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FINGERPRINTING OF CRUDE OIL SPILLS

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SUMMARY

The use of a two-step microcolumn of silica gel and aluminium oxide for the fractionation of crude oil spills is described. The 3-4- and 5-6-ring polynuclear aromatic hydrocarbon fractions were used to identify eleven crude oils according to their areas of origin and individually. Fractionation by thin-layer chromatography was also studied and the results are compared with those obtained on the microcolumn.

INTRODUCTION

The fingerprinting of crude oil spills is a very complex problem, which has been studied by many researchers using refined techniques^{1,2} and several "markers". Aliphatic³⁻⁷ and aromatic^{2,8,9} hydrocarbons and also nitrogen-¹⁰ and sulphur-¹¹ containing polycyclic aromatic hydrocarbons (PAHs) are among the markers used. Aliphatic hydrocarbons have been the most used, but they have the following disadvantages: (a) *n*-alkanes may be quickly degraded in sea water by chemical and biochemical processes¹¹⁻¹³; and (b) iso- and cycloalkanes when used alone do not allow the identification of the origin of crude oil spills with certainty^{5,14,15}.

Better results were obtained by using PAHs with more than two rings, which have a substantial lifetime in sea water and may be identified even after a long period^{13,16-19}. In this work we have studied the composition of crude oil spills by means of fractionation of PAHs according to the number of rings. Column and thin-layer chromatography (TLC), gas chromatography (GC), and GC-mass spectrometry (MS) were the techniques used.

EXPERIMENTAL

Reagents and materials

Eleven crude oils from the Middle East, Africa, Russia and South America were analysed: Light Arabian (LA) from Saudi Arabia; Qatar Marine offshore (QM) from Qatar; Murban (MU) from Abu Dhabi; Dubai (DU) from Dubai; Iranian (IH) from Iran; Emeraude (EM) from Zaire; Mandij (MA) from Gabon; Es Sider Oasis (ES) from Libya; Urals (UR) and Rhomanshiskaya (RH) from Russia; and Tia Juana (TJ) from Venezuela. The crude oils were dissolved in carbon tetrachloride and the solutions (200 mg/ml) were stored in the dark at 5°C.

The solvents used were *n*-pentane (spectroscopy grade) (Merck), carbon tetrachloride (analytical-reagent grade) (Merck), cyclohexane and *n*-hexane (pesticide grade) (Merck) and dichloromethane (Chromasolv) (Riedel de Haën).

A two-step microcolumn of silica gel and aluminium oxide as used previously²⁰ was employed. Ready-for-use plates of silica gel 60 (Merck) were used for TLC.

Fractionation of crude oils

The hydrocarbons were isolated and fractionated on the two-step microcolumn according to the number of rings using a 50- μ l solution of crude oil in carbon tetrachloride and with the same eluents used in the analysis of hydrocarbons dissolved in sea water²⁰. The fractionation in *n*-pentane-dichloromethane containing PAHs with 3-4 rings (including dibenzothiophenes) were concentrated to 1 ml according to the previously described method²⁰. The time required for the analysis was 1 h. The fraction of PAHs with 5-6 rings (concentrated to 0.1 ml) was purified to remove more condensed products by passing the solution through an RP-18 (LiChroprep, 40-60 μ m) (Merck) microcolumn (3 \times 0.6 cm I.D.) and eluting it with 1 ml of *n*-hexane.

Thin-layer chromatography. For each solution of crude oil in carbon tetrachloride a volume of 50 μ l was placed on the plate at the origin along a line 2 cm long. The plate was eluted with cyclohexane up to a migration distance of 10 cm, air-dried and developed again by eluting with methanol up to 7 cm. With a third elution with dichloromethane (migration distance 7 cm) it was possible to collect the fluorescent PAHs near the first solvent front. In this same zone of the layer the sulphur-containing compounds were also collected. Resins and more condensed products remained at the application point. The time required for the fractionation was 2 h. The part of the layer containing fluorescent PAHs was scraped off and extracted with 1 ml of *n*-hexane. The final recovery of extraction was 60% for methylnaphthalenes and acenaphthylene and more than 90% for fluoranthene, pyrene, chrysene, benzo[*a*]pyrene, dibenz[*a,h*]anthracene and benzo[*ghi*]perylene.

Analysis of PAHs

GC analysis was performed on a Carlo Erba HRGC 5160 Mega Series instrument, equipped with a flame-ionization detector and coupled with a Spectra-Physics 4100 computing integrator. For PAHs with 3-4 rings and those obtained by TLC fractionation, the on-column injection technique was used with the following temperature programme: 50°C for 1 min, increased to 300°C at 8°C/min. The carrier gas was hydrogen at a linear velocity of 50 cm/sec. The silica capillary column used (Carlo Erba, Mega) was coated with SE-52 (length 25 m, phase thickness 0.15 μ m). For PAHs with 5-6 rings, a splitless injection technique was employed with a 20 ml/min head flow and an injector temperature 270°C. The temperature programme was 50°C for 1 min, then a sudden increase to 200°C and subsequently a linear increase to 280°C at 3°C/min. A Supelco SE-54 glass capillary column (length 30 m, phase thickness 0.30 μ m) was used.

GC-MS analysis was performed on a VG 7070 EQ mass spectrometer (VG Analytical, Manchester, U.K.) with the following operating conditions: 6 kV acceleration voltage and 100 μ A source current at 70 eV ionizing voltage.

RESULTS AND DISCUSSION

Fractionation with a two-step micro-column

The fingerprinting of crude oils, using PAHs as markers, is generally based on extraction with solvents and/or fractionation on-column in order to obtain a solution of total PAHs^{5,12,19,21}. Our fractionation method, in contrast, is able to separate the PAHs according to their ring number (2, 3-4 and a third fraction mainly containing PAHs with 5-6 rings). The two-ring fraction has not been examined because such compounds are more easily biodegraded than are more condensed PAHs¹⁹. In addition, they give the same GC profile and therefore cannot be used for identification of crude oil spills⁹.

3-4-Ring fraction. Fig. 1 compares the GC profiles of the same 3-4-ring fraction of four crude oils from (a) Murban (Middle East), (b) Urals (Russia), (c) Tia

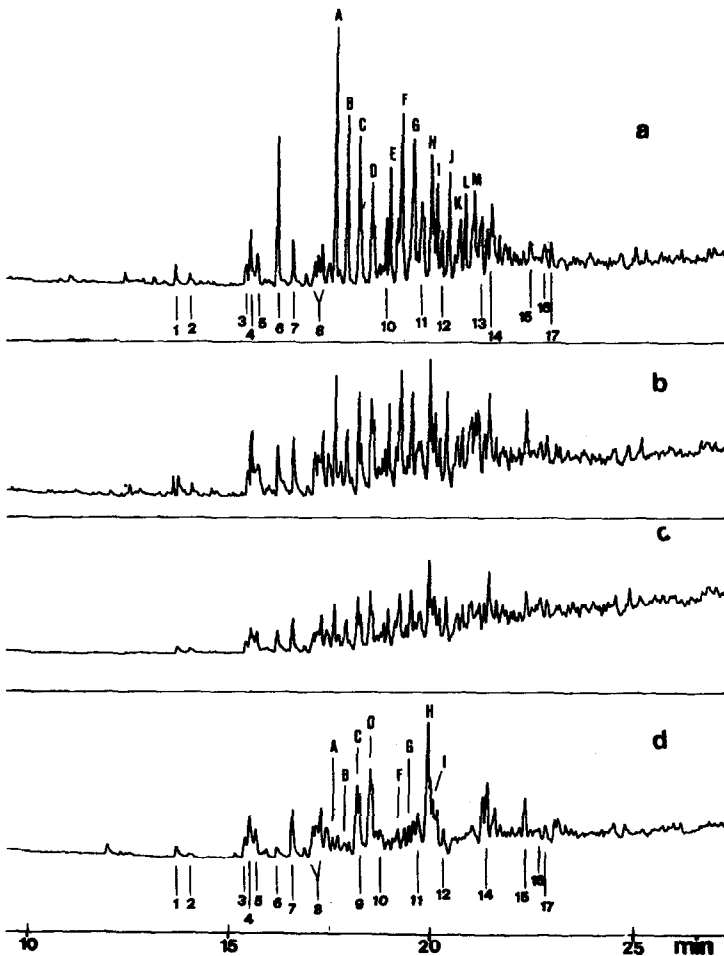


Fig. 1. Gas chromatograms of 3-4-ring fractions: (a) Murban; (b) Urals; (c) Tia Juana; (d) Es Sider Oasis crude oil. See Table I for PAH assignments.

TABLE I
PAHs IDENTIFIED BY GC-MS IN THE 3-4-RING FRACTION OF CRUDE OILS

Peak	Crude oil	
	Middle East/Russian/Venezuelan	African
1	Fluorene	Fluorene
2	9-Methylfluorene	9-Methylfluorene
3	2-Methylfluorene	2-Methylfluorene
4	1-Methylfluorene	1-Methylfluorene
5	3- + 4-Methylfluorene	3- + 4-Methylfluorene
6	Dibenzothiophene	Dibenzothiophene
7	Phenanthrene	Phenanthrene
8	Dimethylfluorene	Dimethylfluorene
A	4-Methyldibenzothiophene	4-Methyldibenzothiophene
B	2- or 3-Methyldibenzothiophene	2- or 3-Methyldibenzothiophene
C	1-Methyldibenzothiophene	1-Methyldibenzothiophene
9		2-Methylphenanthrene
D	4- or 9-Methylphenanthrene	4- or 9-Methylphenanthrene
10	3-Ethyldibenzothiophene	3-Ethyldibenzothiophene
E	4,6-Dimethyldibenzothiophene	
F	Dimethyldibenzothiophene	Dimethylphenanthrene
G	Dimethyldibenzothiophene	Dimethylphenanthrene
11	1,7-Dimethyldibenzothiophene	Dimethylphenanthrene
H	Dimethylphenanthrene	Dimethylphenanthrene
12	Dimethylphenanthrene	Dimethylphenanthrene
I	Trimethyldibenzothiophene	Dimethylphenanthrene
J	Trimethyldibenzothiophene	
K	Trimethyldibenzothiophene	
L	Trimethyldibenzothiophene	
M	Trimethyldibenzothiophene	
12	Trimethyldibenzothiophene	
14	Tetramethyldibenzothiophene + trimethylphenanthrene	Trimethylphenanthrene
15	Trimethylphenanthrene	Trimethylphenanthrene
16	Tetramethylphenanthrene	Tetramethylphenanthrene
17	Tetramethylphenanthrene	Tetramethylphenanthrene

Juana (Venezuela) and (d) Es Sider Oasis (Libya). Profiles (a), (b) and (d) are like those of the other crude oils from the same area. Most of the compounds present in this fraction had been identified by GC²² and GC-MS²³. The 3-4-ring fraction of Middle East, Russian and Venezuelan crude oils contains the same compounds, but in different concentrations. Such a difference in concentration is sufficient to distinguish the area of origin of the three crude oils.

Among these compounds, methyl-substituted dibenzothiophenes are prevalent (see Table I). In contrast, the fraction of Libyan crude oil contains mostly methyl-substituted phenanthrenes (Table I) and its GC profile (Fig. 1d) is consequently different from that of the others. In Table I the identified PAHs are marked with a number or letter that indicates the corresponding peak on the chromatograms (a) and (d) in Fig. 1. The different composition of Libyan crude oil compared with the others, allows the identification of crude oils from Africa.

Peak-height ratios as fingerprints. In order to remove ambiguities in the com-

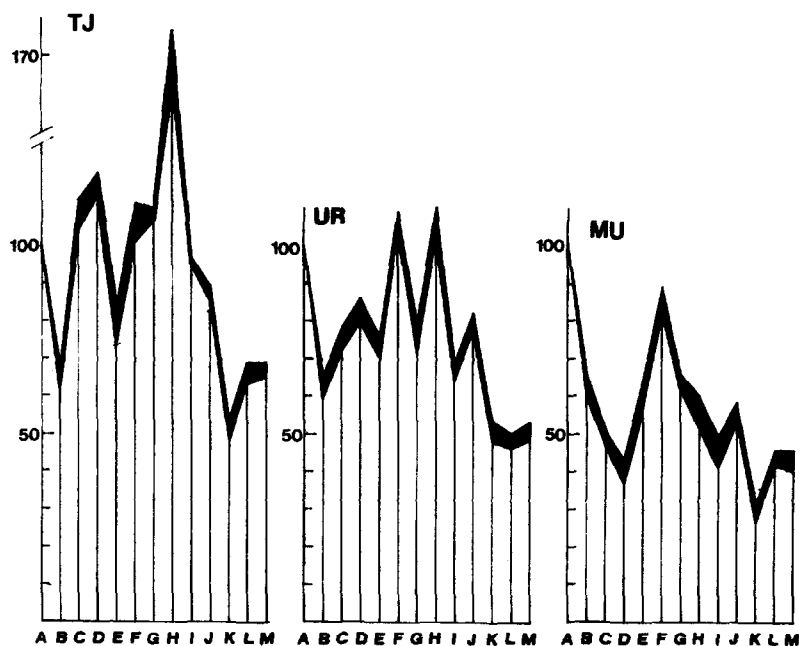


Fig. 2. Fingerprinting graphs of Tia Juana (TJ), Urals (UR) and Murban (MU) crude oils, obtained plotting peak-height ratios for 3-4-ring PAHs.

parison of Middle East, Russian and Venezuelan crude oils, we calculated the ratios between the heights of selected peaks and that of 4-methylthiophene. Peak height rather than peak area was used because the height is more reproducible when the resolution is low²⁴, and may be correlated with weathering processes¹. The following identified PAHs were chosen: (A) 4-methylthiophene, (C) 1-methylthiophene, (D) 4- or 9-methylthiophene, (E) 4,6-dimethylthiophene, (F,G) dimethylthiophene, (H) dimethylphenanthrene and

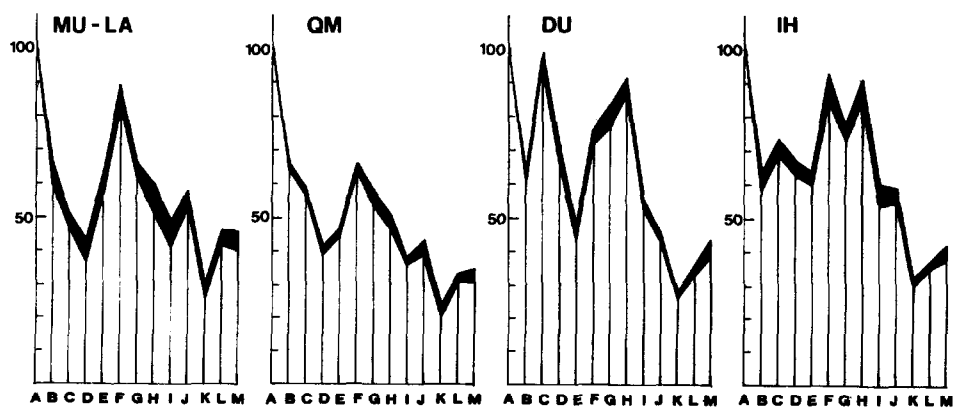


Fig. 3. Fingerprints graphs of Murban and Light Arabian (MU-LA), Qatar Marine (QM), Dubai (DU) and Iranian (IH) crude oils.

(I,J,K,L,M) trimethyldibenzothiophene (see Table I). Such compounds are generally used for identification of crude oil spills^{2,11,18,19,21}.

In Fig. 2 peak-height ratios are plotted for the corresponding PAHs for the Venezuelan (TJ), Russian (UR) and Middle Eastern (MU) crude oils. These graphs were obtained using data from triplicate analyses of each fraction isolated from three samples of the same crude oil. The thickness of the line indicates the standard deviation relative to the nine determinations made on each crude oil. The different trends of the graphs clearly show the differences in the PAHs concentrations among the three crude oils and give the possibility of identifying precisely their area of origin, keeping in mind the low median standard deviation (*ca.* 3%).

The application of the method showed further differences among crude oils from the same area. The graphs in Fig. 3 relate to Middle Eastern crude oils: Murban and Light Arabian (MU-LA), Qatar Marine (QM), Dubai (DU) and Iranian (IH). The first two show the same trend, whereas for the other crude oils different trends

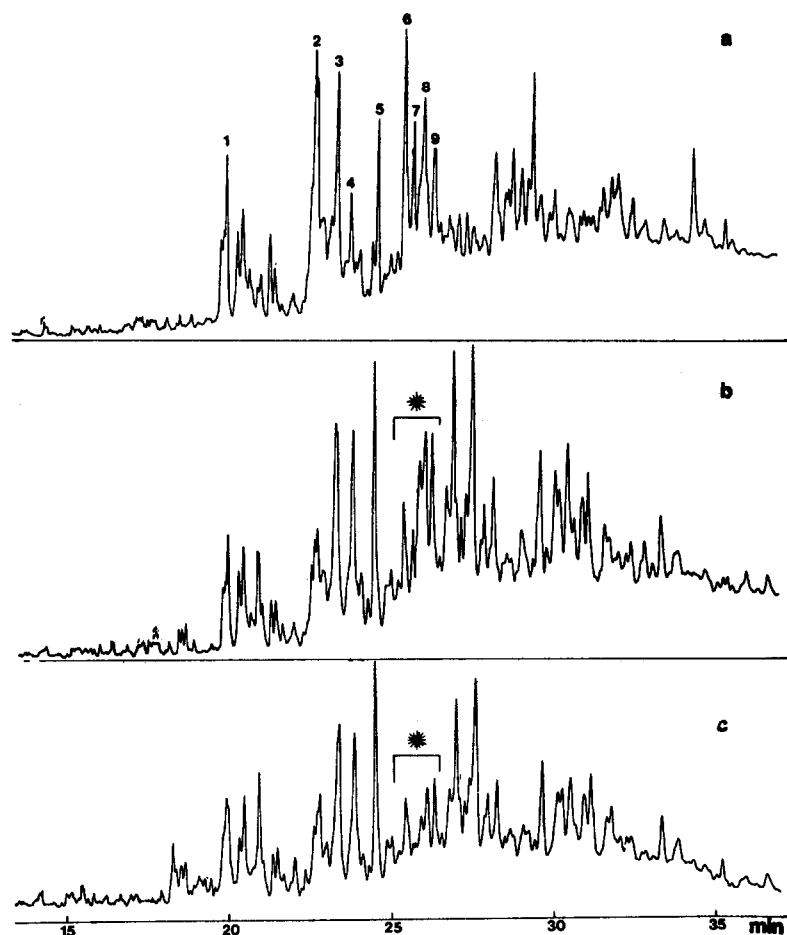


Fig. 4. Gas chromatograms of 5-6-ring fractions: (a) African; (b) Murban; (c) Light Arabian crude oil. See text for PAH assignments.

are observed. With this method it is therefore possible to differentiate among crude oils from the same area, and to distinguish those from different areas much better than with the GC profiles.

5-6-Ring fraction. This fraction includes the 5-6-ring PAHs, whose concentration in crude oils is low²³, some 4-ring PAHs (chrysene, methyl derivatives of pyrene and chrysene) and also more condensed compounds. The presence of the last group prevents the use of the on-column injection technique, and therefore it is necessary to employ the splitless technique. The GC profiles of the 5-6-ring fraction allow a more complete characterization of crude oils from the same area, as shown by the gas chromatograms in Fig. 4b and c, relating to MU and LA. The difference (see the zones marked with asterisks) is due to the different percentages of methylchrysenes in these crude oils.

The use of the peak-height ratios for the methylpyrene also demonstrated typical, trends for each African crude oil (see Fig. 5). The following compounds were employed: methylpyrene (1); trimethylpyrenes (3,4); methylchrysene or methylbenz[*a*]anthracene; (6); 5-methylchrysene (7); 6- or 4-methylchrysene (8); 1-methylchrysene or 7-methylbenz[*a*]anthracene (9) (see Fig. 4a). The splitless technique does not give quantitative results²⁴ and the graphs in Fig. 5 only indicate the trends of nine replicate determinations. The absence of 3-methylchrysene from Libyan crude oil should also be noted.

With the data for the 5-6-ring fraction, therefore, it is possible to differentiate among crude oils with similar compositions. Such results can be achieved by other methods, but only with great difficulty.

Fractionation by thin-layer chromatography

This technique does not have a high resolving power for complex mixtures of aromatic hydrocarbons, but it can separate the polycyclic fraction from the fractionation that includes aliphatic and monoaromatic compounds. For this reason, TLC is often employed in the analysis of petroleum products²⁵, as it is a very simple, fast and repeatable technique.

In order to compare the TLC results with those obtained with the column, the same amounts of crude oil (10 mg) and a volume of extraction solvent equal to the

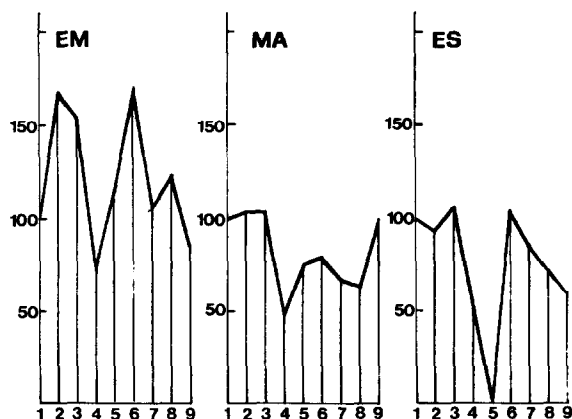


Fig. 5. Fingerprinting graphs of African crude oils obtained plotting peak-height ratios for 5-6-ring PAHs.

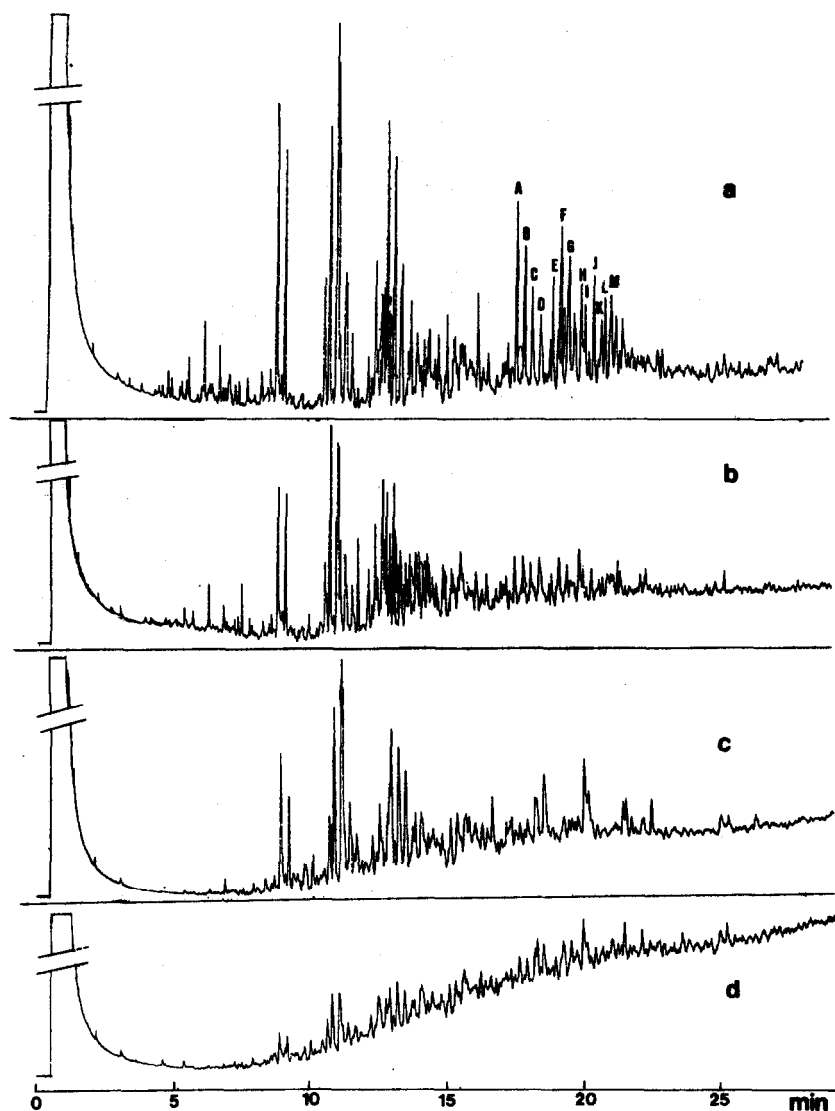


Fig. 6. Gas chromatograms of PAH fraction isolated from crude oils by TLC: (a) Middle East; (b) Russian; (c) African; (d) Venezuelan crude oil.

final volume (1 ml) of the 3-4-ring PAH fraction were used. Fig. 6 shows the gas chromatograms for the whole polycyclic fraction obtained by TLC of the crude oil from the following areas: (a) Middle East, (b) Russia, (c) Africa and (d) Venezuela. With the exception of the fraction isolated from the Venezuelan crude oil, the others contain large proportions of several methyl-substituted naphthalenes. On the basis of the gaschromatograms in Fig. 6, it is possible to differentiate crude oils according to the geographical areas and particularly from the different profile of peaks A-M (see Fig. 6a), which correspond to those of the 3-4-ring fraction isolated with the microcolumn (see Table I).

The composition of the African crude oils is very different from that of the others, as has already been observed with the fractionation on the microcolumn. The large drift of chromatogram (d) is due to the presence of condensed compounds that cannot be removed by TLC.

In order to obtain a better identification of Middle Eastern and Russian crude oils, the peak-height ratio method was applied to peaks A–M. This method cannot be used with Venezuelan crude oil owing to the low resolution of the peaks [see chromatogram (d)]. The resulting graphs show a trend similar to those in Figs. 2 and 3; the greatest differences are shown by the ratios of peaks B, C, D and M, N, which are affected by the presence of two-ring PAHs and by the most condensed compounds, respectively. It should be noted that the median standard deviation is twice that obtained from the 3–4-ring fractions isolated on the microcolumn.

Weathering

Preliminary tests on weathering demonstrated the possibility of applying the above method to the identification of crude oil spilled in sea water. Samples of Murban oil (30 μ l) were placed in 1 l of freshly collected sea water and kept agitated in the open air. After 24 h the solution was extracted with *n*-hexane and analysed by the above method. No differences were observed in the number or heights of the peaks with respect to the original crude oil profile.

CONCLUSIONS

Any analytical method for fingerprinting crude oil spills must use small amounts of sample²⁶, indicate their areas or origin and the pollutant sources and also identify the composition of the oil spill in order to provide information on environmental damage¹³. A very complete fractionation is necessary owing to the chemical complexity of the mixtures²⁷. The fractionation method with the two-step micro column satisfies these requirements because it is possible to correlate the information obtained from the different PAH fractions. For this reason, all the crude oils examined were characterized not only according to their areas of origin but also individually.

The TLC fractionation allows the identification of the Middle Eastern and Russian crude oils and can generally be used as a primary screening method.

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