

Hydrocarbon Balance of a Site which had been Highly and Chronically Contaminated by Petroleum Wastes of a Refinery (from 1956 to 1992)

YVELINE LE DRÉAU*, FRÉDÉRIC JACQUOT*, PIERRE DOUMENQ*, MICHEL GUILIANO*,
JEAN CLAUDE BERTRAND† and GILBERT MILLE*

**Faculté des Sciences et Techniques de St Jérôme, Laboratoire de Chimie Analytique de l'Environnement, Av. Escadrille Normandie Niemen, Boite 312, F-13397 Marseille Cedex 20, France*

†*Faculté des Sciences de Luminy, Centre d'Océanologie de Marseille, Laboratoire d'Océanographie et de Biogéochimie, Case 901, F-13288 Marseille Cedex 9, France*

Petroleum hydrocarbon pollution in the Gulf of Fos (South France) was studied following the cessation of petroleum waste discharge from an oil refinery in the Lavera area. Sediments were collected in core samples of 30 cm at eight stations along a radial from the refinery to the open sea. Petroleum contamination has been detected up to the beginning of the open sea but decreased gradually (from 40 to 3 g kg⁻¹ sediment dry wt). GC profiles (aliphatics and aromatics) are characteristic of petroleum. They reveal the disappearance of short chain *n*-alkanes (up to C₂₅) and of one and two aromatic ring compounds, the increase and the modification of the UCM depends on the depth and the situation, resulting from weathering processes. Biomarkers and three to five aromatic ring compounds are unaltered whatever the depth and the location. If some petroleum hydrocarbons are present for the open sea stations (perhaps from other sources), natural and pyrolytic hydrocarbons are also detected. Sediments from the open sea could be considered to be unpolluted (less than 0.1 g kg⁻¹), and unaffected by the refinery wastes two years after the end of overspills.

© 1997 Elsevier Science Ltd

Keywords: sediments; hydrocarbons; biomarkers; PAHs; GC; GC/SM.

The French Mediterranean coastal region and more particularly the 'Golfe de Fos' area has many petroleum industries. Petroleum treatments by refineries can contribute to increase the organic matter content in sediments. To implement the International Factory Acts, more and more refineries set up high performance waste treatment systems in order to reduce or even to stop petroleum inputs in the marine environment.

This study focused on a site in the 'Golfe de Fos' which had been chronically polluted by petroleum effluents from a refinery (Lavera area) from 1956 to 1992. From the beginning of the refining operations (1956) until 1978 the effluents ranged from 100 to 250 kg a day, carried out by a great quantity of water (200 m³ h⁻¹). In 1978 a purification station was set up and allowed to reduce the quantity of petroleum residues to 90 kg a day. Since 1985 the refinery has reduced its effluent discharge by half and reduced the amount of oil released to 40 kg a day. Finally, since 1992, an internal recycling system for cooling water has been used and the oil content of the effluents has decreased to less than 1 kg a day.

The area has received very irregular quantities of hydrocarbons over the years and their compositions were very variable because of the different origins of the crude oils treated in the refinery.

This site has not received petroleum residues coming from the refinery for 2 years, providing an opportunity to draw up a balance of the petroleum contamination in the site and its close environment in order to estimate its future evolution.

Materials and Methods

Sample collection

The petroleum residues were dumped in a canal flowing into a deep creek opening into the 'Golfe de Fos'. A series of samples was collected by core sampling along a 1000-m long transect running from the outlet of the refinery to the open sea (Fig. 1). The location, the water depth and the nature of sediments of the eight stations are given in Table 1.

In each sampling station four sediment cores were collected in May 1994 and stored frozen (-18°C) until analysis. The 0-2 cm, 2-7 cm, 7-17 cm, 17-27 cm

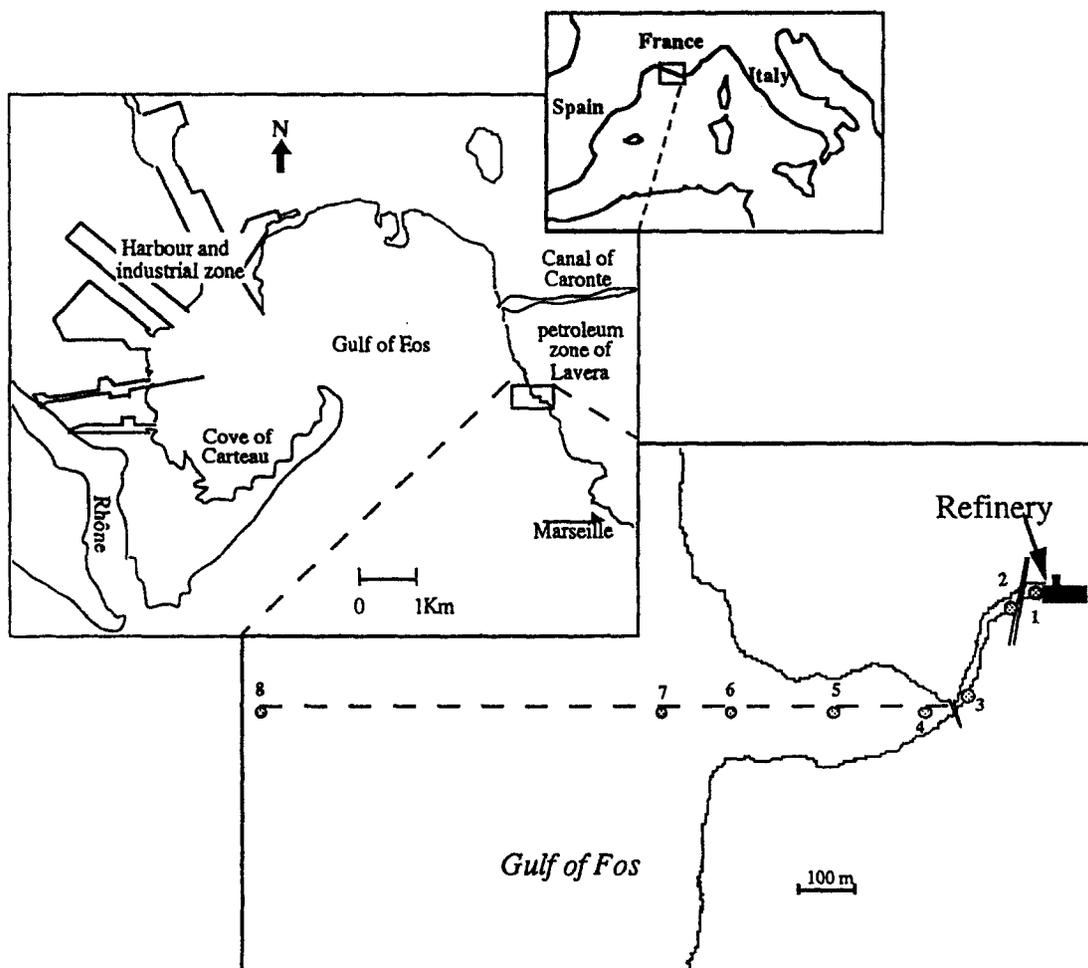


Fig. 1 Location of sampling stations.

TABLE 1

Location and the nature of sediments of the eight stations.

| Stations | Location | Depth (m) | Sediment type |
|----------|------------------------|-----------|-------------------------------------|
| 1 | Outlet of the refinery | < 1 | Fine sandy sediment |
| 2 | Canal | < 1 | Muddy sediment |
| 3 | Canal | < 1 | Coarser and more permeable sediment |
| 4 | Deep creek | 1 | Coarse sand sediment |
| 5 | Deep creek | 2 | Sandy sediment |
| 6 | Deep creek | 5 | Muddy sediment |
| 7 | Open sea (100 m) | 8 | Fine sand |
| 8 | Open sea (1000 m) | 14 | Coarse sand |

layers of each of the four cores were respectively joined together and homogenized before analysis.

Chemical analyses

Hydrocarbons were extracted from wet sediment (about 50 g accurately weighed) by direct saponification via alkaline hydrolysis. Samples were heated under reflux for 4 hours in a mixture of 0.5 N KOH in 95% methanol/toluene 2/1. After filtration on a glass fibre filter (Whatman GF/C) and separation of the two

phases, the aqueous phase was extracted with 3×50 ml of toluene. All toluenic phases containing hydrocarbon compounds were combined, dried on anhydrous $MgSO_4$, evaporated to a residue and weighed to obtain the total extractable organic matter (EOM).

All or part of the EOM was dissolved in a minimum of *n*-hexane and applied to a half silica (8 g) and a half alumina (8 g) (both of these 5% deactivated with water) chromatography column (30×1 cm). Aliphatic fraction (F1) was eluted with 30 ml of *n*-hexane on the column. Elutions with 20 ml of *n*-hexane/dichloromethane (9/1) and 40 ml of *n*-hexane/dichloromethane (4/1) yield an aromatic fraction (F2). Each fraction was evaporated on a rotary evaporator, and under a stream of nitrogen and residues were quantified gravimetrically. The sum of the aliphatic fraction and the aromatic fraction gave the total hydrocarbon content.

Procedural blanks were carried out between each batch of four samples and no contamination was found.

Instrumental analyses

Both F1 and F2 fractions were diluted in an adequate quantity of *n*-hexane and were analysed by capillary gas chromatography under the following conditions: GC

6000 Vega serie Carlo Erba chromatograph, on-column injection, JW Scientific DB5 column, (30 m×0.32 mm (i.d.) ×0.25 μm), helium as carrier gas, temperature programme from 70 to 285°C at 5°C min⁻¹ and at 285°C for 30 min, flame ionization detector at 290°C.

Fractions were also analysed by GC/MS (5890 II HP gas chromatograph and 5989 A HP mass spectrometer) in full scan and selected ions recording (SIR) modes under the following conditions: splitless injection (45 s), HP5-MS (low bleed) capillary column, 30 m ×0.25 mm (i.d.)×0.25 μm, helium as carrier gas, temperature programme from 70 to 110°C at 50°C min⁻¹ then from 110°C to 295°C at 5°C min⁻¹ and 295°C for 30 min.

The aromatic fractions were analysed by UV synchronous fluorescence spectroscopy. Synchronous fluorescence spectra were recorded on a 3000 Perkin Elmer spectrometer. The interval Δλ between λ_{ex} and λ_{em} was constant at 23 nm. Solutions of samples in *n*-hexane were analysed using a 1-cm optical pathlength quartz cell.

Results and Discussion

Gravimetric results

Gravimetric data are given in Table 2 for each station. They point out two tendencies:

1. The distribution of hydrocarbon concentrations in the sediments (g kg⁻¹ sediment dry wt) is dependent on the depth. An accumulation zone in depth is observed for the distant stations (3 to 7). For example the hydrocarbon levels vary from 0.28, 0.17 and 0.05 g kg⁻¹ sediment dry wt for the 0–2 cm core to 2.11, 1.53 and 1.88 g kg⁻¹ sediment dry wt for the 2–27 cm core (average values), respectively, for the stations 4, 5 and 6. For the most contaminated stations (stations 1 and 2) near the refinery a decrease of hydrocarbon concentrations with depth down to 17 cm and an important concentration in the deepest layer are observed.
2. The hydrocarbon levels decrease according to the distance from the pollution source. The average values for the 0–27 cm core vary from 19.37 and 26.64 g kg⁻¹

(sediment dry wt) for respectively, stations 1 and 2 to 0.12 g kg⁻¹ (sediment dry wt) for station 8 (the most remote one).

Station 2 at the beginning of the canal is more contaminated than station 1 at the exit of the refinery. It is a stagnant zone, which is not submitted to the influence of the sea and it represents an accumulation zone. On the contrary, station 3, at the end of the canal, which is under the influence of waves, is far less contaminated (about 15 time less) than station 2. The hydrocarbon concentrations are much lower (<1 g kg⁻¹) near the surface than at depth at stations 4 and 5 in the deep creek, and show the dispersing effect of the sea and the percolation through sediments. The breaking zone between the deep creek (a still sheltered area) and the open sea is station 6. Nevertheless the hydrocarbon concentrations in the 2–27 layers at this station are still relatively high. The hydrocarbon concentrations for station 7 are very low, even if higher at depth than at the surface. Beyond these two stations the hydrocarbon concentrations greatly decrease from 2.28 g kg⁻¹ (sediment dry wt) (station 6) and 1.05 g kg⁻¹ (sediment dry wt) (station 7) to 0.12 g kg⁻¹ (sediment dry wt) for station 8 (average values for 0–27 cm layer). The concentrations of station 8 are analogous to those found in uncontaminated sediments (Volkman *et al.*, 1992) and in Mediterranean coastal sediments (Mille *et al.*, 1992).

Distribution between saturated and aromatic hydrocarbons is not fraction of depth (Table 2). In station 8 the proportion of saturated hydrocarbons is between 34 and 50% (about 80% in the other stations). This could be explained either by a different origin of the polluting hydrocarbons or by a greater alteration of the same aliphatic hydrocarbons. These hypotheses will be discussed later in the GC/MS results.

GC and GC/MS results

The GC/FID chromatograms of the saturated hydrocarbon fractions (Fig. 2) show a fairly clear Unresolved Complex Mixture (UCM). Station 1 shows a regular wide range (from C₁₂ to C₃₀) of odd

TABLE 2
Gravimetric data (g kg⁻¹ sediment dry wt).

| STATIONS | F1 | | | | F2 | | | | THC | | | | F1/THC% | | | | |
|----------|--------|--------|---------|----------|--------|--------|---------|----------|--------|--------|---------|----------|---------|-------|-------|-------|----------|
| | 0-2 cm | 2-7 cm | 7-17 cm | 17-27 cm | 0-2 cm | 2-7 cm | 7-17 cm | 17-27 cm | 0-2 cm | 2-7 cm | 7-17 cm | 17-27 cm | | | | | |
| 1 | 0-2 cm | 2-7 cm | 7-17 cm | 17-27 cm | 17.24 | 12.59 | 6.15 | 27.66 | 2.65 | 2.60 | 3.25 | 5.31 | 19.90 | 15.19 | 9.40 | 32.98 | 87836584 |
| 2 | 0-2 cm | 2-7 cm | 7-17 cm | 17-27 cm | 43.83 | 20.40 | 12.57 | 17.40 | 3.75 | 2.43 | 2.92 | 3.24 | 47.58 | 22.83 | 15.49 | 20.64 | 92898184 |
| 3 | 0-2 cm | 2-7 cm | 7-17 cm | 17-27 cm | 1.00 | 0.94 | 2.55 | 1.43 | 0.32 | 0.34 | 0.48 | 0.33 | 1.32 | 1.28 | 3.03 | 1.76 | 76738481 |
| 4 | 0-2 cm | 2-7 cm | 7-17 cm | 17-27 cm | 0.20 | 0.87 | 1.40 | 1.43 | 0.08 | 0.24 | 0.34 | 0.33 | 0.28 | 1.11 | 1.74 | 1.76 | 72798081 |
| 5 | 0-2 cm | 2-7 cm | 7-17 cm | 17-27 cm | 0.12 | 1.57 | 1.47 | 1.49 | 0.05 | 0.45 | 0.46 | 0.20 | 0.17 | 2.02 | 1.94 | 1.68 | 72787688 |
| 6 | 0-2 cm | 2-7 cm | 7-17 cm | 17-27 cm | 0.02 | 3.50 | 2.07 | 1.67 | 0.03 | 1.00 | 0.75 | 0.08 | 0.05 | 4.50 | 2.82 | 1.75 | 40787395 |
| 7 | 0-2 cm | 2-7 cm | 7-17 cm | 17-27 cm | 0.09 | 0.13 | 2.81 | 0.44 | 0.03 | 0.02 | 0.56 | 0.10 | 0.12 | 0.15 | 3.37 | 0.54 | 82868381 |
| 8 | 0-2 cm | 2-7 cm | 7-17 cm | 17-27 cm | 0.05 | 0.10 | 0.05 | 0.02 | 0.09 | 0.11 | 0.05 | 0.02 | 0.14 | 0.21 | 0.10 | 0.04 | 34474950 |

F1 = Saturated hydrocarbon fraction.

F2 = Aromatic hydrocarbon fraction.

THC = total hydrocarbons.

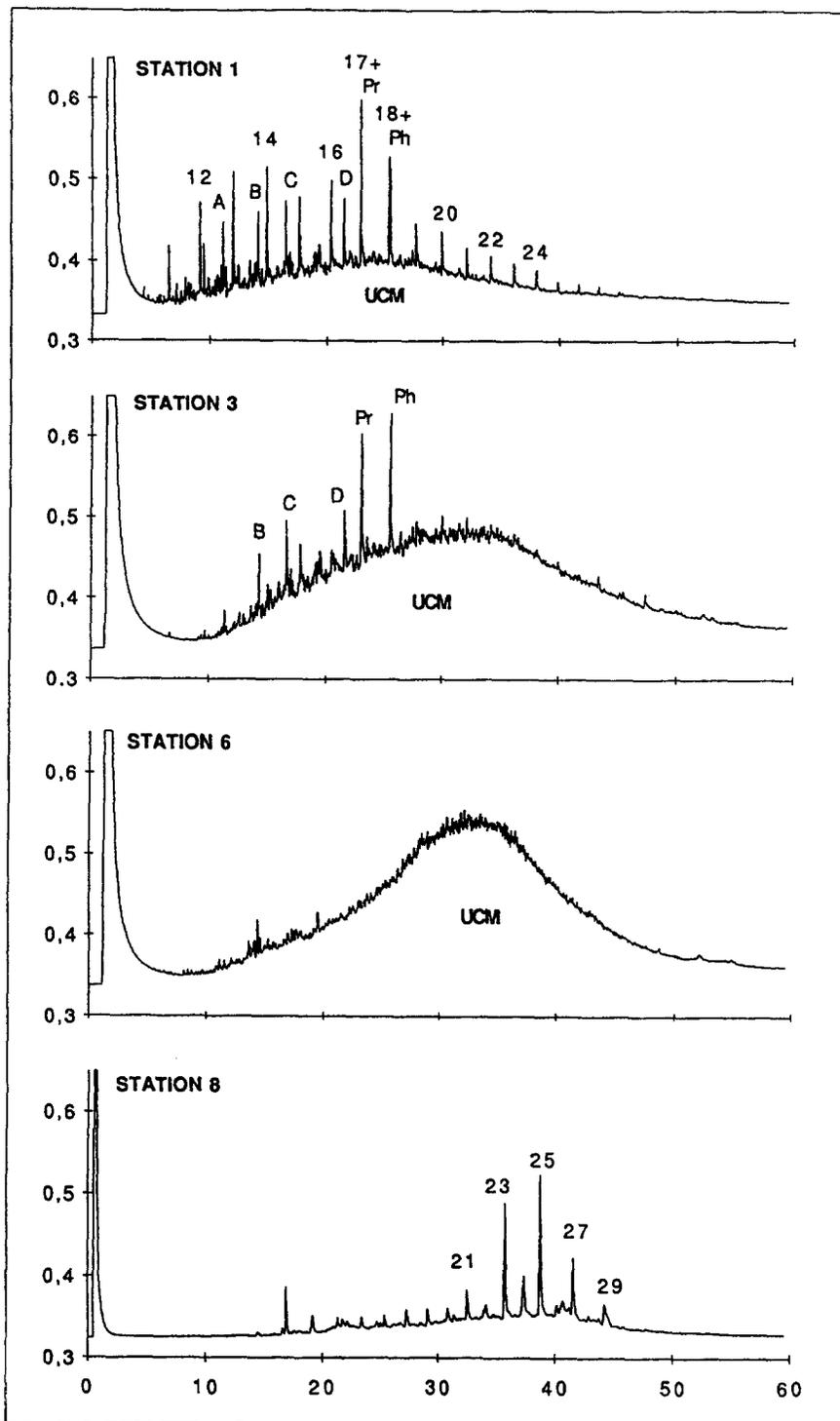


Fig. 2 Gas chromatograms of saturated hydrocarbon fractions (stations 1, 3, 6, 8; 2-7 cm layer). UCM = Unresolved Complex Mixture, *n* (12 to 29) = alkanes with '*n*' atoms of carbon, Pr = Pristane (2, 6, 10, 14 C₁₉), Ph = Phytane (2, 6, 10, 14 C₂₀), A = Norfarnesane (2, 6, 10 C₁₄), B = Farnesane (2, 6, 10 C₁₅), C = Isoprenoid (2, 6, 10 C₁₆), D = Norpristane (2, 6, 10 C₁₈).

and even *n*-alkanes, without a predominance of either which monotonically decreases with the increase of the molecular weight. The presence of pristane, phytane and other lighter branched alkanes as norfarnesane, farnesane, norpristane (Gassmann, 1981) is detected and identified by GC/MS. A medium UCM is observed. These chromatographic characteristics correspond to criteria mentioned by Requejo and Boehm (1985) and by Kennicutt *et al.* (1991), which are specific to petroleum hydrocarbons. Chromatograms relative to stations 2 and 3 do not show any evidence of peaks corresponding to *n*-alkanes. Only peaks corresponding to isoprenoid compounds (pristane and phytane especially) which emerge from a large UCM. No compounds were identifiable and only a large UCM was visible on chromatograms for stations 4 to 6 (in the deep creek).

The GC profiles for stations 7 and 8 (in the open sea) are notably different from the others. They show a strong predominance of odd *n*-alkanes between C₂₁ to C₂₉, which are characteristic of hydrocarbons of terrestrial origin. A similar distribution of *n*-alkanes has also been found in sediments from the Derwent River (Volkman *et al.*, 1992) and attributed to plant wax inputs. A far smaller UCM, in which the maximum is in the higher carbon atom numbers (about C₂₅) zone than for the other stations, is also observed for stations 7 and 8.

A UCM on the saturated hydrocarbon gas chromatogram is often attributed to degraded petroleum contamination (Jones *et al.*, 1983) although an alternative source can be the degraded algal detritus and other microorganisms (Johnson and Calder, 1973). The UCM is usually said to contain a multitude of branched and cyclic compounds that cannot be resolved by capillary columns. However Gough and Rowland (1990) have shown using chemical degradation techniques that the UCM primarily consists of linear chains connected to branched points giving rise to 'T-shaped' molecules. These molecules are resistant to biodegradation and thus accumulate in sediments. The UCM is shifted to the higher carbon atom number zone when

the alteration degree increases. This phenomenon is clearly observed for stations 1–6.

The indices given in Table 3 were calculated from the chromatographic analyses. The Carbon Predominance Index (CPI) represents the relative abundance of odd numbered linear alkanes in front of even numbered linear alkanes (Bray and Evans, 1961). This CPI is around 1 for the first four stations, which characterizes petroleum hydrocarbons (Morel *et al.*, 1991). However these indices are higher (around 2) for stations 7 and 8 and indicate the presence of biogenic compounds (Volkman *et al.*, 1981).

Pristane and phytane are common isoprenoids detected in coastal marine sediments (Clark and Blumer, 1967) with an abundance depending on the local environment, but they are also present in most crude oils. In most cases it has been shown that they derive from the phytyl side chain of chlorophyll, either under reduction conditions (phytane) or oxidation conditions (pristane) (Blumer *et al.*, 1963), but sometimes pristane and phytane may have other sources. For example, pristane can originate as a direct input from the lipids of certain zooplankton and phytane has been identified in the lipids of archeobacteria such as methanogens (Risatti *et al.*, 1984).

The ratios of *n*-C₁₇ to pristane and *n*-C₁₈ to phytane (calculated from the GC/MS data) are very low ($0 < C_{17}/Pr < 1$) for the stations located in the canal and at the mouth of the deep creek, which reveal a weathering process on linear alkanes rather than on branched alkanes (Seifert and Moldowan, 1979; Wang *et al.*, 1994). These ratios are higher ($0.5 < C_{17}/Pr < 2.1$) for stations 7 and 8 showing biogenic inputs.

In contrast to petroleum distributions, *n*-C₁₇ is predominant in algae lipid distributions (Gelpi *et al.*, 1970; Wakeham, 1990) and in lipids of several marine bacteria (Han and Calvin, 1969; Shiea *et al.*, 1991), whereas *n*-C₂₉ is abundant in terrestrial plants (Simoneit, 1978). Thus the *n*-C₂₉:*n*-C₁₇ ratio gives information about the relative contribution of terrestrial inputs and marine inputs to the repartition of sedimental biogenic hydrocarbons (Eglinton and

TABLE 3
Indices calculated on the basis of GC/MS analysis of acyclic alkanes.

| STATIONS | CPI | | | | C ₁₇ /Pr | | | | C ₁₈ /Ph | | | | C ₂₉ /C ₁₇ | | | | | | | |
|----------|--------|--------|---------|----------|---------------------|------|------|------|---------------------|------|------|------|----------------------------------|------|------|------|------|------|------|------|
| | 0-2 cm | 2-7 cm | 7-17 cm | 17-27 cm | 0.00 | 0.37 | 0.67 | 0.34 | 0.00 | 0.36 | 0.21 | 0.00 | 0.24 | 0.39 | 0.61 | 0.36 | | | | |
| 1 | 0-2 cm | 2-7 cm | 7-17 cm | 17-27 cm | 0.67 | 0.77 | 0.91 | 1.75 | 0.22 | 0.32 | 0.66 | 0.36 | 0.14 | 0.20 | 0.26 | 0.28 | 0.74 | 0.48 | 0.64 | 1.02 |
| 2 | 0-2 cm | 2-7 cm | 7-17 cm | 17-27 cm | 0.99 | 0.92 | 1.12 | 0.67 | 0.97 | 0.71 | 0.12 | 0.00 | 1.11 | 0.38 | 0.00 | 0.00 | 0.23 | 0.22 | 0.85 | ND |
| 3 | 0-2 cm | 2-7 cm | 7-17 cm | 17-27 cm | 0.59 | 0.98 | 0.53 | 0.00 | 0.50 | 0.50 | 0.44 | 0.46 | 0.25 | 0.18 | 0.21 | 0.18 | 2.77 | 0.68 | 0.76 | 0.84 |
| 4 | 0-2 cm | 2-7 cm | 7-17 cm | 17-27 cm | 0.84 | 1.09 | 1.18 | 1.32 | 1.02 | 0.98 | 0.84 | 0.96 | 0.29 | 0.31 | 0.57 | 0.43 | 0.43 | 0.34 | 0.80 | 1.68 |
| 5 | 0-2 cm | 2-7 cm | 7-17 cm | 17-27 cm | 0.74 | 1.40 | 1.10 | 0.92 | 1.23 | ND | ND | ND | 0.38 | ND | ND | ND | 1.47 | ND | ND | ND |
| 6 | 0-2 cm | 2-7 cm | 7-17 cm | 17-27 cm | 0.65 | ND | ND | ND | 2.24 | 1.43 | 1.41 | 1.14 | 1.50 | 1.50 | 1.36 | 1.69 | 2.47 | 3.00 | 2.63 | 3.32 |
| 7 | 0-2 cm | 2-7 cm | 7-17 cm | 17-27 cm | 1.93 | 1.86 | 1.21 | 1.97 | 1.71 | 1.85 | 1.21 | 1.13 | 0.89 | 1.55 | 1.66 | 1.85 | 0.74 | 0.52 | 0.44 | 3.56 |
| 8 | 0-2 cm | 2-7 cm | 7-17 cm | 17-27 cm | 2.11 | 2.01 | 2.62 | 4.17 | | | | | | | | | | | | |

C₁₇, C₁₈, C₂₉ = *n*-alkanes with 17, 18, 29 carbon atoms.

Pr = Pristane, Ph = Phytane.

CPI = Carbon Predominance Index (from *n*-C₂₀ to *n*-C₃₂).

ND = Not determined.

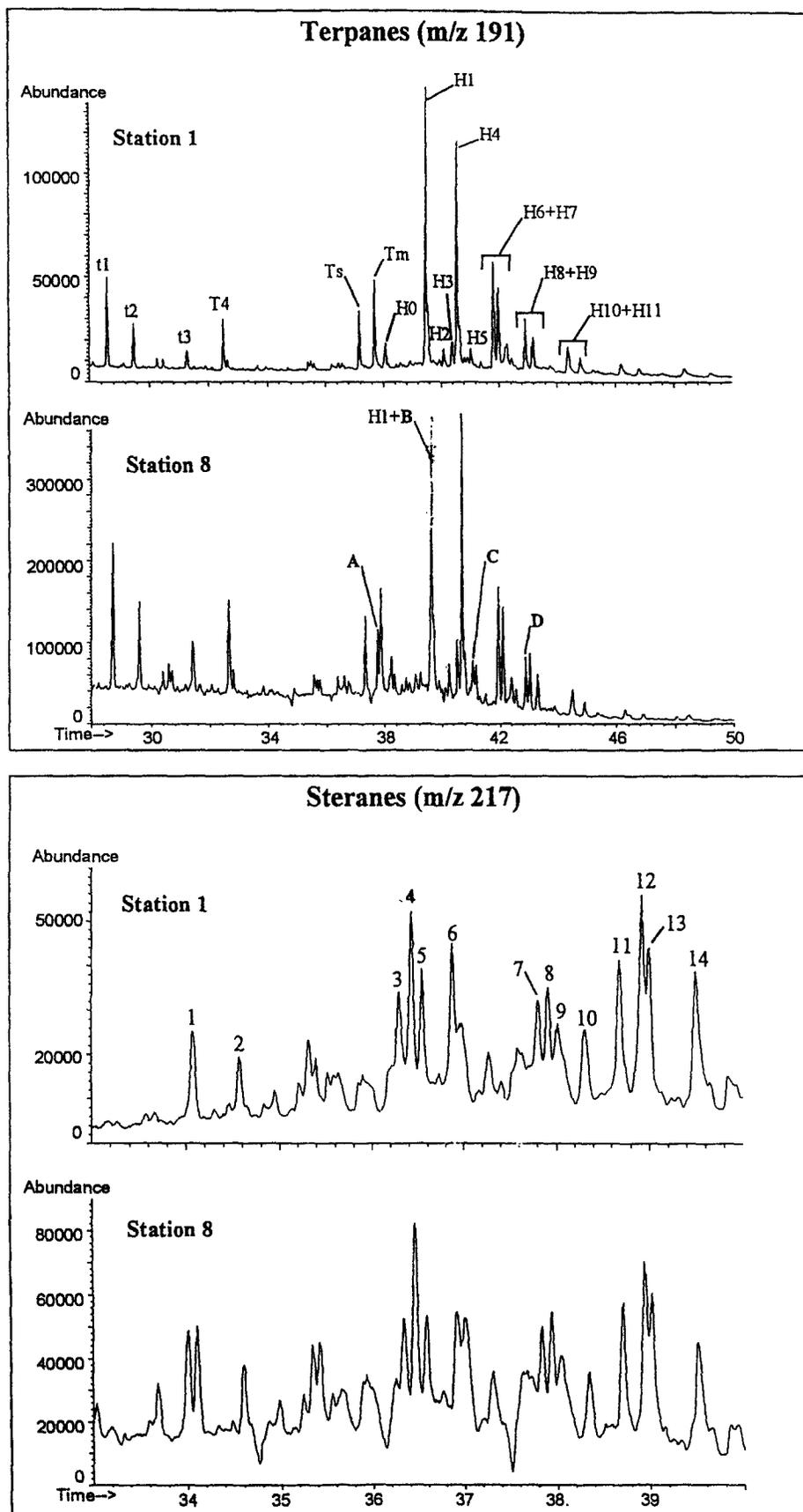


Fig. 3 Chromatograms of tricyclic terpanes, tetracyclic terpanes and pentacyclic terpanes (hopanes) (m/z=191), steranes and diasteranes (m/z 217) (stations 1 and 8; 2-7 cm layer).

TABLE 4
Terpanes and steranes attributions (see Fig. 3).

| Peak | Assignment |
|-----------------|---|
| Terpanes | |
| t1 | C23 tricyclic terpane |
| t2 | C24 tricyclic terpane |
| t3 | C25 tricyclic terpane |
| T4 | C24 tetracyclic terpane |
| Ts | C27 18 α (H) 22,29,30 trinorneohopane |
| Tm | C27 17 α (H) 22,29,30 trinorhopane |
| H0 | C28 17 β (H) 21 α (H) 29,30 bisnormoretane |
| H1 | C29 17 α (H) 21 β (H) 30 norhopane |
| H2 | C29 17 β (H) 21 α (H) 30 normoretane |
| H3 | C30 oleanane |
| H4 | C30 17 α (H) 21 β (H) hopane |
| H5 | C30 17 β (H) 21 α (H) 30 moretane |
| H6 | C31 17 α (H) 21 β (H) homohopane 22S |
| H7 | C31 17 α (H) 21 β (H) homohopane 22R |
| H8 | C32 17 α (H) 21 β (H) bishomohopane 22S |
| H9 | C32 17 α (H) 21 β (H) bishomohopane 22R |
| H10 | C33 17 α (H) 21 β (H) trihomohopane 22S |
| H11 | C33 17 α (H) 21 β (H) trihomohopane 22R |
| A | C27 17 α (H) 22,29,30 trinorhop17(21)ene |
| B | C30 olean12ene |
| C | C30 hopene |
| D | C30 hop22(27)ene |
| Steranes | |
| 1 | C27 13 β (H) 17 α (H) diacholestane 20S |
| 2 | C27 13 β (H) 17 α (H) diacholestane 20R |
| 3 | C27 5 α (H) 14 α (H) 17 α (H) cholestane 20S |
| 4 | C27 5 α (H) 14 β (H) 17 β (H) cholestane 20R + C29 13 β (H) 17 α (H) ethyl (24) diacholestane 20S |
| 5 | C27 5 α (H) 14 β (H) 17 β (H) cholestane 20S |
| 6 | C27 5 α (H) 14 α (H) 17 α (H) cholestane 20R |
| 7 | C28 5 α (H) 13 β (H) 17 β (H) ethyl (24) cholestane 20R |
| 8 | C28 5 α (H) 14 β (H) 17 β (H) ethyl (24) cholestane 20S |
| 9 | C29 13 α (H) 17 β (H) ethyl (24) diacholestane 20R |
| 10 | C28 5 α (H) 14 α (H) 17 α (H) ethyl (24) cholestane 20R |
| 11 | C29 5 α (H) 14 α (H) 17 α (H) ethyl (24) cholestane 20S |
| 12 | C29 5 α (H) 14 β (H) 17 β (H) ethyl (24) cholestane 20R |
| 13 | C29 5 α (H) 14 β (H) 17 β (H) ethyl (24) cholestane 20S |
| 14 | C29 5 α (H) 14 α (H) 17 α (H) ethyl (24) cholestane 20R |

Hamilton, 1967). The n -C₂₉: n -C₁₇ ratio was greater than 1 for station 7. This suggests a rather terrestrial biogenic origin for the detected hydrocarbons. The n -C₂₉: n -C₁₇ ratio was less than 1 for station 8. It indicates a rather marine (algae and bacterial) biogenic origin of the sedimentary organic matter. Because the detected hydrocarbons were mainly petroleum hydrocarbons this ratio could not be reasonably used for stations 1 to 6.

Cycloalkanes are less susceptible to degradation than non-cyclic alkanes and present the same molecular skeleton as their biological precursors. Some families (hopanes, steranes ...) are often used in petroleum geochemistry in order to determine the alteration and maturity degree of petroleum in the deposition basins (Moldowan *et al.*, 1991; Ten Haven *et al.*, 1986). Parameters based on these compounds are also the major discriminants which allow the identification of crude oil spilled in the natural environment and are used in some environmental studies (Page *et al.*, 1988; Porte *et al.*, 1990; Hostettler *et al.*, 1992; Kvenvolden *et al.*, 1993; Hostettler and Kvenvolden, 1994; Vandermeulen and Singh, 1994). On the basis of these works these

TABLE 5
Indexes calculated on the base of GC/MS analyses of terpanes and steranes.

| STATIONS | Tm/Ts | | | | | | I | II | III | IV | V | VI | | | | | | | | | | | | |
|----------|--------|--------|---------|----------|---------|----------|----|----|-----|----|----|----|----|----|----|----|-----|----|----|-----|----|----|----|----|
| | 0-2 cm | 2-7 cm | 7-17 cm | 17-27 cm | 7-17 cm | 17-27 cm | | | | | | | | | | | | | | | | | | |
| 1 | 0-2 cm | 2-7 cm | 7-17 cm | 17-27 cm | 55 | ND | 36 | 52 | 48 | 53 | 54 | 55 | 0 | 26 | 46 | 54 | 69 | 46 | 29 | 27 | 68 | 54 | 39 | 35 |
| 2 | 0-2 cm | 2-7 cm | 7-17 cm | 17-27 cm | 49 | 56 | 49 | 72 | 58 | 70 | 61 | 65 | 65 | 57 | 60 | ND | 42 | 34 | 45 | 100 | 31 | 43 | 50 | ND |
| 3 | 0-2 cm | 2-7 cm | 7-17 cm | 17-27 cm | 54 | 53 | 26 | ND | 55 | 59 | 29 | ND | 53 | 49 | 27 | ND | 27 | 41 | 18 | ND | 28 | 50 | 15 | ND |
| 4 | 0-2 cm | 2-7 cm | 7-17 cm | 17-27 cm | 54 | 54 | 52 | 52 | 60 | 57 | 56 | 61 | 51 | 52 | 53 | 54 | 36 | 39 | 38 | 43 | 44 | 48 | 45 | 46 |
| 5 | 0-2 cm | 2-7 cm | 7-17 cm | 17-27 cm | 53 | 52 | 53 | 52 | 59 | 59 | 53 | 57 | 26 | 39 | 51 | 52 | 68 | 58 | 47 | 39 | 51 | 51 | 49 | 47 |
| 6 | 0-2 cm | 2-7 cm | 7-17 cm | 17-27 cm | 52 | 55 | 53 | 53 | 58 | 60 | 48 | 53 | 4 | 71 | 32 | 38 | 100 | 76 | 56 | 36 | 58 | 56 | 54 | 48 |
| 7 | 0-2 cm | 2-7 cm | 7-17 cm | 17-27 cm | 53 | 55 | 53 | 52 | 53 | 59 | 56 | 55 | 53 | 53 | 56 | 62 | 29 | 38 | 49 | 37 | 43 | 35 | 32 | 43 |
| 8 | 0-2 cm | 2-7 cm | 7-17 cm | 17-27 cm | 54 | 54 | 53 | 51 | 59 | 58 | 58 | 63 | 52 | 47 | 40 | 43 | 30 | 31 | 54 | 59 | 43 | 45 | 50 | 50 |

Tm/Ts = C₂₇ 17 α (H) 22, 29, 30 trinorhopane/C₂₇ 18 α (H) 22, 29, 30 trinorneohopane.

I (%) = C₃₁S/C₃₁(S+R) hopanes.

II (%) = C₂₃ tricyclic terpane/C₃₀ hopane.

III (%) = C₂₉ norhopane/C₃₀ hopane.

IV (%) = C₂₉ sterane S/(S+R).

V (%) = C₂₇ diasteranes (S+R)/C₂₉ sterane.

VI (%) = C₂₈ steranes (S+R)/C₂₉ sterane.

ND = Not determined.

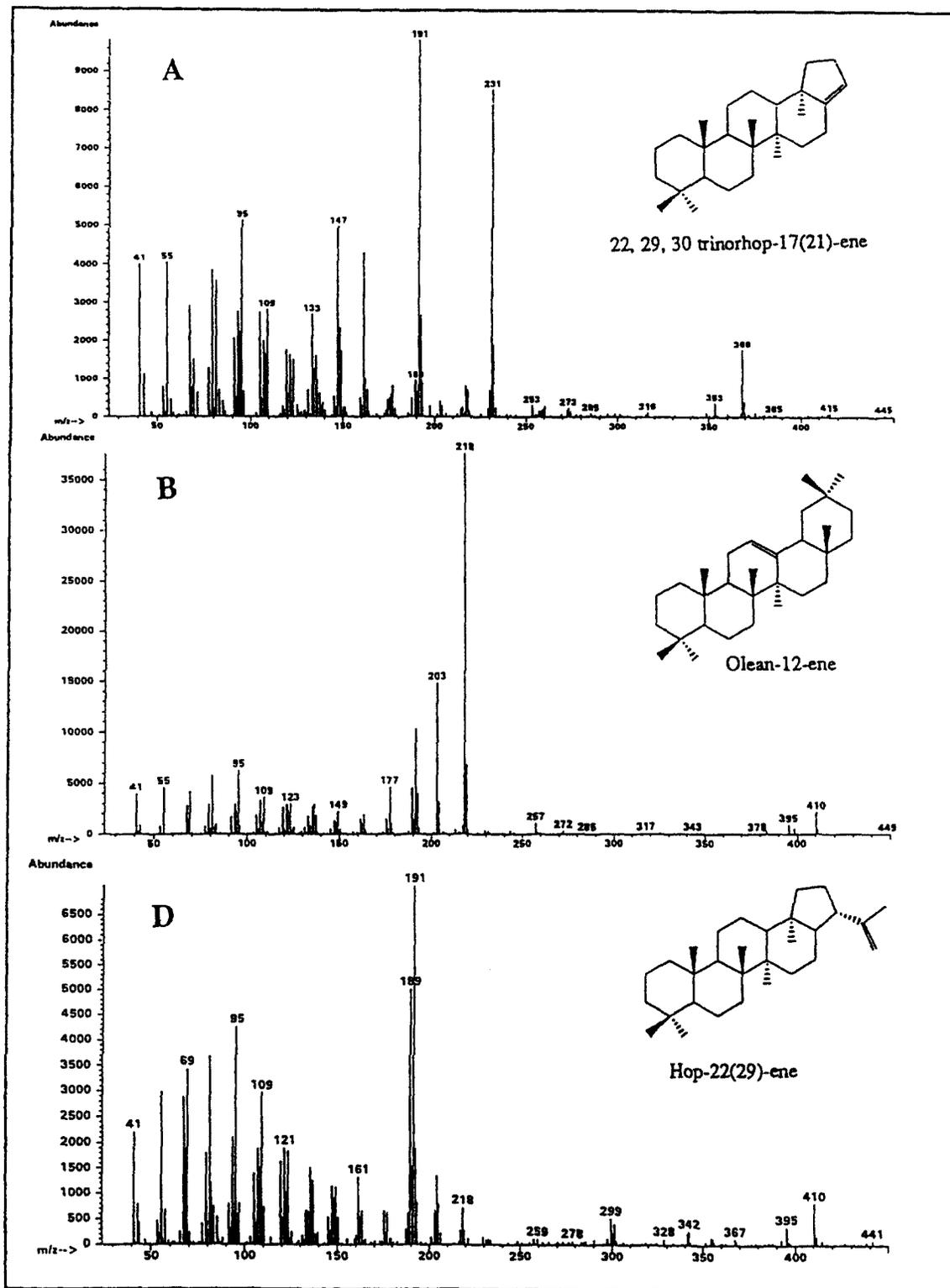


Fig. 4 Mass spectra of compounds A, B, D detected in Fig. 3.

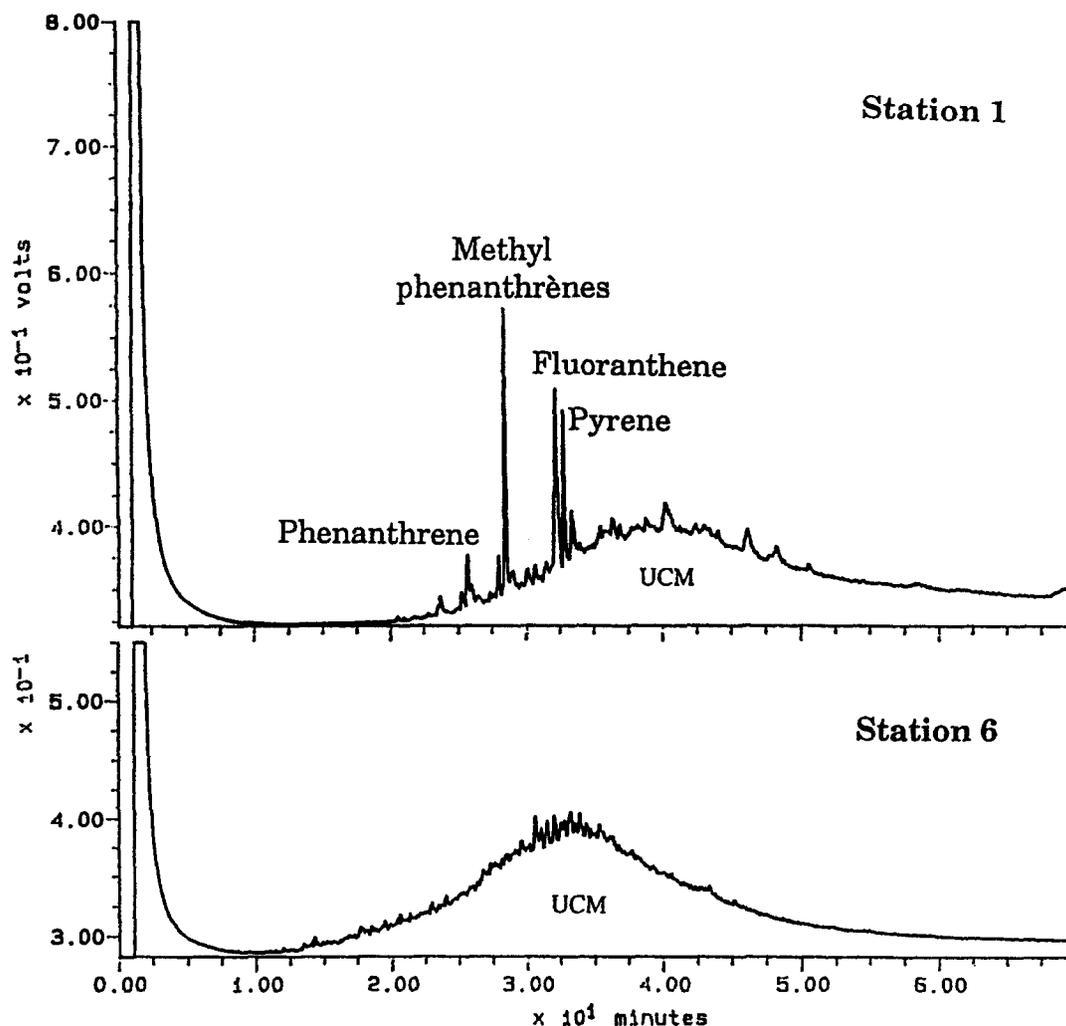


Fig. 5 Gas chromatograms of aromatic hydrocarbon fractions (stations 1 and 6; 2-7 cm layer).

compounds are also analysed (Fig. 3) by GC/MS in SIR mode in order to obtain fingerprints characteristic of hopane and sterane families. Attributions are made using retention times and previous works quoted above and are given in Table 4.

The terpane distributions (Fig. 3) for each sample range from C_{19} to C_{35} with the predominance of C_{30} hopane and C_{29} norhopane. In some samples, C_{23} and C_{24} tricyclic terpanes are relatively abundant. The sterane distributions (Fig. 3) for each sample ranges from C_{27} diasterane to C_{29} sterane, but the abundance of each compound differ from sample to sample. These distributions are specific to petroleum (Hostettler and Kvenvolden, 1994).

Considering the complexity of effluents, there is no characteristic profile which allows us to find identification of the pollutant crude oil. This obstacle has already been met, notably by Vandermeulen and Singh (1994) during the study of the sites contaminated by the Arrow oil but which underwent other anthropogenic inputs after the oil spill. Nevertheless profiles shown in Fig. 3 are quite different. For example C_{27} steranes are more abundant than C_{29} steranes for station 8 and the

opposite for station 1. It can suggest either that the hydrocarbons detected did not originate from the same petroleum mixture, or that the petroleum mixture has been altered. The second hypothesis does not seem possible because Peters and Moldowan (1993) showed that, in altered petroleum, the C_{29} steranes are predominant compared to C_{27} .

Indices generally used in geochemistry and in environmental studies have been also calculated for all samples (Table 5): C_{23} tricyclic terpane/ C_{30} hopane (Williams *et al.*, 1986), C_{29}/C_{30} hopane (Palacas *et al.*, 1986), C_{31} S/(S+R) hopanes (Ensminger *et al.*, 1974), C_{29} S/(S+R) steranes (Mackenzie *et al.*, 1980, Brault and Simoneit, 1988), C_{27} diasterane/ C_{29} sterane (Jiamo *et al.*, 1990), C_{28}/C_{29} sterane (Grantham and Wakefield, 1988). They are stable for all the stations. Consequently no relationship can be reasonably established neither depending on the depth nor on the distance from the effluent source.

Whereas the GC/FID profiles corresponding to stations 7 and 8 show only *n*-alkanes characteristic of a biogenic origin, the GC/MS (full scan mode) analysis reveals distributions of steranes (from C_{27} diasteranes to

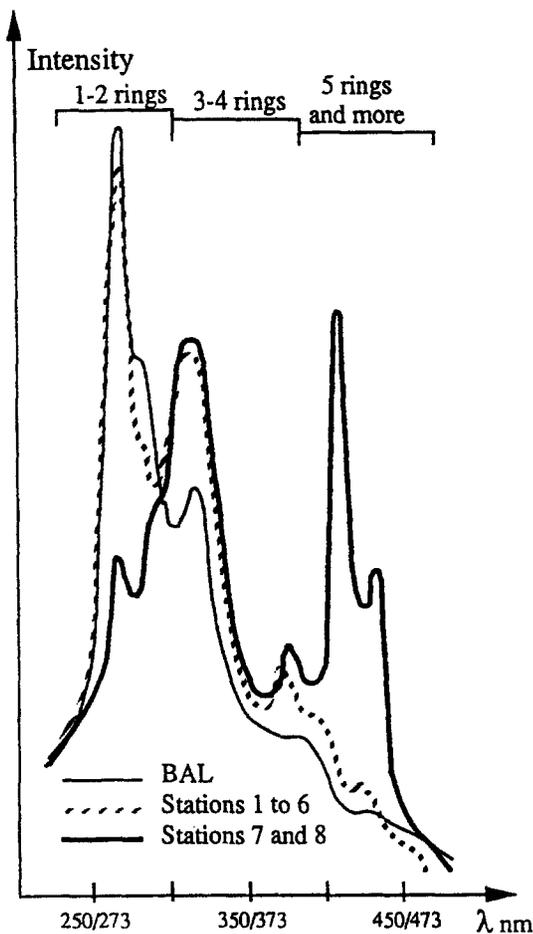


Fig. 6 UV synchronous ($\Delta\lambda = 23$ nm) fluorescence spectra (stations 1-8).

C_{29} steranes) and terpanes (from C_{19} to C_{35}) characteristic of petroleum contamination as for the other stations. However, in the hopane distribution other compounds (peaks A, B, C, D in Fig. 3) have a suspected biological origin. The mass spectra of compounds A, B and D (Fig. 4) are identified by comparison with literature data (Philp, 1985). For example peak 'A' (Fig. 3) is certainly a diplotene which is a bacterial biogenic compound.

Aromatic hydrocarbons contained in sediments may have a petrogenic, pyrolytic and diagenetic or biogenic origin (Laflamme and Hites, 1978). As in the case of a saturated fraction the chromatograms for aromatic fractions (Fig. 5) show a UCM and very few resolved peaks. This distribution pattern confirms that these hydrocarbons have mainly a petroleum origin (Wakeham *et al.*, 1980; Jones *et al.*, 1983).

The UV fluorescence spectrometry analyses of aromatic fractions (Fig. 6) show profiles for each station compared to a specific crude oil [Brut Arabian Light (BAL) 250] used as a reference, because it has been treated by the Lavera refinery. Profiles are similar for the first six stations. PAH mixtures contain large amounts of two-ring (naphthalene derivatives characteristic of recent effluents) and three-ring (phenanthrene

derivatives) compounds and very few four-ring compounds (pyrene derivatives). These four-ring compounds were not detected in crude oil. Profiles obtained for stations 7 and 8 are very different from the others. The concentrations of light compounds (two rings) are lower than the concentrations of three-ring compounds. Four-ring compounds are also detected. Heavy compounds (five or more rings) such as benzopyrene derivatives, which have pyrolytic origins, and perylene derivatives, which have terrigenous origins, (Laflamme and Hites, 1978; Parlanti, 1990) are present.

The search of selected polycyclic aromatic hydrocarbons by GC/MS, or the estimation of the relative proportion between methylated derivatives and parent compounds for a given class of compounds, are often carried out for the identification of hydrocarbon origins and allow a characterization of pollution (Kennicutt *et al.*, 1992). Fig. 7 shows the phenanthrene (P) ($m/z = 178$), methyl phenanthrenes (MP) ($m/z = 192$) and dimethyl phenanthrenes (DMP) ($m/z = 206$) fingerprints for station 1 and station 8 which are the most representative of the different stations according to the UV fluorescence spectrometry profiles. In the samples collected from station 1 mono and di-alkylated phenanthrene are present in amounts comparable to those of the parent phenanthrenes, as in typical crude oils (Prah and Carpenter, 1983).

In samples collected from station 8, 3 MP have a slight abundance relatively to 9+4 and 1 MP. The same observation has been made by Bayona *et al.* (1986) who indicated that this isomer is lost before 9 MP in condition of biodegradation. Concerning the dimethyl phenanthrenes (DMPs), we observe an important decrease of these compounds in station 8 compared to station 1. DMPs have a slighter abundance relatively to MPs in station 8 than in station 1. This observation seems different from what Hostettler and Kvenvolden (1994) found in their study about the *Exxon Valdez* oil spill and from the order of oxic degradation established by Volkman *et al.* (1984) in favour of monomethylated derivatives relatively to dimethylated derivatives. These observations could be due to different weathering conditions from those described by Hostettler and Kvenvolden (1994) and also to the overlapping of different sources of hydrocarbons.

Anomalous important abundance of anthracene (A) is observed at station 8. P:A ratio calculated for stations 8 and 1 are respectively equal to 0.25 and 1.4. P:A ratio is usually close to 50 for crude oil and ranges from 3 to 26 in sediment samples (Colombo *et al.*, 1989). The obtained value for station 8 is atypical but it has been found in some sediments (Giger and Blumer, 1974). Anthracene is generally attributed to pyrolytic sources (petroleum products and/or forest fires) (Soclo, 1986). Observation made for station 8 could be explained by the fact that the study area surroundings are urban and industrial, and forest fires are common in the Mediterranean coastal zone.

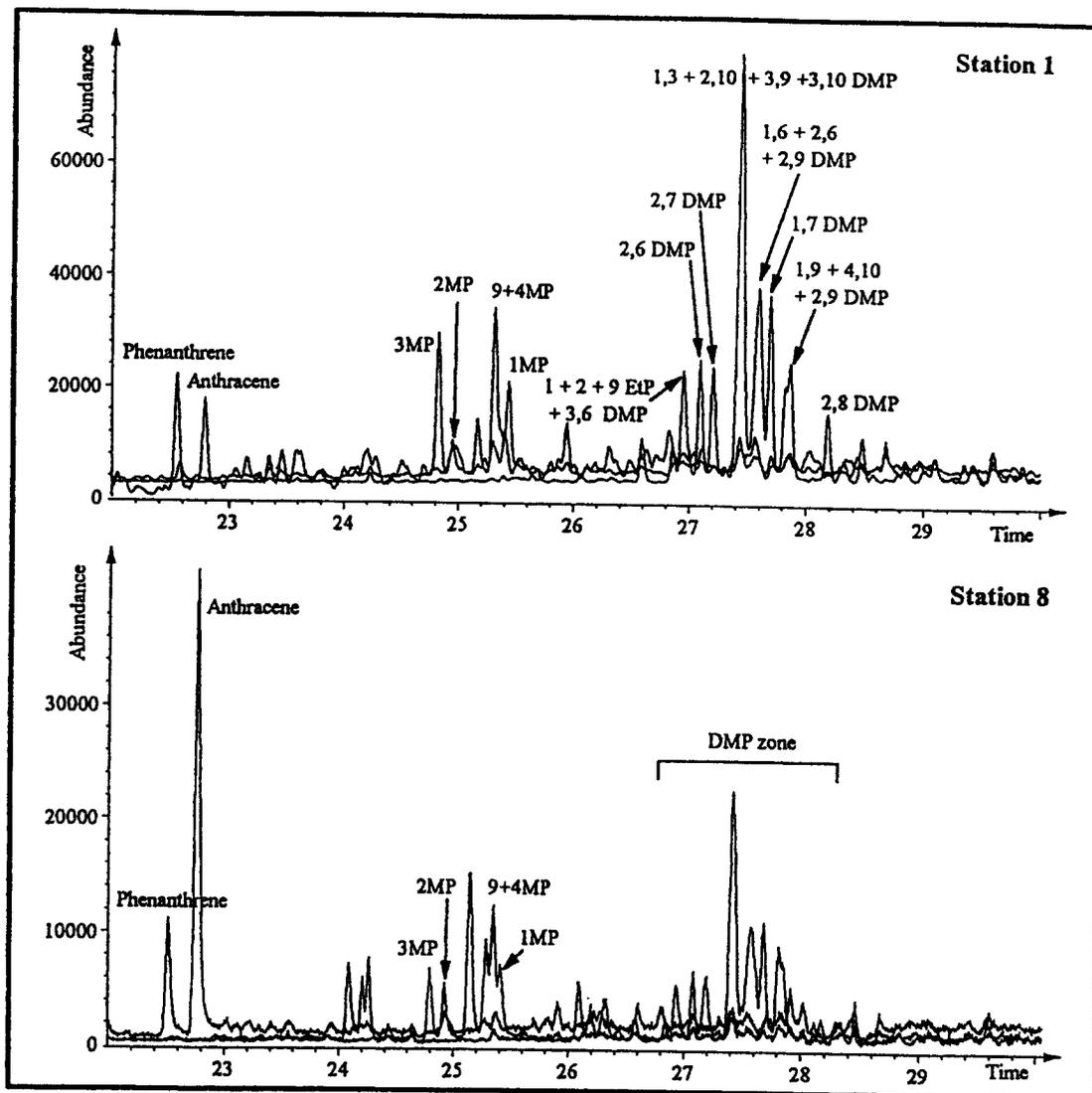


Fig. 7 Mass chromatograms of phenanthrene ($m/z=178$), methylphenanthrenes ($m/z=192$) and dimethylphenanthrenes ($m/z=206$) (stations 1 and 8; 2–7 cm layer).

The relative abundances of different classes of phenanthrenic compounds (parent, mono and dimethylated derivatives) are presented in Fig. 8. The relative repartition of methyl phenanthrenes in comparison with phenanthrene is similar for stations 1 to 6 even if monomethylated derivatives are more abundant in station 1 than in the other stations. This profile corresponds to a petrogenic origin of these compounds (Garrigues *et al.*, 1988). In contrast, for stations 7 and 8, phenanthrene predominates compared with methylated and dimethylated derivatives. This profile is characteristic of pyrolytic PAHs resulting from atmospheric combustion residues (forest fires and fossil fuels) and confirms the lower petroleum influence shown by the saturated compound analysis.

Conclusion

This work illustrates the present state of the site for hydrocarbon pollution two years after refinery effluent

discharges have been stopped. It is a starting point to for subsequent studies. It will allow better comprehension of the degradation hydrocarbons and will determine the degree to which this site has been rehabilitated.

The petroleum contamination influence has been detected at up to station 6, even if the hydrocarbon concentration had decreased. This decrease is not regular but gradual. Three zones are observed:

A very highly contaminated zone (about 50 g kg^{-1} sediment dry wt) near the refinery (stations 1 and 2),
A less contaminated zone (about 3 g kg^{-1} sediment dry wt) in the deep creek (stations 3 to 6).

A slightly contaminated zone (about 0.1 g kg^{-1} sediment dry wt) in the open sea (stations 7 and 8), which corresponds to rates found in most of the Mediterranean coastal sediments (Mille *et al.*, 1992).

These contamination rates are lower than those found by Mille *et al.* (1985) in the same stations (from 1 to 4)

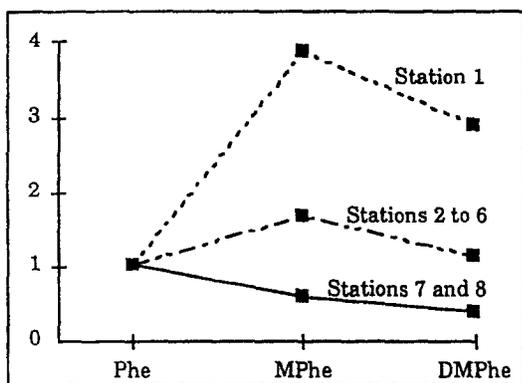


Fig. 8 Relative abundances of phenanthrene and mono- and di-methylated derivatives.

during overspills (from 3 to 10 times less), especially at the surface.

Moreover sediments of stations 7 and 8 mainly contained biogenic hydrocarbons and some pyrolytic poly-aromatic hydrocarbons. Nevertheless the GC/MS analyses of hopanes and steranes specific to petroleum, indicate that traces of petrogenic hydrocarbons are detected in these samples. Pyrolytic aromatic hydrocarbons are also detected and altered petrogenic aromatic hydrocarbon distributions were suspected. It is possible that the hydrocarbons detected do not come from the same petroleum mixture found near the refinery (steranes distribution were quite different). These observations are not surprising since the studied area is an urban and industrial zone and many forest fires have occurred in the surroundings in summer time. Moreover the 'Golfe de Fos' is also a navigation zone that is submitted to hydrocarbon contamination sources.

This work has been undertaken in the framework of the GDR Elf Aquitaine/CNRS 'Hycar' no. 1123.

- Bayona, J. M., Albaiges, J., Salanas, A. M., Pares, R., Garrigues, P. and Ewald, M. (1986) Selective aerobic degradation of methyl substituted-aromatic hydrocarbons in petroleum by pure microbial cultures. *International Journal of Environmental Analytical Chemistry* **23**, 239-303.
- Blumer, M., Mullin, M. M. and Thomas, D. W. (1963) Pristane in zooplankton. *Science* **140**, 974-981.
- Brault, M. and Simoneit, B. R. T. (1988) Steroid and triterpenoid distributions in Bransfield Strait sediments: hydrothermally enhanced diagenetic transformations. *Advances in Organic Geochemistry* **13**(4-6), 697-705.
- Bray, E. E. and Evans, E. D. (1961) Distribution of *n*-paraffins as a clue to recognition of source beds. *Geochimica Cosmochimica Acta* **22**, 2-15.
- Clark, R. J. and Blumer, M. (1967) Distribution of *n*-paraffins in marine organisms and sediment. *Limnology and Oceanography* **12**, 79-87.
- Colombo, J. C., Pelletier, E., Brochu, C., Khalil, M. and Cotoggio, J. A. (1989) Determination of hydrocarbon sources using *n*-alkanes and polyaromatic hydrocarbon distribution indexes. Case study: Rio de la Plata Estuary, Argentina. *Environmental Science Technology* **23**, 888-894.
- Eglinton, G. and Hamilton, R. J. (1967) Leaf epicuticular waxes. *Science* **156**, 1322-1335.

- Ensminger, A., Van Dorsselaer, A., Spikerelle, C., Albrecht, P. and Ourisson, G. (1974) Pentacyclic triterpenes of the hopane type as ubiquitous geochemical markers. Origin and significance. *Adv. Org. Geochem.* 245-260.
- Garrigues, P., Connan, J., Parlanti, E., Bellocq, J. and Ewald, M. (1988) Relationship between rank and distribution of methylaromatic hydrocarbons for condensates of different origins. *Organic Geochemistry* **13**(4-6), 1115-1121.
- Gassmann, G. (1981) Chromatographic separation of diastereomeric isoprenoids for the identification of fossil oil contamination. *Marine Pollution Bulletin* **12**(3), 78-84.
- Gelpi, E., Schneider, H., Mann, J. and Oro, J. (1970) Hydrocarbons of geochemical significance in microscopic algae. *Phytochemistry* **9**, 603-612.
- Giger, W. and Blumer, M. (1974) Polycyclic aromatic hydrocarbons in the environment: isolation and characterisation by chromatography, visible, ultraviolet, and mass spectrometry. *Analytical Chemistry* **46**, 1663-1671.
- Gough, M. A. and Rowland, S. J. (1990) Characterisation of unresolved complex mixtures of hydrocarbons in petroleum. *Nature* **344**, 648-650.
- Grantham, P. J. and Wakefield, L. L. (1988) Variations in sterane carbon number distributions of marine source derived crude oils through geologic time. *Organic Geochemistry* **12**, 61-73.
- Han, J. and Calvin, M. (1969) Hydrocarbon distribution of algae and bacteria and microbiological activity in sediments. *Proceedings of the National Academy of Science USA* **64**, 436-443.
- Hostettler, F. D., Rapp, J. B. and Kvenvolden, K. A. (1992) Use of geochemical biomarkers in bottom sediment to track oil from a spill, San Francisco bay, California. *Marine Pollution Bulletin* **24**, 15-20.
- Hostettler, F. D. and Kvenvolden, K. A. (1994) Geological changes in crude oil spilled from the Exxon Valdez supertanker into Prince William Sound, Alaska. *Organic Geochemistry* **21**, 927-936.
- Jiamo, F., Guoying, S., Jiayou, X., Eglinton, G., Gowar, A. P., Rongfen, J., Shanfa, F. and Pingan, P. (1990) Application of biological markers in the assessment of paleoenvironments of Chinese non-marine sediments. *Organic Geochemistry* **16**, 769-779.
- Johnson, R. W. and Calder, J. A. (1973) Early diagenesis of fatty acids and hydrocarbons in a salt marsh environment. *Geochimica Cosmochimica Acta* **37**, 1943-1955.
- Jones, D. M., Douglas, A. G., Parkes, R. J., Taylor, J., Giger, W. and Schaffner, C. (1983) The recognition of biodegraded petroleum derived aromatic hydrocarbons in recent marine sediments. *Marine Pollution Bulletin* **14**, 103-108.
- Kennicutt, M. C. Jr II, Sweet, S. T., Fraser, W. R., Stockton, W. L. and Culver, M. (1991) The grounding of the Bahia Paraiso, Arthur Harbour - I Antarctic. I. Distribution and fate of oil spill related hydrocarbons. *Environmental Science Technology* **25**, 509-518.
- Kennicutt, M. C. Jr II, McDonald, T. J., Denoux, G. J. and McDonald, S. J. (1992) Hydrocarbon contamination on the Antarctic peninsula I. Arthur Harbor-subtidal sediments. *Marine Pollution Bulletin* **24**, 499-506.
- Kvenvolden, K. A., Hostettler, F. D., Rapp, J. B. and Carlson, P. R. (1993) Hydrocarbons in oil residues on beaches of islands of Prince William Sound, Alaska. *Marine Pollution Bulletin* **26**, 24-29.
- Laflamme, R. E. and Hites, R. A. (1978) The global distribution of polycyclic aromatic hydrocarbons in recent sediments. *Geochimica Cosmochimica Acta* **42**, 289-303.
- Mackenzie, A. S., Patience, R. L., Maxwell, J. R., Vandembroucke, M. and Durand, B. (1980) Molecular parameters of maturation in the Toarcian Shales, Paris Basin, France - I. Changes in the configuration of acyclic isoprenoid alkanes, steranes and triterpanes. *Geochimica Cosmochimica Acta* **44**, 1709-1721.
- Mille, G., Chen, J. Y., Dou, H., Azoulay, E., Colin, M. and Bertrand, J. C. (1985) Evolution des hydrocarbures dans des sédiments côtiers méditerranéens prélevés à proximité d'un émissaire d'une raffinerie de pétrole. *Marine Environmental Research* **17**, 65-80.
- Mille, G., El Jammal, T., Doumenq, P. and Bertrand, J. C. (1992) Hydrocarbons and fatty acids in surficial sediments (French mediterranean coastal zones). *Science of the Total Environment* **113**, 209-228.
- Moldowan, J. M., Fago, J. F., Carlson, R. M. K., Young, D. C., Duyn, G. V., Clardy, J., Schoell, M., Pillinger, C. T. and Watt, D. S. (1991) Rearranged hopanes in sediments and petroleum. *Geochimica Cosmochimica Acta* **55**, 3333-3353.

- Morel, G., Samhan, O., Literathy, P., Al-Hashhash, H., Moulin, L., Saeed, T., Al-Matrouk, K., Martin-Bouyer, M., Saber, A., Paturel, L., Jarosz, J., Vial, M., Combet, C., Fachinger, C. and Suptil, J. (1991) Evaluation of chromatographic and spectroscopic methods for the analysis of petroleum-derived compounds in the environment. *Fresenius Journal of Analytical Chemistry* **339**, 699-715.
- Page, D. S., Fostez, J. C., Fickett, P. M. and Gilfillan, E. S. (1988) Identification of petroleum sources in an area impacted by the Amoco Cadiz oil spill. *Marine Pollution Bulletin* **19**, 103-115.
- Palacas, J. G., Monopolis, D., Nicolaou, C. A. and Anders, D. E. (1986) Geochemical correlation of surface and subsurface oils, Western Greece. *Organic Geochemistry* **10**, 417-423.
- Parlanti, E. (1990) Utilisation des hydrocarbures comme traceurs d'origine et d'évolution de la matière organique sédimentation en milieu marin. Etude du Golfe du Lion et du Golfe de Gascogne. Thesis, Université de Bordeaux I, no. 495.
- Peters, K. E. and Moldowan, J. M. (1993) The biomarker guide: interpreting molecular fossils in petroleum and ancient sediments, pp. 363, Prentice-Hall Ed.
- Philp, R. P. (1985) Fossil fuel biomarkers, applications and spectra, pp. 294, Elsevier Ed.
- Porte, C., Barcelo, D., Tavares, T. M., Rocha, V. C. and Albaiges, J. (1990) The use of mussel watch and molecular marker concepts in studies of hydrocarbons in a tropical bay (Todos Os Santos, Bahia, Brazil). *Archives of Environmental Contamination and Toxicology* **19**, 263-274.
- Prahl, F. G. and Carpenter, R. (1983) Polycyclic aromatic hydrocarbons (PAH)-phase associations in Washington coastal sediment. *Geochimica Cosmochimica Acta* **47**, 1013-1023.
- Requejo, A. G. and Boehm, P. D. (1985) Characterisation of hydrocarbons in a subsurface oil-rich layer in the Sargasso Sea. *Marine Environmental Research* **17**, 45-64.
- Risatti, J. B., Rowland, D. A., Yon, D. A. and Maxwell, J. R. (1984) Stereochemical studies of acyclic isoprenoids - XII. Lipids of methanogenic bacteria and possible contributions to sediments. *Organic Geochemistry* **6**, 93-104.
- Seifert, W. K. and Moldowan, J. M. (1979) The effect of biodegradation on steranes and terpanes in crude oils. *Geochimica Cosmochimica Acta* **43**, 111-126.
- Shiea, J., Brassel, S. C. and Ward, D. M. (1991) Comparative analysis of extractable lipids in hot spring microbial mats and their component photosynthetic bacteria. *Organic Geochemistry* **17**, 309-319.
- Simoneit, B. R. T. (1978) *Chemical Oceanography*, Eds J. P. Riley and R. Chester, pp. 233-311. Academic Press, London.
- Soclo, H. H. (1986) Etude de la distribution des hydrocarbures aromatiques polycycliques (HAP) dans les sédiments récents. Identification des sources. Thesis, Université de Bordeaux I, no. 50.
- Ten Haven, H. L., De Leeuw, J. W., Peakman, T. M. and Maxwell, J. R. (1986) Anomalies in steroid and hopanoid maturity indices. *Geochimica Cosmochimica Acta* **50**, 853-855.
- Vandermeulen, J. H. and Singh, J. G. (1994) Arrow oil spill, 1970-90: Persistence of 20-year weathered Bunker C fuel oil. *Canadian Journal of Fisheries and Aquatic Science* **51**, 845-855.
- Volkman, J. K., Farrington, J. W., Gagosian, R. B. and Wakeham, S. G. (1981) Lipid composition of coastal marine sediments from the Peru upwelling region, *Adv. Org. Geochem.* 228-240.
- Volkman, J. K., Alexander, R., Kagi, R. I., Rowland, S. J. and Shappard, P. N. (1984) Biodegradation of aromatic hydrocarbons in crude oils from the Barrow sub-basin of Western Australia. *Organic Geochemistry* **6**, 619-632.
- Volkman, J. K., Holdsworth, D. C., Neil, G. P. and Bavor, H. J. Jr (1992) Identification of natural, anthropogenic and petroleum hydrocarbons in aquatic sediments. *Science of the Total Environment* **112**, 203-219.
- Wakeham, S. G., Schaffner, C. and Giger, W. (1980) Polycyclic aromatic hydrocarbons in recent lake sediments. I. Compounds having anthropogenic origins. *Geochimica Cosmochimica Acta* **44**, 403-413.
- Wakeham, S. G. (1990) Algal and bacterial hydrocarbons in particulate matter and interfacial sediment of the Cariaco Trench. *Geochimica Cosmochimica Acta* **54**, 1325-1336.
- Wang, Z., Fingas, M. and Sergy, G. (1994) Study of 22 year-old Arrow oil samples using biomarker compounds by GC/MS. *Environmental Science Technology* **28**, 1733-1746.
- Williams, J. A., BJORoy, M., Dolcater, D. L. and Winters, J. C. (1986) Biodegradation in South Texas Eocene oils - effects on aromatics and biomarkers. *Organic Geochemistry* **10**, 451-461.