UCM suggests neither biodegradation nor severe weathering, but it indicates that the samples lost the lower molecular weight components by processes other than weathering or biodegradation. UCM is not observed in the present tar ball samples (Fig. 2), explaining that the samples are not biodegraded. During long transportation journey, paraffin wax (n-alkanes) of crude oil sticks to the tanker walls. This n-alkane is discharged into the sea during tanker washing. Therefore, tanker derived tar balls are rich in higher molecular weight alkanes (Zakaria et al., 2001). In addition, there were no tanker accidents, oil well blow outs and other significant sources that cause oil spills in the Sea, prior to the tar ball deposition. Therefore, we concluded that the tar balls might have been originated from the tank-wash. Therefore, it is apparent that all the tar ball samples are chemically consistent with similar properties, and have come from the same source. Thus, the absence of UCM and low L/H ratio suggest that the tar balls might have been formed due to tanker wash.

Elmendorf et al. (1994) suggested that the ratio of PAH degradation in the environment decreases with the number of rings and increase in the degree of branches within a homologous series. Further, **Hegazi et al., (2004)** suggested that highly branched PAHs are useful for the identification of original oil present in the marine environment. Petroleum consists of various PAHs, alkylated PAHs and their configured isomers. In the present study, we have monitored only selected 16 EPA priority PAHs. The results of our analysis are presented in **Fig. 3**. The results are in consistent with our n-alkane analysis. Except for the samples collected from Calangute, other tar balls are abundant in LMW components (Dibenzothiophine, Phenanthrene, 3-Methyl-phenanthrene, 2-Methyl-phenanthrene, 9-Methyl-phenanthrene, 1-Methyl-phenanthrene, 2-Methylanthracene, Fluoranthene, Pyrene), suggesting that these samples were weathered slightly, whereas, the Calangute sample weathered severely. However, there still exists a gap in understanding the depletion of HMW components (1-MethylPyrene, Chrysene, Benzo(k)fluoranthene, Benzo(e)pyrene, Benzo(a)pyrene). Detailed study is required to understand this feature observed in the PAH analysis.

3.3. Source Specific Biomarkers-Outcome

Zakaria et al., (2000) showed the characteristics of MECO and SEACO, wherein the $17\alpha(H)21\beta(H)C_{29}$ -norhopane is dominant over $17\alpha(H)21\beta(H)C_{30}$ -hopane in MECO and $17\alpha(H)21\beta(H)C_{30}$ -hopane is dominant over $17\alpha(H)21\beta(H)C_{29}$ -norhopane in SEACO. Similar patterns were observed in the diagnostic ratios of crude oil (**Table 1**). The diagnostic ratios, C_{29}/C_{30} and $\sum C_{31}-C_{35}/C_{30}$ could be the reliable source identifiers. The present tar ball analysis showed that the $17\alpha(H)21\beta(H)C_{30}$ -hopane is abundant than any other compounds in all the samples. The hopane chromatograms for the samples of Calangute, Mandrem-1 and BH are shown in **Fig. 4**. This feature was also observed in the diagnostic ratio of C_{29}/C_{30} . This ratio is much lower for MECO and closer to the SEACO (**Fig.5a**), indicating the SEACO signature. Oleanane is prolific in some of the SEACO (**Sosrowidjojo et al., 1994**). No Oleanane, however, was present in MECO (**Zakaria et al., 2000**). Interestingly, the analysis of present tar ball samples shows significant amount of Oleanane (**Fig. 5c & Table 1**). This also supports the fact that the tar balls must have been originated from the SEACO. However, the BH crude is also abundant in Oleanane, so the source could be BH as well. When the cross-plot diagram (**Fig. 6**) of diagnostic ratios of C_{29}/C_{30} vs $\sum C_{31}-C_{35}/C_{30}$ were compared with the data of MECO and SEACO, it was observed that all the tar ball samples fall under the SEACO category. BH crude oil also falls in this same category.

The source specific biomarker diagnostic ratios that we obtained remain inconclusive as they fail to differentiate between the two crude oils SEACO and BH. Therefore, in order to confirm the source, the tar ball samples were subjected to compound-specific carbon isotope analysis.

3.4. Compound Specific Isotope $\delta^{13}\text{C}$ analysis

The compound specific carbon isotope analysis was carried out using GC/IRMS (Gas Chromatography/ Isotope Ratio Mass Spectrometry). GC/IRMS is a powerful tool to identify the source of the spilled oil. Though our tar ball samples have significant amount of biomarker signatures and also yielded the possible diagnostic ratios, yet we are unable to differentiate the actual source (SEACO or BH). Therefore, we subjected all the six tar ball samples and one BH sample to carbon isotope analysis of individual alkanes. The results showed that the average $\delta^{13}\text{C}$ value of alkanes of all the tar ball samples fall within the range of -28.05 to -30.49, whereas, for BH, the average value is -33.05 (Fig. 7). Thus, we concluded that the present tar balls are not originated from the Bombay High crude oil. Also, **Fig. 7** shows that the tar balls are consistent with each other, indicating that the tar balls have originated from a single source. Therefore, we can now confirm through the cross-plot diagram (**Fig. 6**) that the tar ball samples that are deposited along the Goa beaches during 2010 and 2011 are originated from the SEACO.

4. Conclusions

Based on the diagnostic ratios of n-alkane and hopane analysis, it is concluded that the tar balls that were deposited along the Goa coast during 2010 and 2011 are formed from the tanker-wash derived oil spills and have the characteristics of floating tar balls. The diagnostic ratios of tar balls, BH crude oil and SEACO are nearly the same. Compound Specific Isotope $\delta^{13}\text{C}$ analysis brought out a clear representation that all the tar balls samples are from a single source, but not from BH. The cross-plot of hopane biomarker diagnostic ratios also confirms that the source is SEACO.

The Middle East countries on the north west of the Arabian Sea are rich in oil producing fields. But, there is a good demand for SEACO in these countries (Zakaria et. al, 2001). The SEACO oil is transported to the Middle East countries through the Arabian Sea. It is possible that the tankers that are going to the Middle East with SEACO are likely to clean the tanks in the Arabian Sea. The resulting oil undergoes weathering process, leading to tar ball formation. With the arrival of SW monsoon, all the floating tar balls start moving towards the west coast of India, and eventually deposit on the nearby beaches in consistence with the circulation pattern. The International Maritime Organization (IMO) has totally prohibited deballasting enroute. With strict enforcement of national and international pollution laws and alert vigilance by national authority, it is possible to control the occurrence of tar balls on the Goa coast.

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References

- Atlas, R. M., 1995. Petroleum biodegradation and oil spill bioremediation. *Marine Pollution Bulletin*. 31, 178-182.
- Barakat, A. O., Qion, Y., Kim, M., Kennicutt, M.C., 2001. Chemical characterization of naturally weathered oil residue in arid terrestrial environmental in Al-Alamein, Egypt. *Environmental Science and Technology* 27, 291–310.
- Betti, M., F. Boison, M. Eriksson, I. Tolosa., E. Vasileva., 2011. Isotope analysis for marine environmental studies. *International Journal of Mass Spectrometry*, 307(1-3), 192-199.
- Bence, A. E., Kvenvolden, K. A and Kennicut II, M. C., 1996. Organic geochemistry applied to environmental assessments of Prince William Suond, Alaska, after Exxon Valdez oil spill-a review. *Organic Geochemistry*24, 7-42.
- Bray, E. E., Evans. E.D., 1961. Distribution of n-paraffin's as a clue to recognition of source bed. *Geochim.Cosmochim.Acta* 22, 2.
- Boehm, P. D., G. A. Douglas., W. A. Burns., P.J.Mankiewicz., D.S.Page., and A. E. Bence., 1997. Application of petroleum hydrocarbon chemical fingerprinting and allocation techniques after the Exxon valdez oil spill. *Marine Pollution Bulletin*. 34, 599-613.
- Booth, A. M., Paul, A., Sutton, C., Anthony Lewis, Alastair, C. Lewis, Alan Scarlett, Wing Chau, John Widdows and Steven J Rowland., 2007. Unresolved complex mixtures of aromatic hydrocarbons: thousands of overlooked persistent, bio accumulative and toxic contaminants in mussels. *Environmental Science and Technology*, 41, 457-64.
- Chandru, K., Mohamad Pauzi Zakaria., Sofia Anita., Azadeh Shahbazi., Mahyar Sakari., Pourya Shah poury Bahry., Che Abd Rahim Mohamed., 2008. Characterization of alkanes, hopanes and polycyclic aromatic hydrocarbons (PAHs) in tarballs collected from the east coast of peninsular Malaysia. *Marine Pollution Bulletin*. 56, 950-962.
- Christensen, J.H., and Giorgio Tomasi., 2007. Practical aspects of chemometrics for oil spill fingerprinting. *Journal of chromatography. A*, 1169, 1-22.

- Dhargalkar, V. K., T.W. Kureishy and M.V. Bhandare., 1977. Deposition of tar balls (Oil residue) on beaches along the west coast of India. *Mahasagar: Bulletin of the National Institute of Oceanography*, 10 (3 & 4), 103-108
- Dutta, T.K., Harayama, S., 2000. Fate of crude oil by the combination of photo oxidation and biodegradation. *Environmental Science and Technology* 34, 1500-1505.
- Elmendorf, D. L; Haith, C. E; Douglas, G. S; Prince, R. C; 1994. Bioremediation of Chlorinated and polycyclic aromatic hydrocarbon compounds. In: Hincee, R. E; Leeson, A.E; Semprini, L; Ong, S. K. Lewis Publishers, Ann Arbor, MI, 188-202.
- Fayad, N. M., 1986. Identification of tar balls following the Nowruz oil spill. *Marine Environmental Research* 18, 155-163.
- Hegazi, A. H., J. T. Andersson., M. A. Abu-Elgheit., M. Sh. El-Gayar., 2004. Source diagnostic and weathering indicators of tar ball utilizing acyclic, polycyclic and S-heterocyclic components. *Chemosphere* 55, 1053-1065.
- Kvenvolden, A. K., F.D. Hostettler, P. R. Carlson, J. B. Rapp, C.N. Threlkeld and A. Warden., 1995. Ubiquitous tar balls with a California-source signature on the shrelines of Prince William Sound, Alaska. *Environmental Science and Technolgy*, 2684-2694.
- Li. Y., Yongqiang Xiong., Wanying Yang., Yueliang Xie., Siyuan Li., Yongge Sun., 2009. Compound Specific stable carbon isotopic composition of petroleum hydrocarbons as a tool for tracing the source of oil spills. *Marine Pollution Bulletin* 58, 114-117.
- Mazeas, L. and Budzinski, H., 2001. Polycyclic aromatic hydrocarbon $^{13}\text{C}/^{12}\text{C}$ ratio measurement in petroleum and marine sediments application to standard reference materials and sediment suspected of contamination from the Erikaoil spill. *Journal of chromatography. A*, 923, 165-76.
- Mazeas, L., and Budzinski, H., 2002. Molecular and stable carbon isotopic source identification of oil residues and oiled bird feathers sampled along the Atlantic Coast of France after the Erika oil spill. *Environmental science & technology*, 36, 130-7.
- Mansuy, L., R. Paul Philip and John Allen., 1997. Source Identification of oil spills based on the isotopic composition of individual components in weathered oil samples. *Environmental Science and Technology*, 31, 3417-3425.
- Oulhote. Y; B. Le Bot; S. Deguen, P. Glorennec., 2011. Using and interpreting isotope data for source identification. *Trends in Analytical Chemistry*, 30, 302-312.
- Peters, K. E., Moldwan, M. J., 1993. *The Biomarker guide*. Prentice Hall, New Jersey.
- Sosrowidjojo, I. B., Alexander, R and Kagi, P., 1994. The biomarker composition of some crude oils from Sumatra. *Organic Geochemistry* 21, 303-312.
- Teal, J.M., Farrington, J.W., Burst, K.A., Stegeman, J., Tripp, B.W., Woodin, B., Phinney, C., 1992. The West Falmouth oil spill after 29 years: fate of fuel oil compounds and effects on animals. *Mar. Pollut. Bull.* 24, 607.
- Volkman, J. K, A. T. Revil and A. P. Murray., 1997. Application of biomarkers for identifying sources of natural and pollutant hydrocarbons in aquatic environments, In: R. P. Eganhouse (ed), *Molecular Markers in Environmental Geochemistry*, American Chemical Society, Washington DC, 83-89

- Volkman, J. K., 1984. Biodegradation of aromatic hydrocarbons in crude oils from the Barrow Sub-basin of western Australia. *Organic Geochemistry*, 619-632.
- Wang, Z., Chun Yang., MervFingas., Bruce Hollebone., Un HyukYim and Jae Ryoung Oh., 2007. Petroleum Biomarkerfingerprinting for oil spill characterization and source Identification. In *Oil Spill Environmental forensics*. Zhendi Wang and Scott A. Stout. Academic Press; USA.
- Wang, Z.D., M. Fingas., and K. Li., 1994a. Fractionation of ASMB oil, identification and quantitation of aliphatic, aromatic and biomarker compounds by GC/FID and GC/MSD. *Journal of Chromatography Science*. 32, 361–366 (Part I) and 367–382 (PartII).
- Wang, Z.D, Fingas, M and Sergy. G., 1994. Study of 22- year-old Arrow oil samples using biomarker compounds by GC/MS. *Environmental Science and Technology* 28, 1733–1746.
- Wang, Z., Fingas, M., Michael Landriault., LiseSigouin., Bill Castle., David Hostetter., Dachung Zhang., Brad Spencer., 1998. Identification and linkage of tar balls from the coast of Vancouver Island and Northern California using GC/MS and Isotopic techniques *Journal of High Resolution Chromatography* 21, 383-395.
- Wang, Z.D., Fingas, M., and D. Page., 1999a. Oil spill identification, *Journal of Chromatography* 843, 369–411.
- Wang, Z., Fingas, M., 2003. Development of oil hydrocarbon fingerprinting and identification techniques. *Marine Pollution Bulletin*. 47, 423–452.
- Wang, Z. D., Yang. C., Hollebone. B., Fingas. M., 2006. Forensic fingerprinting of diamondoids for correlation and differentiation of spilled oil and petroleum products. *Environmental Science and Technology* 40, 5636–5646.
- Wang, Z., Stout, S. A., 2007. *Oil Spill Environmental Forensics*. Elsevier, San Diego.
- Yim, U.H., Moonkoo Kim, Sung Yong Ha, Sunghwan Kim, and Won Joon Shim., 2012. Oil spill environmental forensics: the Hebei Spirit oil spill case. *Environmental Science and Technology*, 46, 6431-7.
- Zafiriou. O., Blumer. M., and Myers J., 1972. Correlation of oils and oilproducts. Woods Hole Oceanographic Institution, WHOI, 72-75.
- Zakaria, M.P., A. Horinouchi., S. Tsutsumi., H. Takada., S. Tanabe., and A. Ismail., 2000. Oil pollution in the straits of Malacca, Malaysia: Application of Molecular Markers for source identification. *Environmental Science and Technology* 34, 1189-1196.
- Zakaria, M. P., Tomoaki Okuda and Hideshige Takada., 2001. Polycyclic Aromatic Hydrocarbon (PAHs) and Hopanes in stranded Tar-balls on the coast of Peninsular Malaysia: Applications of Bimarkers for Identifying sources of oil pollution. *Marine Pollution Bulletin*. 42, 1357-1366.
- Zsolnay, A., 1978. The Weathering of tar on Bermuda. *Deep Sea Research*, 25, 245-1252.

Figures

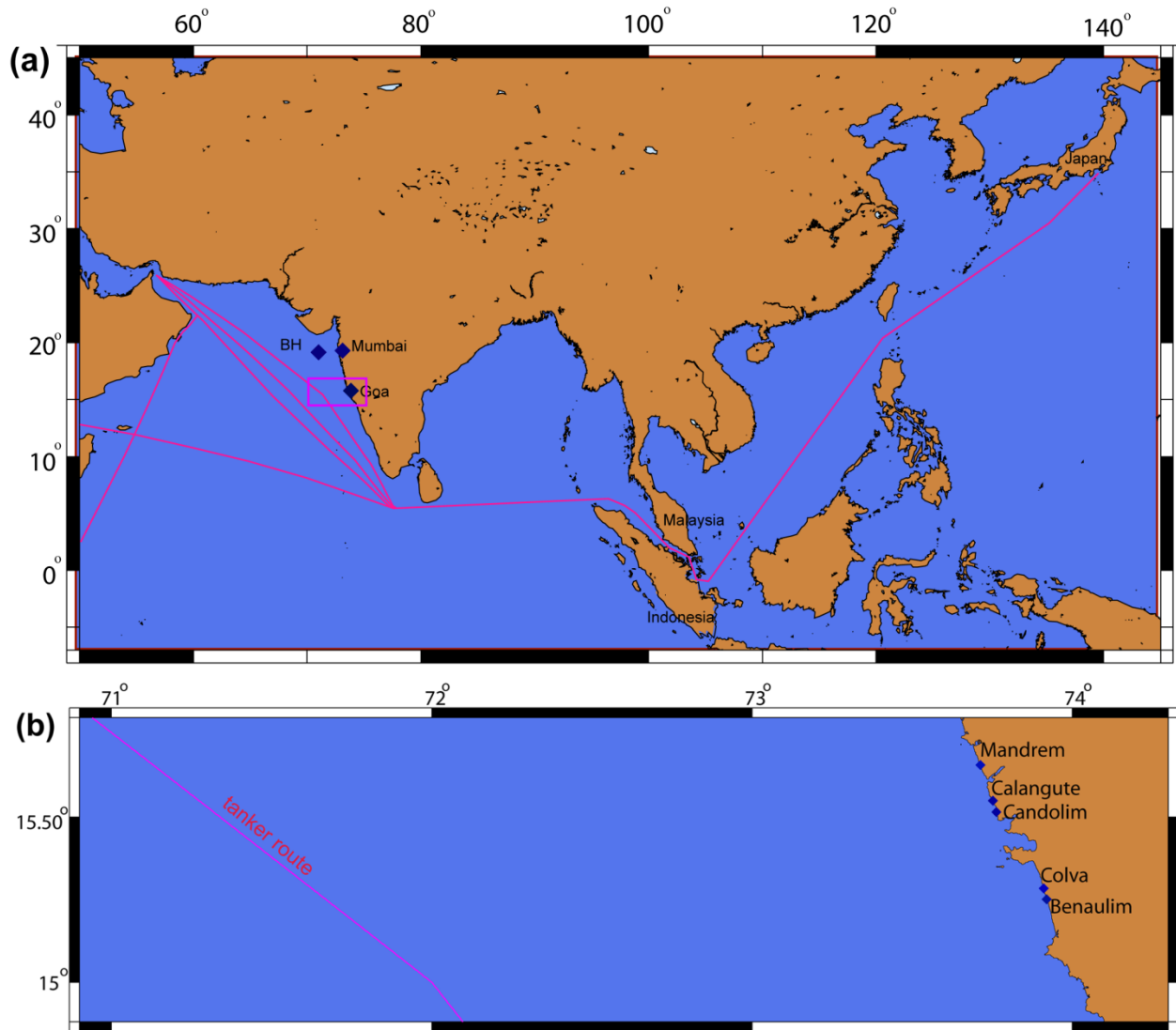


Fig. 1(a). Tanker routes from Middle East to Japan and Bombay High oil rig (BH) in the Arabian Sea (b). Sampling locations along the Goa coast.

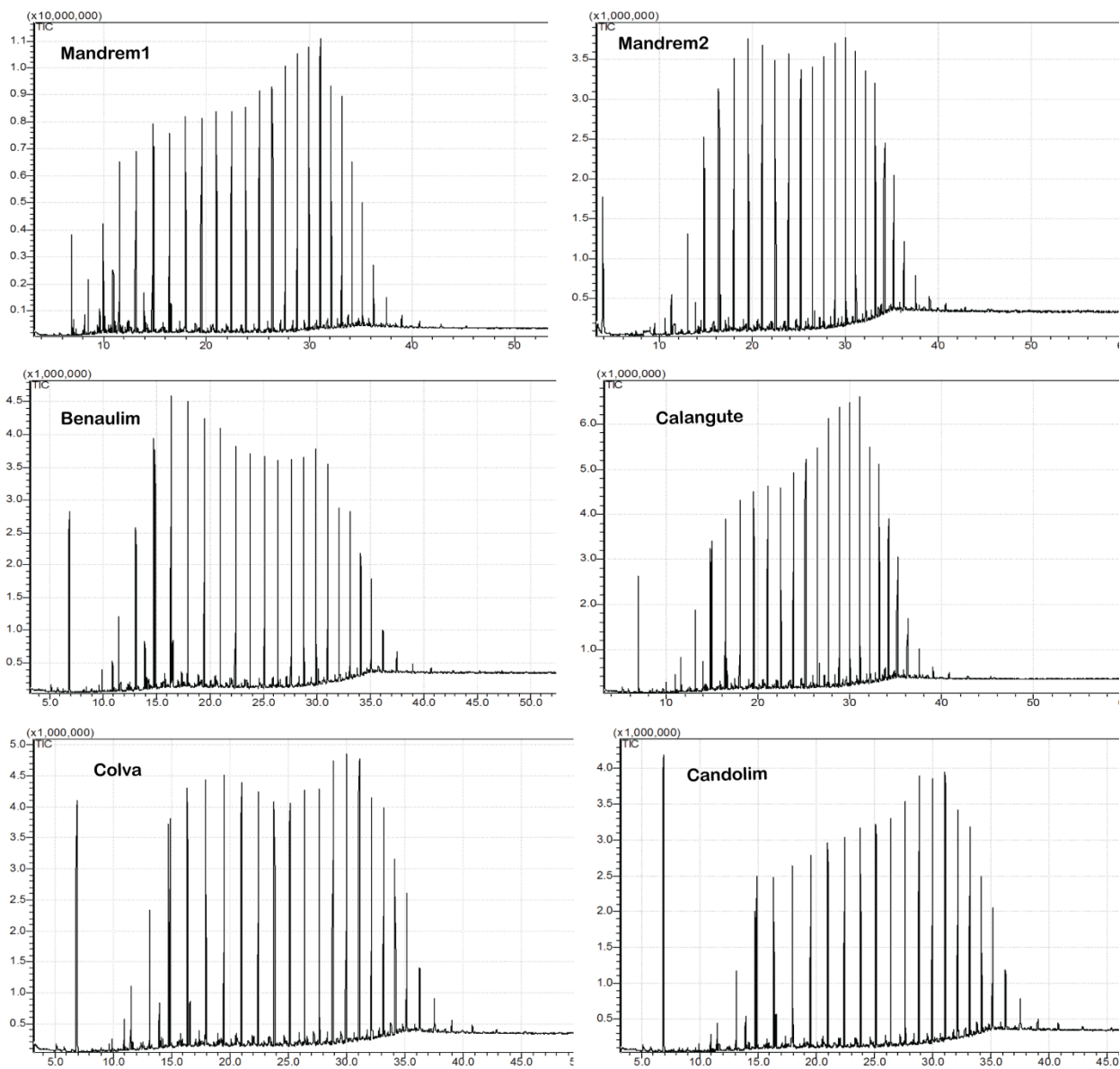


Fig. 2. GC chromatograms for Aliphatic fraction

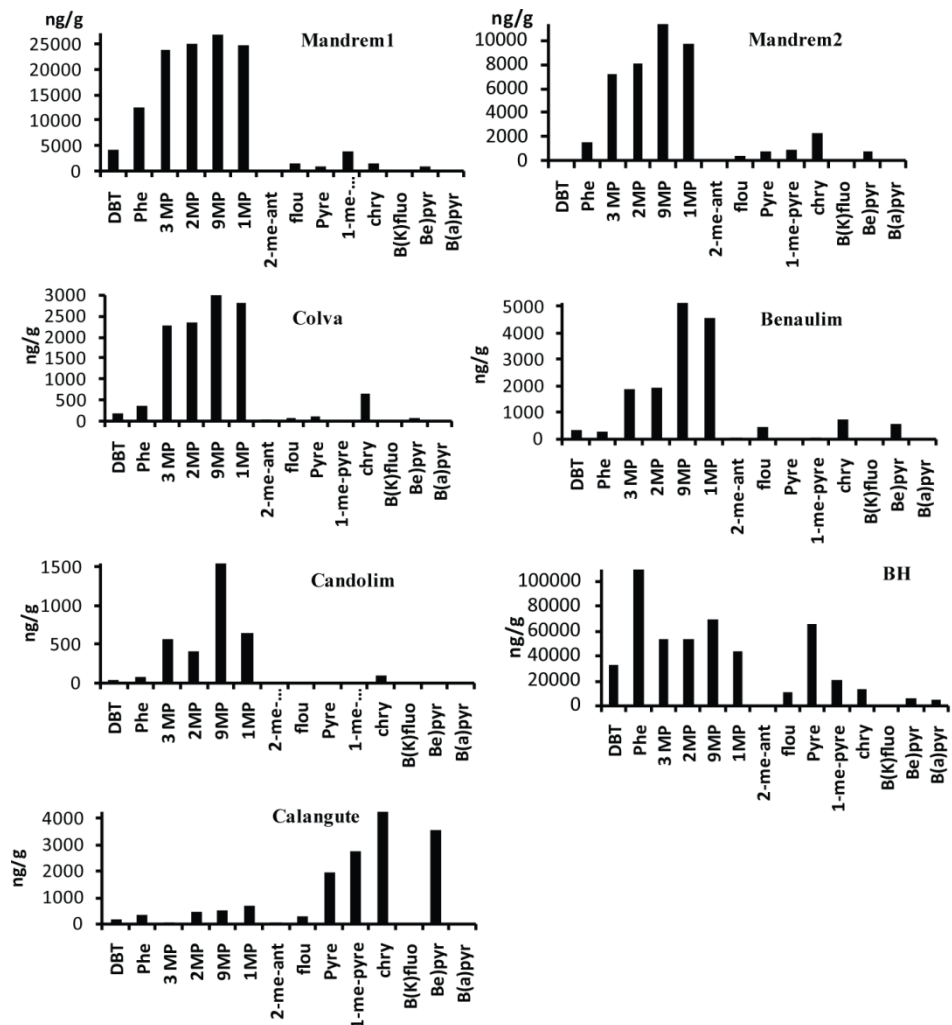


Fig. 3. PAH concentration in tar ball samples and BH crude oil. DBT: Dibenzothiophene, Phe: Phenanthrene, 3-MP: 3-Methyl-Phenanthrene, 2-MP: 2-Methyl-Phenanthrene, 9-MP: 9-Methyl-Phenanthrene, 1-MP: 1-Methyl-Phenanthrene, 2-me-ant: 2-Methyl Anthracene, flou: Fluoranthene, pyre: Pyrene, 1-me-pyre: 1-Methyl Pyrene, chry: Chrysene, B(K)fluo: Benzo(k)fluoranthene, B(e)pyr: Benzo(e)Pyrene, B(a)pyr: Benzo(a)Pyrene

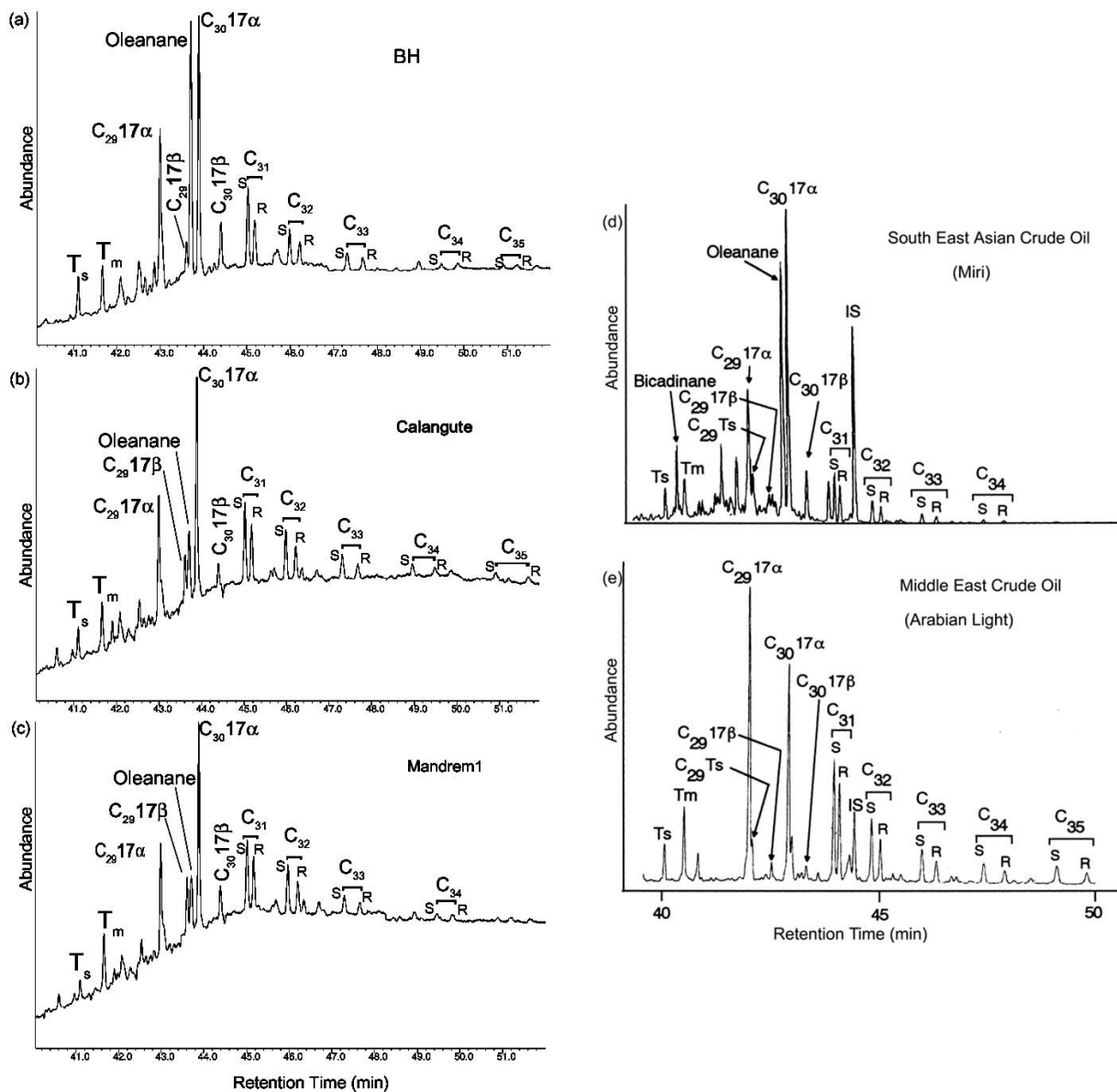


Fig. 4(a), (b), (c), (d) and (e) are the pentacyclic triterpane chromatograms; (a), (b) and (c) are for the present tar ball samples; (d) and (e) are for MECO and SEACO, respectively obtained from Zakaria et al., (2000).

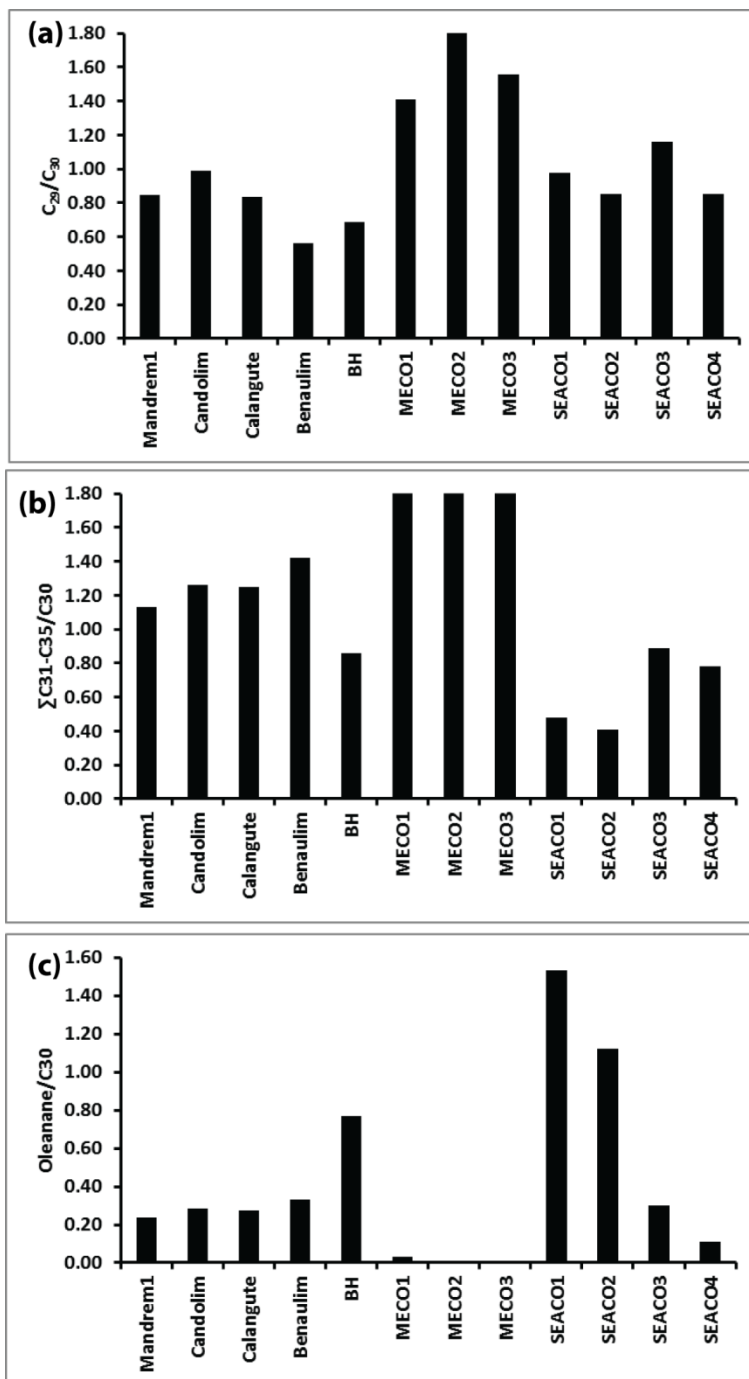


Fig. 5. Pentacyclic triterpane diagnostic ratios for tar ball samples, the MECO and SEACO; (a) C_{29}/C_{30} : ratios of $17\alpha(H)21\beta(H)C_{29}$ hopane to $17\alpha(H)21\beta(H)C_{30}$ -hopane, (b) $\Sigma C_{31-C35}/C_{30}$: sum of homo hopanes ranging from $C_{31}-C_{35}$ to $17\alpha(H)21\beta(H)C_{30}$ -hopane.

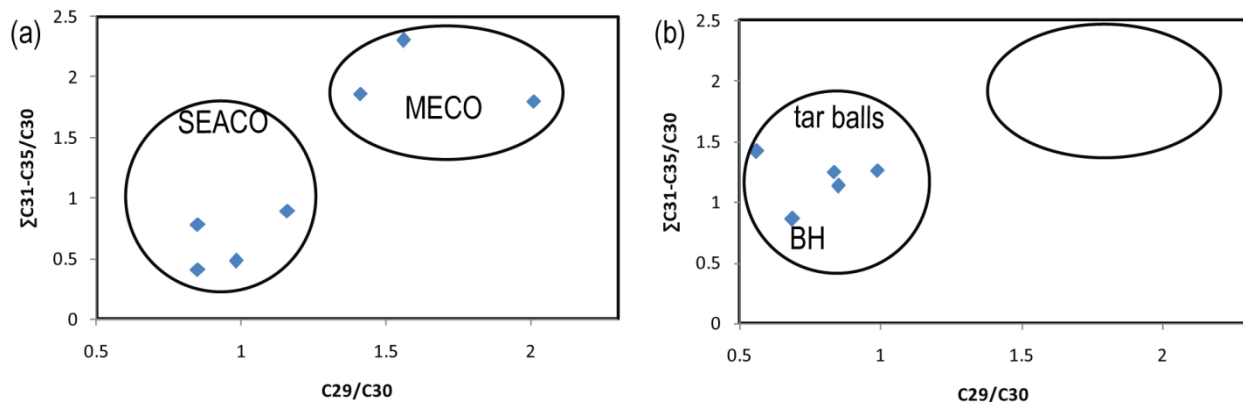


Fig. 6(a). Cross-plot diagram of MECO and SEACO data (Zakaria et al., 2000); (b) Cross-plot diagram for the present analysis of four tar balls and BH.

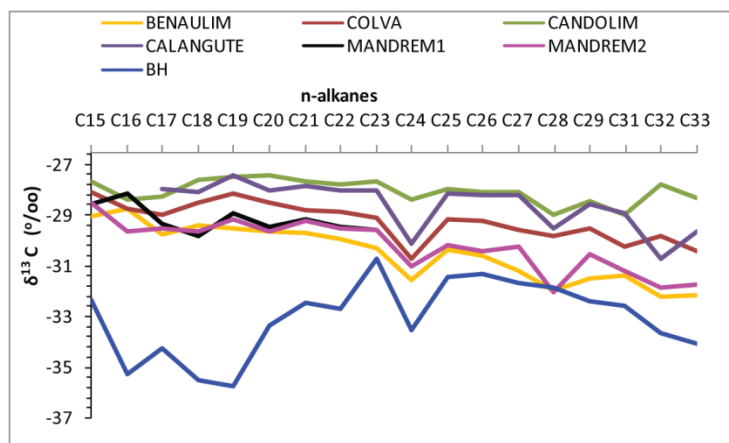


Fig. 7. Compound specification $\delta^{13}\text{C}$ isotope ratio for tar ball and BH

Table

Table 1. Diagnostic ratios of alkanes and triterpanes. Pr/ Ph: ratio of Pristane to Phytane, CPI: Carbon preference Index, L/H: ratio of sum of n-C₁₆ to n-C₁₇ relative to n-C₂₇-n-C₃₆, C₂₉/C₃₀: ratio of 17 α (H),21 β (H)C₂₉-norhopane to 17 α (H)21 β (H)C₃₀-hopane, $\Sigma\text{C}_{31}\text{-C}_{35}/\text{C}_{30}$: ratio of sum 17 α (H)21 β (H)C₃₁ homo hopane to 17 α (H)21 β (H)hopane

Table. 1

Station	Alkanes						Triterpanes			Reference
	Pr/Ph	CPI	L/H	C ₁₇ /C ₁₈	C ₁₇ /Pr	C ₁₈ /Ph	C ₂₉ /C ₃₀	$\frac{\sum C_{31}-C_{35}}{C_{30}}$	Oleanane/C ₃₀	
Manderm1	0.76	1.11	3.92	0.87	1.58	1.38	0.85	1.13	0.24	Present Study
Mandrem2	0.73	1.11	0.84	0.76	1.17	1.13	-	-	-	Present Study
Benaulim	0.76	1.10	0.93	0.82	1.14	1.06	0.56	1.42	0.33	Present Study
Colva	0.81	1.09	0.90	0.82	1.04	1.03	-	-	-	Present Study
Candolim	0.74	1.09	0.93	0.76	1.12	1.09	0.99	1.26	0.29	Present Study
Calangute	0.75	1.11	0.69	0.79	0.98	0.93	0.83	1.25	0.27	Present Study
BHCO	2.98	1.06	2.25	1.04	0.89	2.54	0.69	0.86	0.77	Present Study
MECO1	0.94	0.93	3.92	-	-	-	1.41	1.86	0.03	Zakaria et al. (2000)
MECO2	0.64	0.96	4.6	-	-	-	2.01	1.80	0	Zakaria et al. (2000)
MECO3	0.97	0.96	3.35	-	-	-	1.56	2.30	0	Zakaria et al. (2000)
SEACO1	4.01	1.15	2.43	-	-	-	0.98	0.48	1.53	Zakaria et al. (2000)
SEACO2	4.03	1.34	2.45	-	-	-	0.85	0.41	1.12	Zakaria et al. (2000)
SEACO3	3.66	1.12	4.52	-	-	-	1.16	0.89	0.30	Zakaria et al. (2000)
SEACO4	2.91	1.07	1.62	-	-	-	0.85	0.78	0.11	Zakaria et al. (2000)