ORIGINAL ARTICLE

Even-numbered *n*-alkanes/*n*-alkenes predominance in surface sediments of Gabes Gulf in Tunisia

Fatma Aloulou · Monem Kallel · Mohamed Dammak · Boubaker Elleuch · Alain Saliot

Abstract Hydrocarbons in surface sediments were studied quantitatively and qualitatively in 18 stations along the coastline of Gabes Gulf in Tunisia. The results show that the total hydrocarbon levels vary along a wide range from 90 to 1,800 ppm. The GC–MS profiles of aliphatic hydrocarbons vary according to the stations and show that the hydrocarbons were derived from various sources. A special feature prevalent in several stations was identified: aliphatic hydrocarbons with distinctive chemical features. This includes a high abundance of even-numbered *n*-alkanes (*n*-C14–*n*-C26, maximizing at *n*-C18, *n*-C20 and *n*-C22) and *n*-alk-1-enes (*n*-C14:1–*n*-C24:1, maximizing at *n*-C16:1, *n*-C18:1, *n*-C20:1 and *n*-C22:1). This unusual predominance of even-numbered *n*-alkanes/alkenes is reported for the first time in the Gulf of Gabes and it thus contributes to the information on the rare occurrence of such distributions in the geosphere.

Keywords Even *n*-alkanes · Even *n*-alkenes · Surface sediment · Gulf of Gabes

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Introduction

Hydrocarbons of coastal sediments are produced by varying sources (marine organisms, terrestrial plants, anthropogenic activities…) leading to a complex mixture of these compounds. *N*-alkanes, the most ubiquitous components of hydrocarbons in marine sediments, have been usually investigated to recognize the possible origin of these compounds.

In comparison to the large published works regarding the assessment of potential sources of hydrocarbons in marine sediments, *n*-alkane distributions with odd number preferences (C27, C29, C31) and/or (C15, C17, C19) or with no carbon number preferences, relating, respectively, to vascular plants, marine animals and anthropogenic origin, have been frequently reported (Benlahcen et al. 1997; Baumard et al. 1999; Gogou et al. 2000; Louati et al. 2001; Tsapakis et al. 2003; De Luca et al. 2004; Gogou and Stephanou 2004; Zaghden et al. 2005; Mille et al. 2007; Zrafi-Nouira et al. 2008). However, distribution of *n*-alkanes with even carbon number preferences has been much less reported (Nishimura and baker 1986; Grimalt and Albaigés 1985, 1986, 1987; Debyser et al. 1977; Mille et al. 1992, 2007). Furthermore, in some studies such distribution has been reported together with the occurrence of even carbon number *n*-alk-1-enes, especially in the range of C22–C30 (Grimalt and Albaigés 1985, 1987).

To the best of our knowledge, the only reported occurrence of even alk-1-enes with even alkanes in the range of C14–C22 corresponds to the Calabar River, Nigeria (Ekpo et al. 2005). This unusual distribution is described in the present study at many stations.

We endeavor by this work carried out in collaboration with the International Environmental Technology Center in Tunis (CITET) and being part of the overall project entitled "Gabes Gulf, Marine and Coastal Resources Protection" to

extend the number of known sedimentary settings in which these even *n*-alkanes/*n*-alkenes distributions occur as well as the data available on the origin of hydrocarbons in marine sediments of the Mediterranean Sea and especially of the Tunisian coastal shelf.
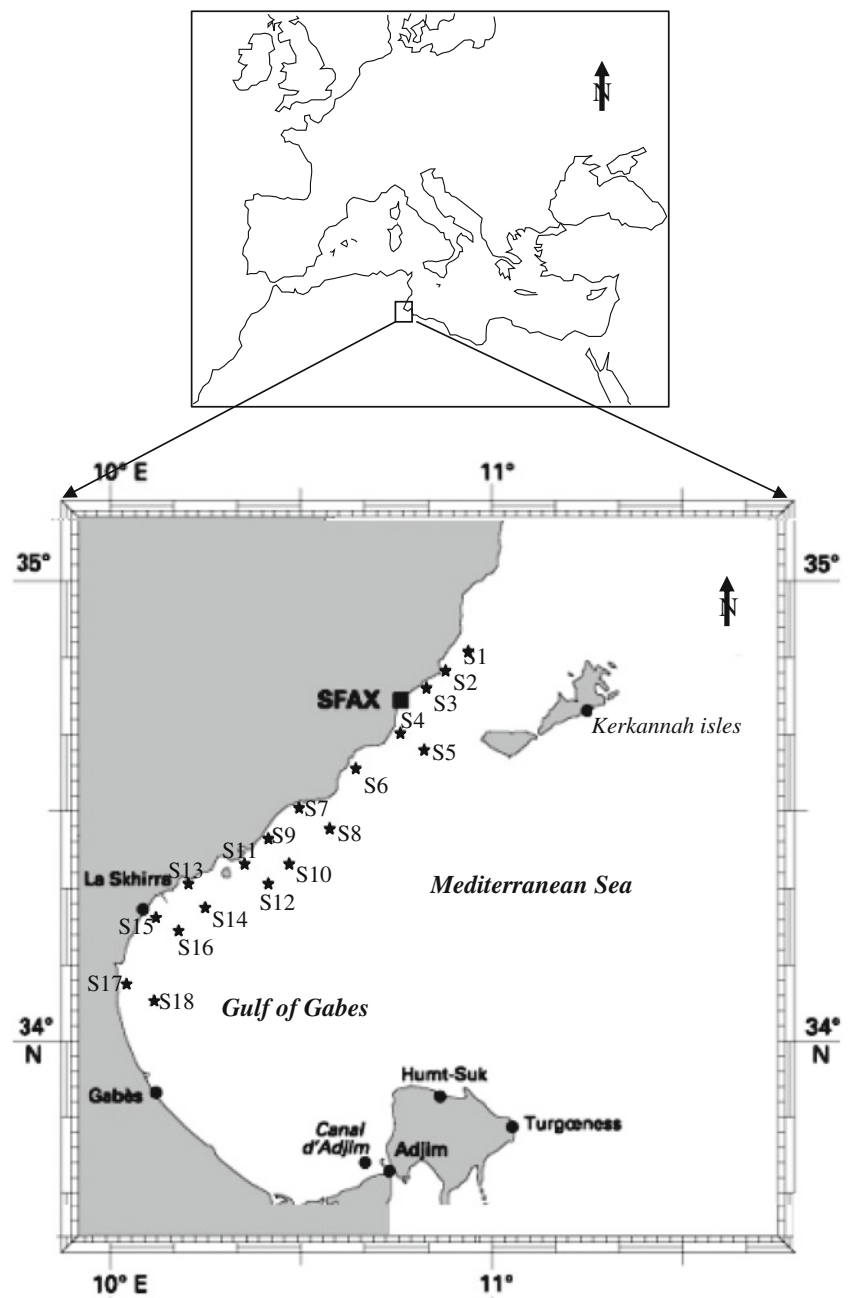
Experiments

Study area and sampling

The Gulf of Gabes is shallow and located in the southwestern part of the Strait of Sicily. It is bounded by

Kerkennah Isles on the northeast and by Djerba Island on the southeast. It is 100-km long and 100-km wide at 34°00′00″N 10°25′00″E (Fig. 1). It has a broad continental shelf. It also has a very soft slope in which the isobath of 200 m is reached only beyond 250 km of the coast. Standing as the site of the highest tides in the Mediterranean (1.7 m) at spring tides, the Gulf of Gabes is most productive in this area. Ben Othmane (1971) described 208 marine species in this area, at a depth of about 0 and 300 m showing the particular ecological and economical importance of this area.

Sampling sites stretch along the northern coast of the Gabes Gulf for about 90 km from Sfax City to Gabes City

Fig. 1 Location map of the sampling sites in the Gulf of Gabes

(Fig. 1). This area has three fishing harbors and two phosphoric acid production plants. Surficial sediments (0–5 cm) were collected from 18 sites (S1–S18) of the coastal shelf of the study area. Stations S1, S7 and S11 were located on a fishing harbor. S3 was localized to the estuary of Oued Ezzit. S2 was located on an urbanized area. S4 was localized opposite to a large gypsum landfill (calcium sulfate), which contained waste from a phosphoric acid production plant and which was close to the outfall of the industrial and domestic wastewater. The other stations were chosen from a presumed low polluted coastal area far from any human activities.

Samples are freeze-dried after collection, ground and sieved to obtain <63-μm fine particles.

Extraction and separation

A sediment sample of about 20 g was extracted after 12 h using Soxhlet with a mixture of dichloromethane/methanol (2:1, v/v). The extraction yield was $90 \pm 6\%$. The extract was then concentrated on a rotary evaporator and reduced using a stream of dry nitrogen gas to a volume of approximately 150 μl. It was then submitted to adsorption chromatography on a column (2-cm i.d., 20-cm length) filled with Florisil (60 mesh). Total hydrocarbons were eluted with dichloromethane and polar compounds with methanol.

The amount of total hydrocarbons was determined by gravimetry. The total hydrocarbons were separated by column chromatography using a two-step scheme, providing two fractions:

- non-aromatic hydrocarbons (F_1), elution with hexane;
- aromatic hydrocarbons (F_2), elution with hexane/dichloromethane 4:1, v/v.

Each fraction was evaporated to 150–200 μl under a stream of dry nitrogen.

Instrumental analysis

Gas chromatography–mass spectrometry (GC–MS) was carried out with Hewlett-Packard 6890 gas chromatograph coupled with a 5973 Mass Selective Detector, using a DB-5MS (*Agilent*) fused silica capillary column (30 m × 0.25-mm i.d., 0.25-μm film thickness) and helium as a carrier

Table 1 Concentrations (mg kg^{-1} sed. dry weight) and geological parameters of *n*-alkanes and *n*-alkenes in surface sediments from the Gulf of Gabes, Tunisia

	THC	*n*-alkanes						Alkenes	
		Range	C_{max}	% <C21	Pr/Ph	%UCM	CPI	%	C_{max}
S1	370	15–25	C17	58	0.75	70	1.12	0	ND
S2	712	14–22	C18	77	0.82	29	0.42	26	C18:1
S3	120	16–26	C18	66	0.4	21	0.56	37	C20:1
S4	1,800	16–26	C20 C21 C22	36	0.31	30	0.98	0	ND
S5	1,167	14–16	C16	85	ND	31	ND	23	C18:1 C20:1
S6	320	16–24	C18 C20 C22	59	ND	26	0.60	6	C18:1
S7	600	16–28	C18	62	0.51	44	0.57	0	ND
S8	830	14–16	C16	83	ND	23	ND	92	C18:1 C20:1
S9	250	18–26	C22	33	ND	16	1.09	24	C20:1
S10	575	14–16	C16	70	ND	38	ND	90	C20:1
S11	110	16–24	C18	51	0.41	66	0.85	19	C18:1 C18:3
S12	1,237	14–18	C18	89	ND	35	ND	91	C18:1 C20:1
S13	190	17–26	C22	23	ND	19	0.67	0	ND
S14	796	14–16	C16	86	ND	26	ND	92	C18:1 C20:1
S15	370	16–28	C22	19	ND	20	0.80	20	C20:1 C24:1
S16	765	16–14	C16	92	ND	22	ND	91	C20:1 C22:1
S17	90	16–25	C20	58	0.31	21	0.31	30	C20:1
S18	332	16–27	C24	20	ND	29	1.29	34	C22:1 C24:1

THC total hydrocarbon content, *Pr* pristane, *Ph* phytane, *UCM* unresolved complex mixture, *ND* not determined

$\% < C21$: $\sum nC14\text{-}nC20/\text{total } n\text{-alkanes}$
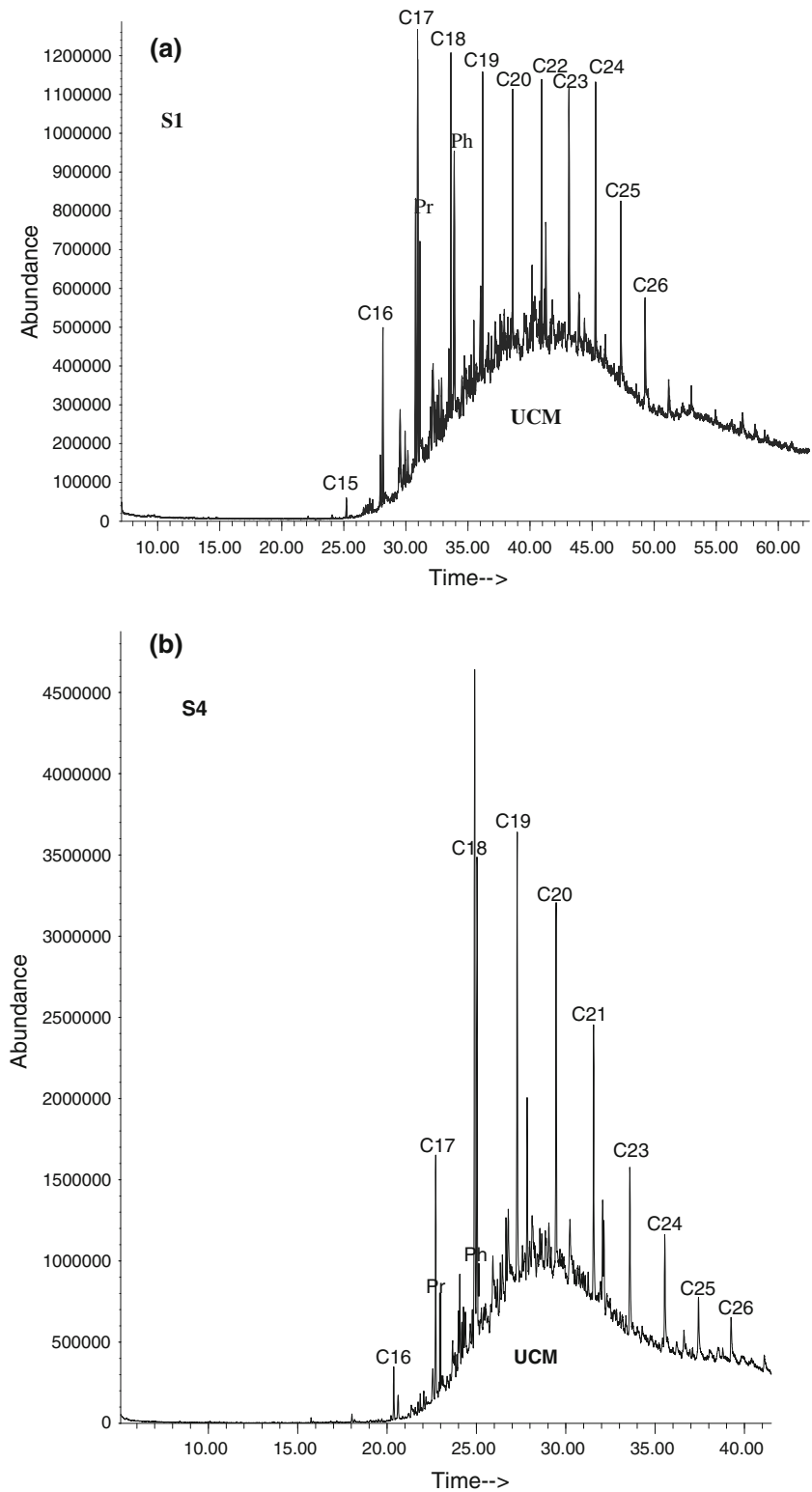
$CPI = (\sum nC17 + nC19 + \cdots + nC27)/(\sum nC16 + nC18 + \cdots + nC26)$

gas. The GC temperature was programmed at the level of 80–290°C at 4°C min⁻¹. The samples were injected in the splitless mode with an injector temperature of 280°C. Analyses were run in the electron impact mode at 70 eV with a 2.9-s scan time over a 50–550-a.m.u. range resolution.

Identification and quantification

The identification of *n*-alkanes was based on the GC–MS data (i.e., m/z 99 fragmentograms and mass spectra). Retention times were compared with those of external

Fig. 2 TIC GC–MS total ion of *n*-alkanes of the surface sediment samples: **a** station 1; **b** station 4

standards and concentrations of each *n*-alkane were calculated through the peak area derived from the ion fragmentogram and the standard calibration curve of each corresponding standard compound.
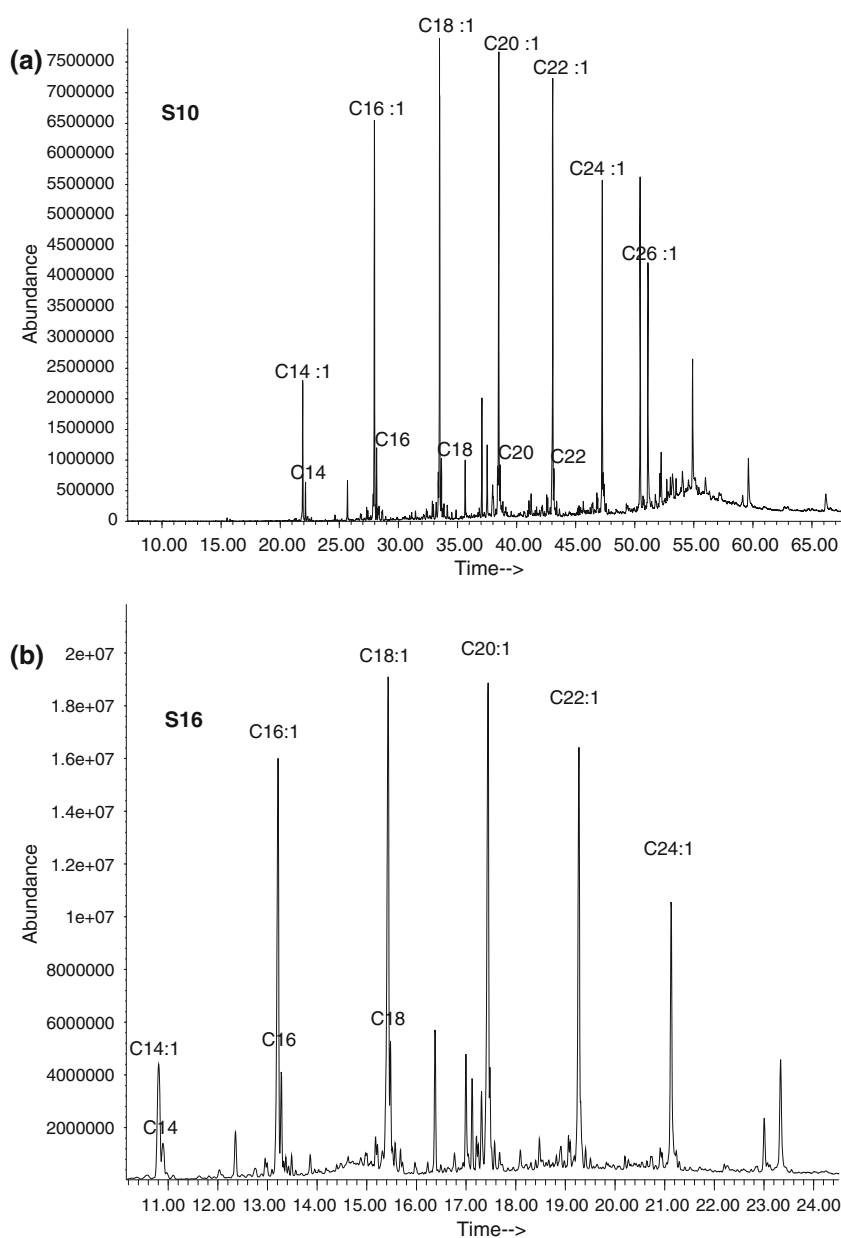
Statistical analyses

Linear discriminant analysis was used for data analysis. This method was chosen among other linear combination methods (principal component analysis) because it not only divides objects into two or more classes, but is also the best to predict the membership of an entity to a later predefined group. The statistical software used is SPSS 13.0

Results and discussion

Total hydrocarbons

Total hydrocarbon concentrations determined by gravimetry varied between 90 and 1,800 mg kg^{-1} of sediment dry weight (Table 1). The lowest value (<100 mg kg^{-1} sed. dry weight) was observed in station S17 and the highest values (>1,000 mg kg^{-1} sed. dry weight) were found in stations S4, S5 and S12. These values were much lower than those already reported in the Sfax coastline area (Louati et al. 2001; Zaghden et al. 2005).

Fig. 3 TIC GC–MS total ion of *n*-alkanes of the surface sediment samples: **a** station 10; **b** station 16

Aliphatic hydrocarbons

GC–MS traces of aliphatic hydrocarbons consist of a series of resolved compounds n-alkanes, branched alkanes, cycloalkanes and alkenes. The carbon maximum number C_{max} of the most abundant n-alkane can be used as an indicator of relative source input (Simoneit 2002). On the basis of GC–MS profile and C_{max}, stations can be separated into several groups.
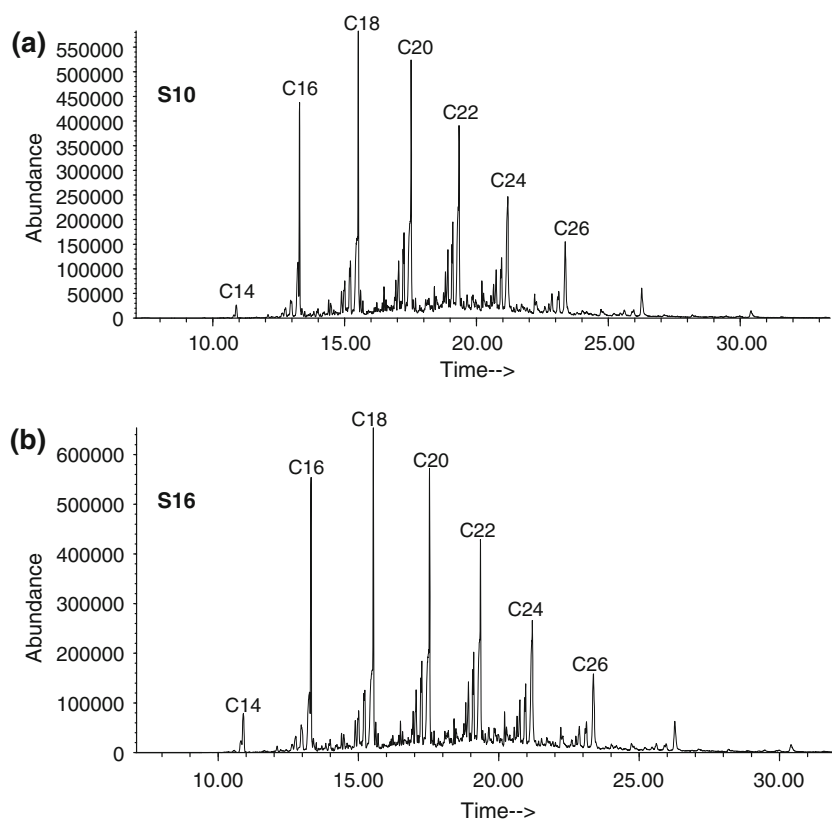
Group 1 (stations S1, S4 and S11): GC–MS profiles show a regular distribution of n-alkanes in the range of C16-C25 with no predominance of odd- or even-numbered n-alkanes (Fig. 2). This feature is common in many coastal environments and ascribed to petroleum contamination (Albaigés and Cuberes 1980; Aboul-Kassim and Simoneit 1995; Medeiros et al. 2005). The occurrence of isoprenoid n-alkanes pristane (Pr) and phytane (Ph) coupled with such distribution generally can support the contamination by residues from petroleum and petroleum products. Though Pr is common in planktons and various marine animals and Ph occurs also from diagenetic sources (Blumer and Snyder 1965), it is often reported as a major component of petroleum (Simoneit 1984, 1985). The Pr/Ph ratio equal or less than 1 indicates petroleum-derived hydrocarbons (Broman et al. 1987) and values from 1.4 to 6.7 indicate biogenic hydrocarbons (Lecaros et al. 1991). For stations

S1, S4 and S11, values of Pr/Ph ratio range between 0.31 and 0.75 confirming petroleum inputs.

Group 2 (S5, S8, S10, S12, S14 and S16): the main feature observed in the GC–MS profile is the dominance of even alkenes over alkanes. These hydrocarbons are monolinear olefins (C16:1 to C22:1 and C24:1) (Fig. 3). These alkenes are not present in crude oil, but have rather a biogenic origin. In general, n-alkenes in sediments have a dual biogenic origin. First, n-alkenes (<C22) composed of a mixture of n-C15:1, n-C17:1 and n-C19:1 originate from algal (Winters et al. 1960; Youngblood et al. 1971) and zooplanktonic (saliot 1981) inputs. Second, higher plants produce long chain n-alkenes that parallel the distribution of n-alkanes in the C22–C35 range in the cuticular waxes (Giger and Schaffner 1977; Giger et al. 1980; Albaigés et al. 1984). As far as we know, the occurrence of even alk-1-ene is reported only in two cases in the diatomaceous oozes from the Cerdanya basin (Grimalt and Albaigés 1987) and in the surficial sediments of the Clabar River (Ekpo et al. 2005). In fact, no biogenic source for these even carbon number predominated n-alk-1-enes has been described.

Another striking feature displayed by the corresponding GC–MS profile is the depletion of n-alkanes. These n-alkanes with much lower concentrations than those of n-alkenes appear simultaneously with n-alkenes. This suggests the single origin for these hydrocarbon compounds.

Fig. 4 Examples of typical GC–MS key ion plots for n-alkanes in the sediment samples, (m/z 99): **a** station 10; **b** station 16

Furthermore, *m/z* 99 fragmatograms are marked by the occurrence of only even *n*-alkanes in the range of *n*-C16–*n*-C26 with a maximum at *n*-C18 and *n*-C20, suggesting microbial origin of *n*-alkanes as well as of *n*-alkenes (Fig. 4). This feature is reported for the first time in coastal sediments and no similar distribution has been described.

Group 3 (stations S2, S3, S6, S7, S9, S13, S15, S17 and S18): resolved aliphatic hydrocarbons consist mainly of C12-C26 *n*-alkanes. The short chain *n*-alkanes (<C21) show an even C_{max} at C16, C18 and C20 (Fig. 5). Nevertheless, the GC/MS chromatograms of these stations are slightly different. The stations S9, S13, S15 and S17 display a depletion of Pr and Ph (Fig. 5a, b), whereas the stations S2, S3, S7 and S18 exhibit Pr and Ph (Fig. 5c, d) with Pr/Ph ratios less than 1, between 0.31 and 0.82, which suggests petroleum input at these stations.

However, the predominance of even *n*-alkanes in the range C12–C20 with C_{max} at C16 and C18 has been reported in many coastal sediments such as in the Arabian Gulf (Grimalt and Albaigés 1985; Mille et al. 1992), the Gulf of Mexico

(Nishimura and Baker 1986) from Makassar Strait (Noor et al. 1989) and the Gulf of Fos (Mille et al. 2007). Therefore, it has been reported that the occurrence of this even *n*-alkanes is related to biogenic origin from microbial direct input (Nishimura and Baker 1986; Grimalt and Albaigés 1987). More recently, Jovančićević et al. (2001) and Ekpo et al. (2005) have reported the same distribution in oil polluted sediments. These authors suggest that these even *n*-alkanes are synthesized by microorganisms living in petroleum-contaminated environments.

Results of a review of the sources and the predominance of even *n*-alkanes in sedimentary environments show that hydrocarbons in stations S9, S13, S15 and S17 come from biogenic microbial input, while hydrocarbons in stations S2, S3, S7 and S18 come from bacteria attributed to petroleum-contaminated sediment. This confirms the hypothesis of the contamination of these stations by petroleum residues.

To better evaluate the relative origin of hydrocarbons in sediments, key parameters such as the carbon preference
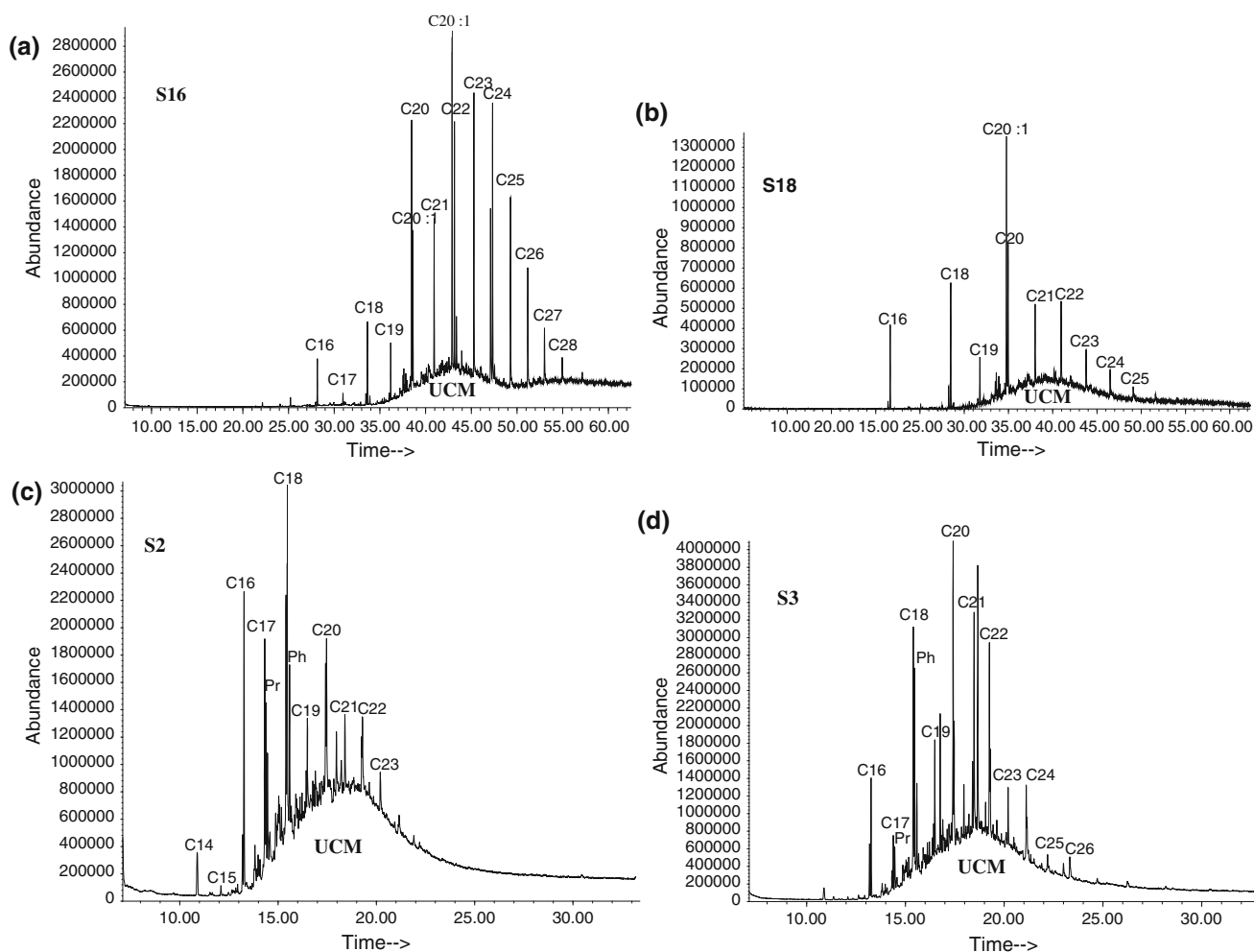

Fig. 5 TIC GC–MS total ion of *n*-alkanes of the surface sediment samples: **a** station 2; **b** station 3; **c** station 16; **d** station 18

index (CPI) and the unresolved complex mixture (UCM) are associated with *n*-alkanes distribution and C_{max}.

Carbon preference index (CPI)

The CPI has been frequently used as a source indicator of *n*-alkanes in marine sediments (Farrington and Tripp 1977; Boehm and Requejo 1986; Colombo et al. 1989; Prahl et al. 1994). It represents the relative abundance of odd-numbered linear alkanes versus even-numbered linear alkanes (Bray and Evans 1961). *N*-alkanes derived from terrestrial vascular plant usually have CPI values ranging from 3 to 6, while petrogenic hydrocarbons show CPI values close to 1. Lower CPI indicates microbial sources of hydrocarbons (Rushdi et al. 2006).

As summarized in Table 1, CPI values are close to 1 for stations S1, S4 and S11, corresponding to the petrogenic sources. Whereas, stations S2, S3, S6, S7, S9, S15, S13, S15 and S17 exhibit CPI values between 0.31 and 0.80 indicating that there are major microbial sources. In fact, the same range of CPI has been reported from the sediments of the Arabian Gulf (Grimalt and Albaigés 1985) and the Calabar River (Ekpo et al. 2005) where direct biological input of even light hydrocarbon is supposed to be present.
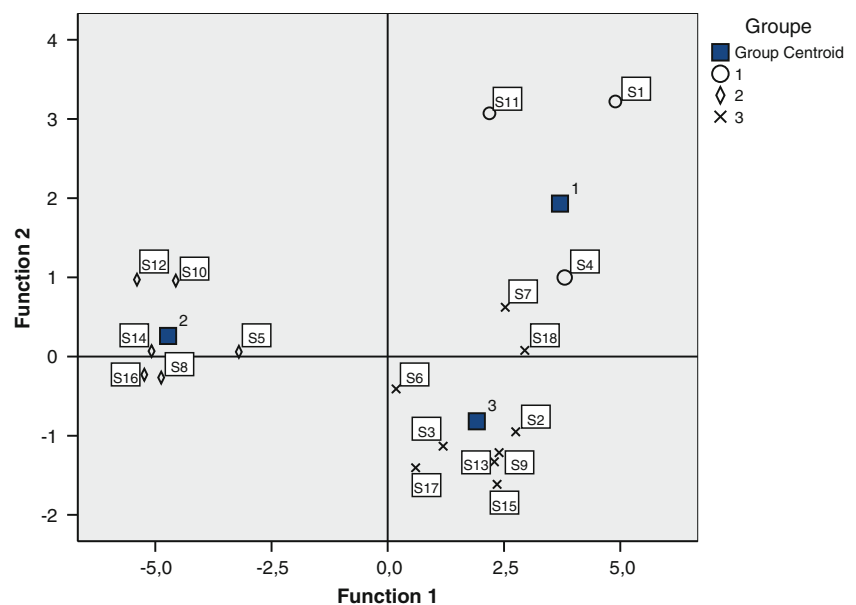
Unresolved complex mixture (UCM)

The unresolved complex mixture (UCM) contains a number of unresolved branched and cyclic hydrocarbons (Simoneit 1984, 1985). These hydrocarbons are derived mainly from oil spills and/or fossil fuel utilization. Biogenic hydrocarbons derived from higher plants

exhibit no UCM (Simoneit and Mazurek 1982). Alteration of microbial detritus can yield a UCM with a C_{max} at 19–22 (Simoneit et al. 1979; Venkatesan and Kaplan 1982; Jarde 2002). From the previous discussion, it is obvious that the stations S1, S2, S3, S4, S7, S11 and S18 show a strong UCM that makes an important contribution especially at stations S1 and S11, which varies between 70 and 66%, respectively, confirming the input of petrogenic hydrocarbons. Whereas, in the stations S9, S13, S15 and S17 with biogenic *n*-alkanes, the contribution of UCM is smaller and varies between 19 and 21% (Table 1).

Thus, the data obtained from compositional parameters of hydrocarbons (CPI, UCM) agree with those obtained through GC/MS spectra analysis (distribution of *n*-alkanes, C_{max}). These data emphasize clearly the potential sources of hydrocarbons in the Gabes Gulf.

To better illustrate that quantitative variables (CPI, Pr/Ph, % <C21, %UCM and % alkenes) support the segregation of the stations according to the three predefined groups, we performed linear discriminant analysis, which identified two major functions explaining 100% of total variance. Function 1 opposes CPI (+0.47) to percentages of alkenes (−0.46), while function 2 is mainly correlated to percentages of UCM (+0.96). The plot of observations (Fig. 6) shows a clear separation of the three groups. The first group (S1, S4 and S11) is identified by function 1 and is characterized by high levels of CPI and low percentages of alkenes. The second group is identified by function 2 and includes stations S5, S8, S10, S12, S14 and S16, which have low percentages of UCM. Finally, group 3, including stations S2, S3, S6, S7, S9, S13, S15, S17 and S18, is located in the lower right quart of the plot and is

Fig. 6 Plot of the stations in the plan of the first two-first discriminant functions

distinguished by high percentages of UCM and alkenes and lower levels of CPI.

Conclusion

Gravimetric data of total hydrocarbons reported on surficial sediments from the Gulf of Gabes show that the levels of hydrocarbons resulting from natural sources are of the same order or sometimes higher than those of hydrocarbons from anthropogenic sources. These results explain that hydrocarbon concentrations in the Gulf of Gabes are not automatically linked to petroleum contamination levels.

Aliphatic hydrocarbons characterized by GC–MS show that the microbial input is the most dominant origin of hydrocarbons in the majority of the stations. This is emphasized by the occurrence of even numbered n-alkanes (C16, C18, C20) and even numbered n-alk-1-enes (C16:1, C18:1, C20:1), the decrease of n-alkanes CPI values and the shift of C_{max} to 18 and 20, which indicates a microbial contribution. The typical distribution of petrogenic n-alkanes is identified only at stations with high anthropogenic pressure (S1, S4 and S11).

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