

Using systematic and comparative analytical data to identify the source of an unknown oil on contaminated birds

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Abstract

A tiered and integrated analytical approach for identification of the source of an unknown oil on contaminated birds by use of systematic and comparative GC–MS and GC–flame ionization detection data is described. Characterization of the unknown oil was achieved through a variety of “standard” analyses including not only individual aliphatic, aromatic and biomarker hydrocarbon analysis, but also “source-specific-marker” diagnostic ratio analysis. Once precise chemical data were obtained and data analysis and comparison with the corresponding data from the known oils was completed, product identification (fingerprinting) techniques were used to identify the type of product present, and to estimate the degree of weathering the product had undergone since release. It was concluded that: (1) the residual oil on the birds was most probably an old Bunker C type oil and not from the suspected barge oil; (2) the oil on contaminated birds was relatively highly weathered and (3) some biodegradation of the oil had occurred. © 1997 Elsevier Science B.V.

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1. Introduction

The environmental concerns and legal issues associated with accidental leakage or chronic release of crude oil and refined petroleum products to the environment grow with each passing year. During 1995, in addition to the thousands of uncounted small spill accidents, over 30 relatively large-scale oil spill accidents occurred worldwide [1]. Among them, the 400 000-gallon oil spill near Yosu, South Korea from the Cypriot tanker *Sea Prince* was the largest vessel spill of the year, and a 4.4-million-gallon oil storage tank spill in Grozny, Russia was

the largest facility spill. The ability to unambiguously identify spilled oil and petroleum products in complex contaminated environmental samples, to link them to the known sources, and to track their transport, alteration and ultimate fate is important in settling questions of environmental impact and legal responsibility or liability. In most cases, the oil, after release into the environment, is immediately subjected to a wide variety of weathering processes [2] including evaporation, dissolution, dispersion, photochemical oxidation, water–oil emulsification, microbial degradation and adsorption onto suspended particulate materials. These processes complicate the unambiguous identification of already complex spilled oil samples.

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Environmental Protection Agency (EPA) methods such as 602, 610, and 624 [3] have been used for quantifying hydrocarbons present in petroleum products. These methods were originally designed for water, industrial water and hazardous waste, and are only sensitive to a limited number of compounds in petroleum, and thus lack the ability to identify the source and to track the fate of spilled oil. However, in recent years, there have been major advances in analytical techniques [4–18]. The Emergencies Science Division (ESD) of Environment Canada in co-operation with US Minerals Management Service has conducted projects to develop a systematic analytical approach to identify, characterize and quantify various crude and refined petroleum products in environmental samples with respect to their composition, nature and sources [19–26]. The characterizations are carried out by a variety of analyses including not only individual aliphatics, aromatics (they are probably the most important analytes in oil-spill natural resource damage assessment) and biomarkers (these highly degradation-resistant hydrocarbons are especially useful for characterization of highly weathered crudes and oil residuals), but also the “source-specific-marker” diagnostic ratio analysis. Once precise chemical characterization data are obtained and data analysis is completed, product identification (fingerprinting) techniques are used to identify the type of product present, and to evaluate the degree of weathering the product has undergone since release. In this paper, a detailed tiered and integrated analytical approach is described by which an unknown oil on contaminated birds was accurately identified and how its chemical composition was characterized.

2. Experimental

2.1. Capillary gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS)

Analyses for *n*-alkane distribution and total petroleum hydrocarbons (TPHs) were performed on a Hewlett–Packard (HP) 5890 gas chromatograph equipped with a flame-ionization detection (FID) system and an HP 7673 autosampler. A 30 m×0.32 mm I.D. (0.25- μ m film) DB-5 fused-silica capillary

column (J&W, Folsom, CA, USA) was used. The carrier gas was helium (2.5 ml/min). The injector and detector temperatures were set at 290 and 300°C, respectively. The oven temperature program was: 2 min hold at 50°C; ramp to 300°C at 6°C/min; and 16 min hold at 300°C. A 1- μ l aliquot was injected in splitless mode with a 1-min purge-off.

Analyses of target polycyclic aromatic hydrocarbons (PAHs) and biomarker compounds were performed on an HP Model 5890 GC equipped with a Model HP 5972 mass-selective detector. System control and data acquisition were achieved with a HP G1034C MS ChemStation (DOS series). The mass-selective detector was operated in the scan and selected ion monitoring (SIM) modes for the identification of components, and in the SIM mode for quantitation of target compounds. A HP-5 fused-silica column with dimensions of 30 m×0.25 mm I.D. (0.25- μ m film) was used. The chromatographic conditions were as follows: carrier gas, helium (1.0 ml/min); injection mode, splitless; injector and detector temperature, 290 and 300°C respectively; temperature program for target PAHs, 90°C for 1 min, ramp to 160°C at 25°C/min and then to 290°C at 8°C/min, and hold 15 min; temperature program for alkylated PAHs and biomarker compounds, 50°C for 2 min, ramp to 300°C at 6°C/min and hold 16 min.

For details of analysis quality control and quantification methodology, please refer to Ref. [20,21,25].

2.2. The oil-contaminated bird samples

Four birds with feathers fouled with an unknown oil were found dead in the region of the National Park of Larchipel-de-Mingan of Quebec on 29 January 1996. The Quebec Region of Environment Canada and the provincial emergency response officers wanted to know the nature of the oil, the type of petroleum hydrocarbons, the age and the extent of weathering and degradation of the spilled oil, and changes in oil character since the occurrence of any possible spill. It was suspected that the oil which had contaminated the birds originated from leakage from a barge which had grounded on Anticosti Island (approximately 100 miles away from the National Park) in early December of 1995.

The barge (more than 25 years old) contains 40

tons of oil/water mixture (approximately 4 tons of oil). An oil sample (black and viscous) was collected from the barge and was found to be an oil/water emulsion. The average water content of the emulsion sample was determined to be 74.5% (3 determinations) by the Karl Fischer titration method.

Crude oils including Bunker C oil were obtained from various sources during the period of 1985–1994 and are currently stored in a cold room of the ESD laboratory.

2.3. Analytical approach

2.3.1. Tier 1: Determination of oil residues

The contaminated birds were weighed, mixed with approximately 20 g of anhydrous sodium sulphate and spiked with an appropriate amount of PAH surrogates [20], and then serially extracted three times with 150 ml hexane–dichloromethane (1:1) (DCM) for 5 min (50 ml of solvent was used for extraction of sample D-4 due to much smaller sample mass) each time using sonication. After each extraction, the extract was simply decanted and fresh solvent added. Upon completion of three extractions, the extracts were combined, filtered through anhydrous sodium sulphate, concentrated to approximately 5 ml using rotary evaporation and solvent-exchanged with hexane. The concentrated extracts were then made up to a final volume of 10.00 ml. An aliquot of the concentrated extract (1.00 ml) was blown down with N₂ to residue and weighed on a microbalance to obtain a total solvent-extractable material mass (TSEM, expressed in mg/g of sample).

The Bunker C and Barge oils were directly dissolved in hexane at a concentration of ~100 mg/ml and spiked with surrogates before the column clean-up.

2.3.2. Tier 2: Determination of *n*-alkanes and TPHs by GC–FID and product screen

The microcolumn fractionation technique [20] was employed for the sample clean-up and fractionation of extracted oil, and also for the suspected source oils controls. Appropriate aliquots of the concentrated extracts (containing TSEM/oil ~20 mg) were applied to a 3-g silica gel column, topped with 1-cm anhydrous sodium sulphate, which had been pre-conditioned with 12 ml of hexane (note that addition-

al sodium sulphate was applied for the Barge oil sample clean-up due to relatively higher water content). Half of the hexane fraction (F1) was used for analysis of aliphatics and biomarker compounds; half of the 50% benzene fraction (F2) was used for analysis of target PAHs and alkylated PAH homologues. The remaining halves of F1 and F2 were combined (F3) and used for the determination of TPHs. These three fractions were concentrated under a stream of nitrogen to appropriate volumes, spiked with internal standards (5- α -androstande and C₃₀- $\beta\beta$ -hopane, [²H₁₄]-terphenyl and 5- α -androstande for F1, F2 and F3, respectively) and then adjusted to accurate pre-injection volumes (0.50 ml) for GC analysis.

The additional analytical clean-up step was repeated for sample D-4 to eliminate the oil-unrelated interfering compounds.

Fractions 1 and 3 were analysed by GC–FID to determine GC-detectable saturates and total petroleum hydrocarbons (GC-TPHs) and to aid the identification of the samples such as gasoline, diesel, or crude oil. Individual *n*-alkanes from C₈ to C₄₀, including important isoprenoids, pristane and phytane, are also quantified at this level to obtain a description of the *n*-alkane distribution in the samples.

2.3.3. Tier 3: Distribution pattern recognition of target PAHs and biomarker components by GC–MS measurement

High-performance capillary GC–MS was used to obtain comprehensive quantitative pictures of PAHs and biomarkers in the samples.

Fraction 2 was analysed by GC–MS in SIM mode for 25 individual PAH compounds, and for 5 target alkylated PAH homologues [10–12,19,25] which are valuable for oil differentiation and monitoring of the oil weathering process. Under certain circumstances (for example, for fresh or lightly-weathered oils), analysis of BTEX (the collective name of benzene, toluene, ethylbenzene and the xylene isomers) and alkyl-substituted benzenes was performed [24] to obtain a distribution pattern of alkylated benzenes. Any major unidentified peaks in the chromatograms in the analyses were flagged for potential product-source identification.

Fraction 1 was analysed by GC–MS for over 50 biomarker terpanes and steranes. The distribution

Table 1
Comparison of diagnostic ratios of "source-specific-markers" of the unknown oil with the suspected source oils

Parameters	D-1	D-2	D-3	D-4	Bunker C	Barge oil
Concentrations						
Total of <i>n</i> -alkanes (mg/g TSEM or oil)	13.9	13.1	11.2	10.3	30.5	25.3
Total of target alkyl PAHs ($\mu\text{g/g}$ TSEM or oil)	14 340	13 239	12 641	9739	16 433	91 293
Total of target terpanes ($\mu\text{g/g}$ TSEM or oil)	343	322	280	298	261	65
Conc. of 3 isomers of M-DBT ($\mu\text{g/g}$ TSEM or oil)	172	168	162	91	247	1514
Diagnostic ratios						
<i>Aliphatics</i>						
C17/pri.	1.90	1.85	1.83	1.72	2.72	2.91
C18/phy.	1.64	1.57	1.61	1.56	2.05	1.80
pri./phy.	0.84	0.80	0.86	0.81	0.79	0.75
CPI	0.99	0.99	0.99	0.99	0.99	1.04
<i>PAHs</i>						
4-M-DBT: 2-/3-M-DBT: 1-M-DBT	1:0.97:0.29	1:1.01:0.31	1:0.98:0.29	1:0.98:0.32	1:1.12:0.29	1:0.65:0.23
(3+2-methyl-P)/(4/9+1-methyl-P)	1.32	1.33	1.32	1.30	1.48	0.95
C0C:C1C:C2C:C3C	0.12:0.33:0.33:0.22	0.11:0.33:0.33:0.22	0.12:0.34:0.33:0.21	0.11:0.34:0.34:0.22	0.13:0.32:0.35:0.20	0.38:0.37:0.20:0.05
C2D/C2P:C3D/C3P	0.13:0.12	0.13:0.13	0.14:0.13	0.12:0.11	0.16:0.19	0.35:0.35
Naphs/Chrys	0.54	0.61	0.90	0.29	2.48	41.3
Phens/Chrys	3.40	3.41	3.66	2.92	3.22	13.1
Dibens/Chrys	0.37	0.38	0.41	0.26	0.48	2.9
Fluos/Chrys	0.24	0.25	0.29	0.19	0.40	2.5
<i>Biomarkers</i>						
Terpane C23/C24	2.03	2.08	2.06	1.93	2.04	2.59
Hopane C29/C30	0.38	0.36	0.40	0.39	0.42	0.46
C32(22S)/C32(22R)	1.50	1.56	1.41	1.53	1.52	1.57
C23/C30	0.21	0.20	0.19	0.19	0.29	1.57
C24/C30	0.09	0.09	0.08	0.10	0.14	0.61

M-DBT, D, P and C represent methyl/dibenzothiophene, dibenzothiophene, phenanthrene and chrysene, respectively.
C23, C24, C29, C30 C32(22S) and C32(22R) represent C23 and C24 terpane, C29 and C30 $\alpha\beta$ -hopane and 22S/22R isomer pairs of C32 hopane, respectively.

patterns of biomarkers, in general, are different from oil to oil and, therefore, can be very useful in identification of the oil source, especially for highly-weathered petroleum products in which most *n*-alkanes and target PAH compounds have been highly degraded or completely lost [11–13].

2.3.4. Tier 4: Determination and comparison of diagnostic ratios of the “source-specific-marker” compounds with the suspected source oil

Source-specific-marker compounds and the ratios of these compounds can provide integrated and unique data for purposes of source identification and differentiation, especially for crudes and petroleum products having similar hydrocarbon composition and from similar origin [10–19,22,23]. These compounds have been grouped as follows: Group 1: aliphatic source-specific-marker compounds; Group 2: alkylated PAH homologous series, methyl-dibenzo-thiophene and methylphenanthrene isomers; Group 3: paired biomarker compounds including C₂₃ and C₂₄-terpane, 17 α (H),21 β (H)-30-norhopane and 17 α (H),21 β (H)-hopane (C₂₉ and C₃₀ $\alpha\beta$ -hopane), 18 α (H),21 β (H)-22,29,30-trisnorhopane (Ts) and 17 α (H),21 β (H)-22,29,30-trisnorhopane (Tm), C₃₂ 22*S*/22*R* $\alpha\beta$ -bishopanes and C₃₃ 22*S*/22*R* $\alpha\beta$ -trishopanes (Table 1).

Based on the analytical data from Tiers 1, 2 and 3, a comparison of the “source-specific-marker” compounds and their diagnostic ratios from the unknown oil with that from the suspected source oils can then be performed to identify the source of contamination (a general database containing detailed chemical composition of oils and petroleum products can greatly aid such comparison and analysis).

2.3.5. Tier 5: Determination of weathered percentages of residual oil

Calculations based on hopanes to estimate weathered percentages of oil can be more accurately performed than the traditional methods [7,22], if the “fresh” source oil is available. However, even if there is no fresh oil, estimation of a range of weathered percentages of the residual oil may be still obtained by analysis of the loss of *n*-alkanes, alteration of PAH distribution patterns and degradation of BTEX and alkylbenzenes.

3. Results and discussion

3.1. Determination of oil residues

Table 2 presents the hydrocarbon analysis results for the bird samples by gravimetric and GC-FID methods. In addition to the TSEM and GC-determined TPHs, the ratios of saturates/TSEM, aromatics/TSEM, the resolved peaks/TPHs, UCM/TPHs and TPHs/TSEM are also listed in Table 2. UCM is defined as the unresolved hydrocarbon mixture detected by GC, which appears as the “envelope” or “hump” area between the solvent baseline and the curve defining the base of the resolved peaks.

Key points from Table 2 can be summarized as follows: (1) the birds were badly contaminated by oil, indicated by high values of TPHs from 9450 to 37 700 ppm; (2) the values of saturates in TPHs and the ratios of resolved peaks/TPHs are approximately 50 and 8% for all 4 bird samples, respectively, which are much lower than the corresponding values for most fresh oils and are often characteristic of weath-

Table 2
Hydrocarbons analysis results for the bird samples

Sample	Description	Sample weight (g)	TSEM (mg/g sample)	GC-TPH (ppm)	GC-TPH (mg/g TSEM)	Saturates in GC-TPH (%)	Resolved peaks /GC-TPH (%)	UCM/GC-TPH (%)
D-1	Oil-contaminated duck 1	99.4	9.95	29 329	293	54	8.0	92
D-2	Oil-contaminated duck 2	113.9	13.99	37 735	307	51	8.0	92
D-3	Oil-contaminated duck 3	179.4	6.70	9449	253	53	7.9	92
D-4 ^a	Oil-contaminated duck 4	28.0	1.90	18 729	276	49	7.8	92

^a The effect of oil-unrelated interference in D-4 on the analytical data has been corrected.

ered oils [5,12,19,22]; (3) for the sample D-4, the peaks with extremely high abundance (approximately 70% of resolved peak area) were detected between 36.78 and 44.74 min in the GC–FID chromatogram of F2 and F3 obtained from the first column clean-up (Fig. 1B). The peaks were not associated with oils and were identified to be fatty alcohols and fatty acid monoesters by GC–MS. These interfering surfactants were effectively removed by successively passing the Fraction 2 eluted from the first column through the second silica gel column, as shown in Fig. 1C.

Note that all results of the sample chemical composition discussed below are expressed on the TSEM basis. Using TSEM as a baseline gives a reasonably equal basis for the determination of the relative compositional changes of hydrocarbon groups in samples. It is only in this fashion that the quantitative results are comparable between the bird samples and the suspected source oils. It should be understood, however, that TSEM includes petrogenic material as well as other non-petrogenic extractable material (such as biogenic hydrocarbons, surfactants and other extractable organics) in some cases, and so can be greater than just the true petrogenic hydrocarbon content of a given sample. In order to lessen the interference of non-petrogenic extractable material on analysis results, careful design of extraction procedures and correction of analytical data is required. The additional clean-up step necessary for sample D-4, described above, is an example of just such a procedure.

3.2. Product type screen and analysis of aliphatic hydrocarbons

The GC–FID chromatograms for TPHs analysis are presented in Fig. 1. The *n*-alkane distribution of sample D-1 is presented in Fig. 2. Samples D-2, D-3 and D-4 had an *n*-alkane distribution pattern very similar to that of sample D-1.

The analysis of aliphatic hydrocarbons demonstrates the following:

(1) A value of the carbon preference index (CPI, defined as the sum of odd- over even-carbon-number *n*-alkanes) near 1.0 indicates petroleum. The CPI values and *n*-alkane distribution patterns can therefore be used to distinguish between petrogenic oils and hydrocarbons from more recent biogenic origin.

In an aliphatic fraction of a sample of biogenic origin, an unusually high CPI is typical, and also *n*-alkanes containing an odd number of carbon atoms predominate [22,27–29].

(2) Weathering causes considerable changes in the chemical composition and physical properties of spilled oils [26,30,31]. The findings that the total of *n*-alkanes was determined to be only from 10 to 14 mg/g of TSEM for samples D1–D4, and that the *n*-alkanes lighter than *n*-C₁₄ were not detected and the abundances of *n*-alkanes after *n*-C₁₄ were dramatically decreased when compared with the “fresh” Bunker C oil, indicate that the oil on the birds has undergone extensive weathering and that the chemical composition of the residual oil has been significantly altered.

(3) Given the effects of biodegradation and weathering, the close similarity of the GC traces for the 4 birds leads to the conclusion that they were contaminated with the same oil. Further, the very close ratios of C₁₇/pristane, C₁₈/phytane and pristane/phytane for 4 birds shown in Table 1 indicates that these 4 birds were probably contaminated by oil at the same time, assuming equal rate of microbiological activity.

The GC traces, the hump profiles and Fig. 2 demonstrate that the oil on the birds is primarily composed of heavy residual oil, with the homologous series of *n*-alkanes peaking at around *n*-C₂₄. This kind of GC trace is characteristic of weathered older type Bunker C oils. “Bunker C” is a term which has been widely used for many years to designate the most viscous (thick, sticky) residual fuels for general land and marine use. Currently, most marine fuels are produced by blending Bunker C oils with diesel fuels in various ratios, to yield fuel of a specified viscosity for different purposes. Depending on production oilfields, production years and blending ratios, Bunker C and marine fuels can have widely different physical properties and chemical compositions [32].

Several different Bunker C controls were analyzed to obtain their fingerprints. For comparison, the *n*-alkane distribution of a Bunker C oil sample and the barge oil are also presented in Fig. 2. The bird samples have GC traces similar to that of the old-type Bunker C oil sample with the exception of larger UCMs and a significantly reduced abundance of *n*-alkanes.

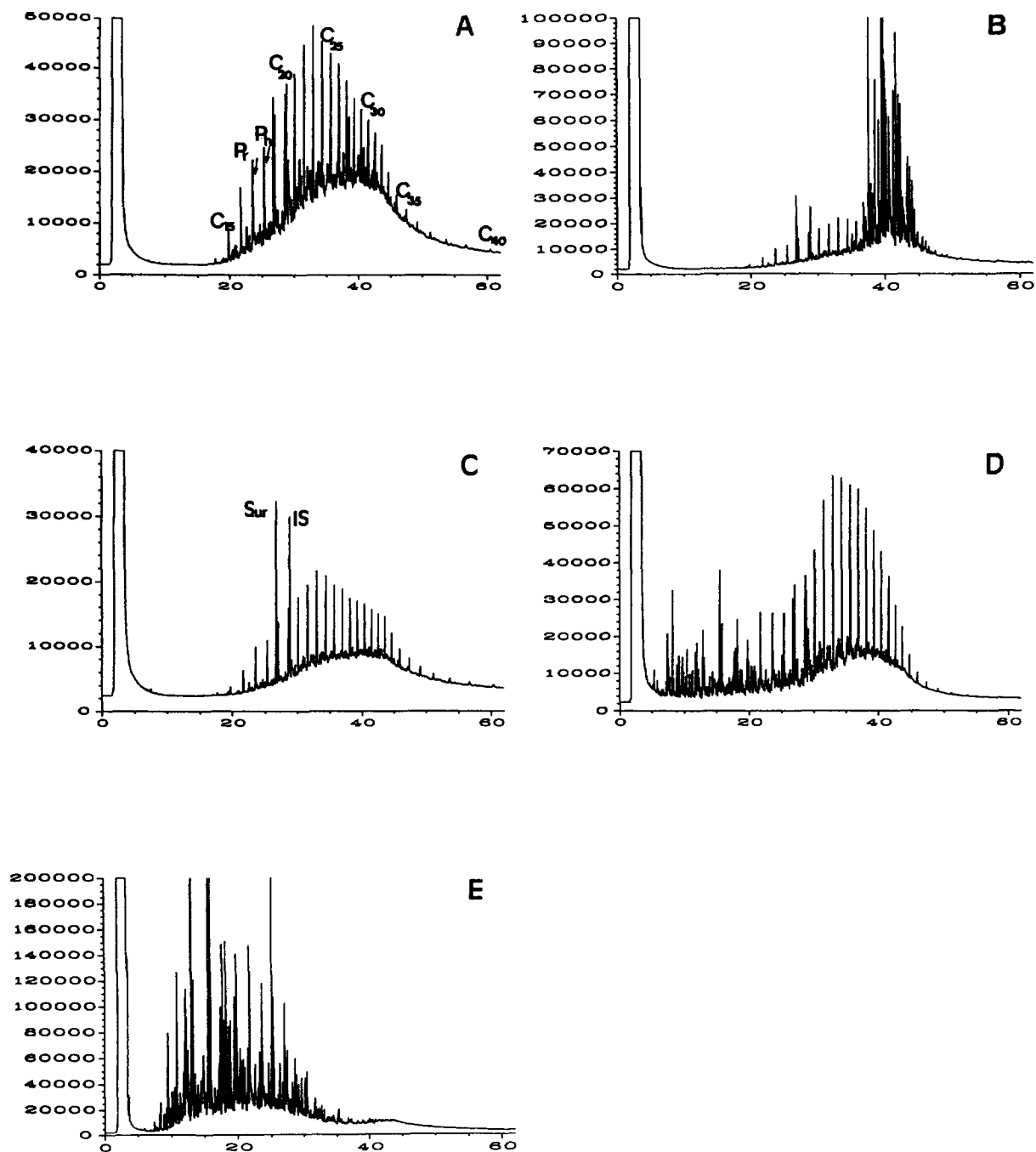


Fig. 1. GC-FID chromatograms for TPH analysis of oil-contaminated bird samples D-1 (A), D-4 after first column cleanup (B), D-4 after two successive column cleanup (C), and the suspected source oil Bunker C (D) and Barge oil (E). Comparison of (B) and (C) demonstrates that the oil-unrelated interference can be effectively removed by an additional clean-up step. IS, Sur, Pr and Ph represent the internal standard 5- α -androstane, surrogate *o*-terphenyl, pristane, and phytane, respectively.

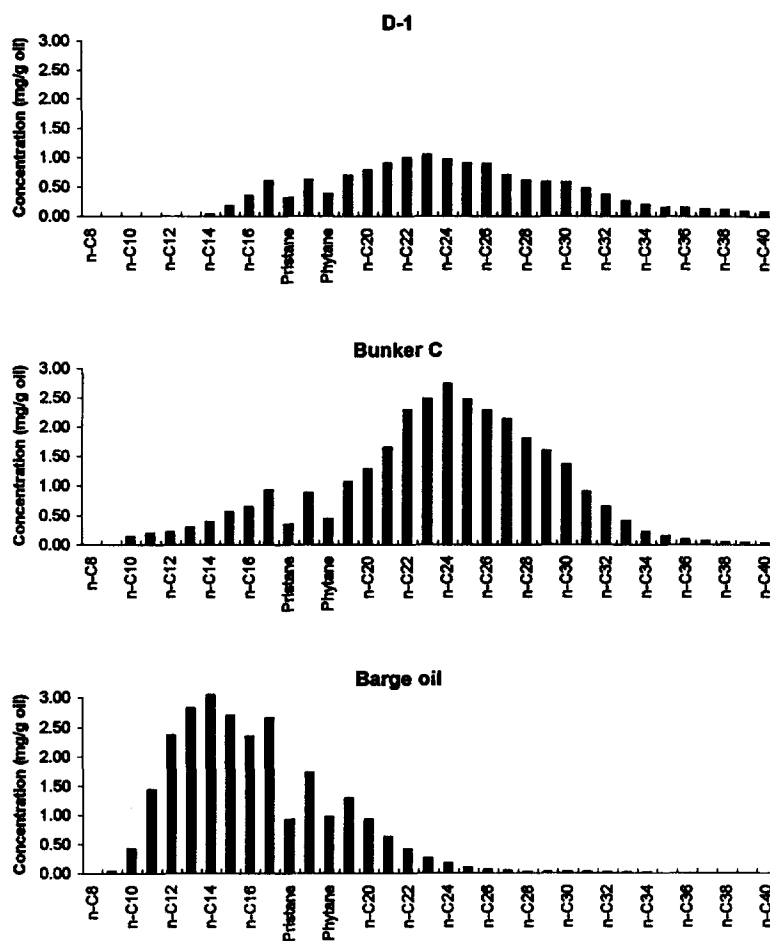


Fig. 2. Comparison of *n*-alkane distribution patterns for the sample D-1, Bunker C and Barge oil.

3.3. Analysis of distribution of target PAHs

PAH compounds are relatively stable and diagnostic constituents of petroleum. Use of the distribution of alkylated PAH homologues as environmental fate indicators and source markers of oil has been reported by several authors [10–12,17–19,22].

The GC–MS total ion chromatograms in the SIM mode for the alkylated PAHs are shown in Fig. 3. Analysis results of the 5 target alkylated PAH series and other target PAHs are summarized in Table 3. The distribution of alkylated PAHs (note the different y-axis scale, 0–20 000, for the barge oil) is depicted in Fig. 4.

The sum of the 5 target alkylated PAHs was

determined to be 14 340, 13 239, 12 641 and 9739 $\mu\text{g/g}$ of TSEM for samples D-1, D-2, D-3 and D-4, respectively. Major compositional changes of PAHs observed for the bird samples are summarized as follows:

(1) No BTEX or lighter alkylbenzene compounds were detected, evidenced by the fact that there were no peaks eluting before a retention time of 17 min (Fig. 3). The lack of volatile compounds, in conjunction with other compositional changes, indicates highly weathered, residual oils [24].

(2) A distribution pattern with the alkyl-naphthalene series being the most abundant among the 5 target alkylated PAH series is observed in many fresh oils. However, a few “fresh” oils (including

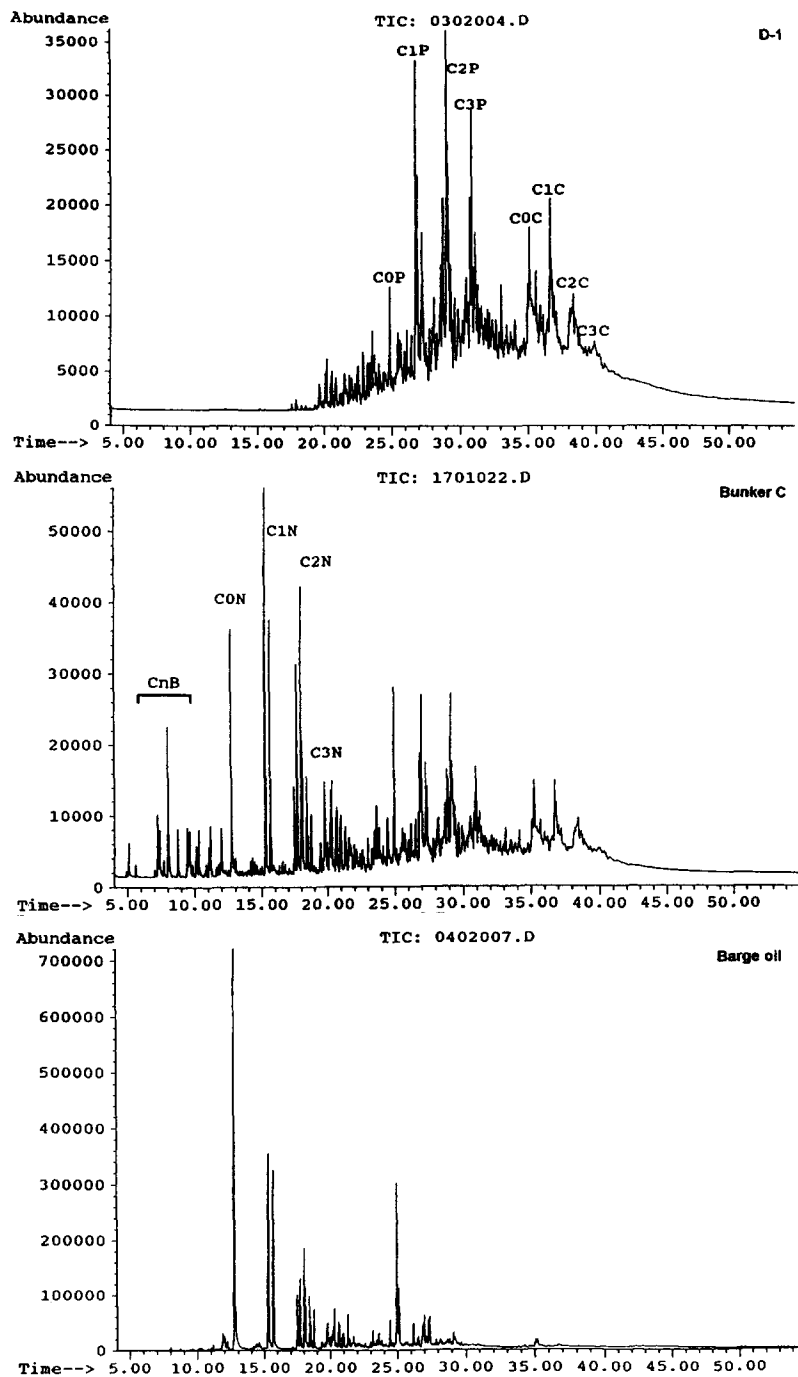


Fig. 3. GC-MS total ion chromatograms (in SIM mode) for the alkylated PAHs analysis of sample D-1, Bunker C and Barge oil. B, N, P and C represent benzene, naphthalene, phenanthrene and chrysene, respectively; n , 0, 1, 2, 3 and 4 represent carbon numbers of alkyl groups in alkylbenzenes and alkylated PAH homologues.

Table 3
Analysis results of alkylated PAHs and other PAHs

PAHs	Sample type				Bunker C ($\mu\text{g/g oil}$)	Barge oil ($\mu\text{g/g oil}$)
	D-1	D-2	D-3	D-4 ^a		
	(μg/g TSEM)					
<i>Naphthalene</i>						
C0-N	1.3	1.5	1.7	5.4	347.7	14939.9
C1-N	4.6	21.7	13.7	18.9	1294.7	20087.0
C2-N	56.8	169.2	208.6	57.3	1914.9	16853.9
C3-N	566.7	562.2	799.2	214.9	1335.2	7892.3
C4-N	762.4	675.4	803.9	316.5	490.9	2192.4
Sum	1391.8	1430.1	1827.0	613.0	5383.3	61 965.6
<i>Phenanthrene</i>						
C0-P	149.6	174.7	171.5	107.1	330.4	6759.6
C1-P	1431.9	1354.4	1304.9	932.9	1609.5	7209.8
C2-P	2865.7	2583.1	2349.5	1876.0	2196.1	3560.0
C3-P	2918.1	2609.2	2398.6	2038.2	1803.1	1549.8
C4-P	1448.4	1266.6	1156.0	1132.7	1050.9	589.5
Sum	8813.7	7988.0	7380.6	6086.8	6990.1	19 668.7
<i>Dibenzothiophene</i>						
C0-D	31.5	34.4	33.5	17.8	93.4	1117.7
C1-D	172.4	168.0	161.9	90.5	232.4	1514.3
C2-D	386.2	340.3	321.8	215.7	365.8	1230.7
C3-D	354.5	337.2	315.9	224.7	354.7	539.6
Sum	944.6	879.9	833.2	548.7	1046.4	4402.3
<i>Fluorene</i>						
C0-F	7.7	13.2	14.6	5.4	98.5	1351.1
C1-F	76.0	84.6	89.7	39.5	200.3	972.6
C2-F	220.8	225.8	222.6	146.6	328.7	944.4
C3-F	308.0	273.7	258.7	214.3	238.3	486.9
Sum	612.5	597.2	585.6	405.8	865.8	3754.9
<i>Chrysene</i>						
C0-C	300.9	263.2	237.1	234.7	282.7	576.7
C1-C	856.0	781.2	683.7	700.4	710.5	548.3
C2-C	858.7	786.4	657.4	696.8	784.7	298.5
C3-C	561.8	512.6	436.9	452.4	440.0	77.9
Sum	2577.4	2343.5	2015.2	2084.2	2218.0	1501.4
Total	14 340	13 239	12 641	9739	16 504	91 293
<i>Other PAHs</i>						
Biphenyl	0.0	0.0	0.0	0.0	22.1	1841.1
Acenaphthalene	0.0	0.0	0.0	0.0	17.7	200.9
Acenaphthene	0.0	4.6	4.9	0.0	44.2	1378.5
Anthracene	13.4	9.2	14.8	0.0	30.9	2065.4
Fluoranthene	17.9	18.4	14.8	10.4	26.5	892.5
Pyrene	143.0	133.4	113.3	62.7	128.1	1303.7
Benz[<i>a</i>]anthracene	111.7	110.4	98.5	83.5	92.8	579.4
Benzo[<i>b+k</i>]fluoranthene	98.3	96.6	83.7	62.7	53.0	247.7
Benzo[<i>e</i>]pyrene	49.2	59.8	44.3	31.3	53.0	130.8
Benzo[<i>a</i>]pyrene	71.5	69.0	29.6	20.9	48.6	271.0
Perylene	13.4	13.8	9.9	10.4	8.8	18.7
Indeno[1,2,3- <i>cd</i>]pyrene	0.0	0.0	0.0	0.0	4.4	121.5
Dibenz[<i>a,h</i>]anthracene	8.9	13.8	9.9	10.4	17.7	14.0
Benzo[<i>ghi</i>]perylene	13.4	13.8	9.9	10.4	26.5	65.4
Total	541	543	433	303	574	9131

^a The effect of oil-unrelated interference in D-4 on PAH data has been corrected.

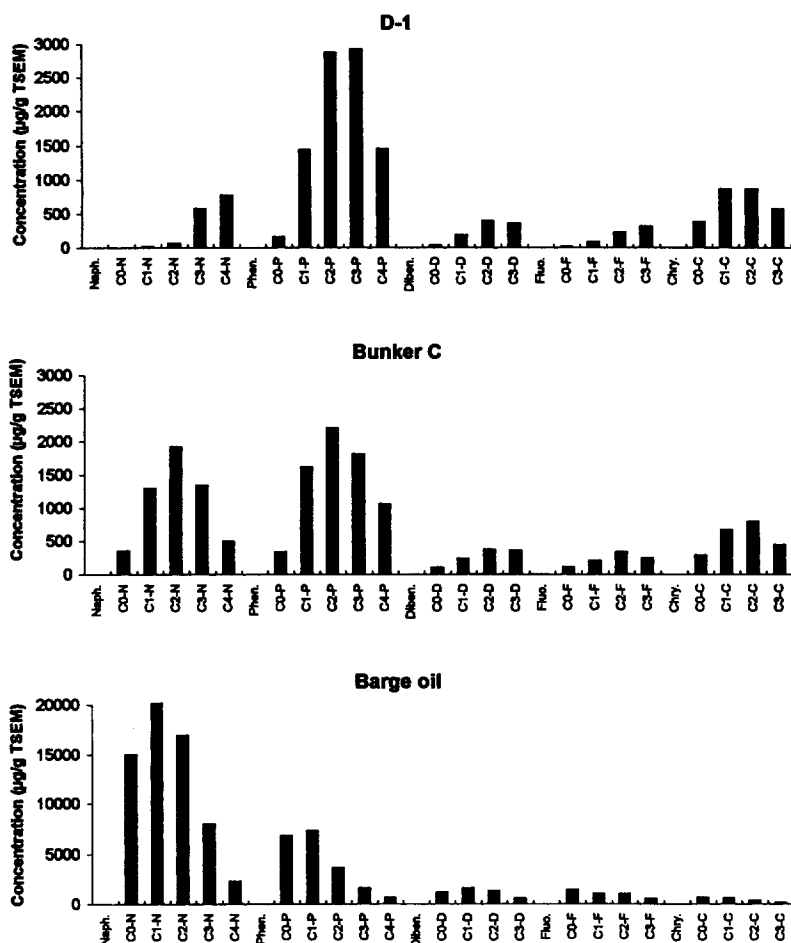


Fig. 4. Target alkylated PAH homologue distribution of sample D-1, Bunker C and Barge oil, illustrating similarity and difference of petrogenic PAH fingerprints between oils. N, P, D, F and C represent naphthalene, phenanthrene, dibenzothiophene, fluorene and chrysene, respectively; 0, 1, 2, 3 and 4 represent carbon numbers of alkyl groups in alkylated PAH homologues.

Bunker C) have a greater concentration of alkylphenanthrenes than alkylnaphthalenes. Compared with Bunker C, the bird samples have a pronounced decrease in the abundances of the alkylnaphthalenes, especially the C_0 -N, C_1 -N, and C_2 -N series, indicating the residual oil has been highly weathered.

(3) Development of a profile showing the composition changes of $C_0 > C_1 > C_2 > C_3$ and the relative percentages of $C_0 < C_1 < C_2 < C_3$ in each PAH group are obvious.

(4) Among other PAHs, the loss of lower-molecular-mass PAHs such as biphenyl, acenaphthalene and

acenaphthene, in comparison to Bunker C, is also obvious.

It can be concluded from the PAH analysis results that: (1) the relative distribution patterns of alkylated PAHs are similar between bird samples (distribution patterns of the other 3 bird samples are not shown here) and Bunker C, but totally different from the barge oil; (2) the unusually high abundances of the alkylphenanthrene and chrysene series relative to other alkylated PAH homologous series are pronounced for bird samples, which is a characteristic feature of Bunker C oil; (3) the effect of weathering on the compositional changes of the residual oil is

apparent, such as the loss of alkyl naphthalenes, dibenzothiophenes and fluorenes, and the simultaneous increase in C_2 -P, C_3 -P and C_4 -P, and the alkylated chrysenes (C_0 - to C_3 -), in comparison to Bunker C.

3.4. Analysis of distribution of biomarkers

Distribution chromatograms of biomarker terpanes and steranes at m/z 191 (left side) and at m/z 217 (right side) for the sample D-1 (other 3ct samples show distribution pattern and profile almost identical to that of sample D-1), Bunker C oil and barge oil are presented in Fig. 5. Typical characteristic features of petroleum biomarker compounds are seen in the residual oil and Bunker C. Only crude oils show these biomarker distributional patterns and profiles [13]. Terpanes are distributed in a wide range from C_{19} to C_{35} with C_{30} and C_{29} $\alpha\beta$ -hopanes being the most abundant. The dominance of C_{27} , C_{28} and C_{29} 20S/20R steranes (with the epimers of $\alpha\beta\beta$ -steranes being more abundant) is apparent. There is also a significant contribution from the diasteranes. The similarity of biomarker distribution profiles between the bird samples and the Bunker C oil is clear in Fig. 5. In addition, the concentrations of the eight most abundant terpanes including C_{23} and C_{24} , C_{29} and C_{30} , C_{32} 22S/22R, and C_{33} 22S/22R terpanes are comparable (see Table 1).

From Fig. 5, it can be also seen that for the barge oil, the distribution pattern and profile of terpanes and steranes is significantly different from the residual oil and Bunker C. This fact, in combination with compositional features of aliphatics and PAHs, clearly identifies the barge oil as a diesel fuel.

3.5. Determination and comparison of diagnostic ratios of the "source-specific-marker" compounds of the unknown oil with the suspected source oils

In addition to quantitative results for major hydrocarbon groups, Table I also summarizes diagnostic ratios of "source-specific-marker" compounds of the unknown oil and the possible source oils.

Analysis of the chromatographic results demonstrates the following:

(1) The bird samples have GC traces and n -alkane

distributions which are similar to those of Bunker C. The only difference observed is that the bird samples show a large UCM, less total n -alkanes, and decreased ratios of C_{17} /pristane and C_{18} /phytane, indicating that all birds were contaminated by the same oil and that the residual oil is weathered and degraded.

(2) The bird samples also have alkyl PAH and biomarker distributional patterns similar to those of the old Bunker C oil.

(3) Among 5 target alkyl PAH homologous series in the bird samples, the alkylphenanthrenes are the most abundant, followed by the alkyl chrysenes series, with the alkyl dibenzothiophene and fluorene series being the least abundant. This is characteristic of weathered Bunker C oil.

(4) The relative ratios of biomarker terpanes C_{23}/C_{24} , C_{29}/C_{30} , $C_{32}(22S)/C_{32}(22R)$ and $C_{33}(22S)/C_{33}(22R)$ for the residual oil and Bunker C are similar, however, the residual oil shows lower ratios of C_{23}/C_{30} and C_{24}/C_{30} than Bunker C. This is most probably due to preferential degradation of the C_{23} and C_{24} vs. C_{30} compounds during weathering and biodegradation [22].

(5) It has been well demonstrated that the chrysenes are the most highly degradation-resistant series among the 5 target alkylated PAH series [4,10–12,17–19,22]. The relative distribution of alkylchrysene homologues was determined to be approximately 0.12:0.33:0.33:0.22 ($C_0C:C_1C:C_2C:C_3C$) for all of the bird samples. These values are very close to the relative ratios of chrysenes for Bunker C, but significantly different from the barge oil.

As weathering proceeds, the relative ratios of less degradation-resistant alkylfluorene and dibenzothiophene series to alkylchrysene series is expected to decrease. The bird samples demonstrate this trend when compared to Bunker C oil: the relative ratios of alkylfluorene and dibenzothiophene series to alkylchrysene series decreased from 0.48 and 0.40, respectively, for Bunker C to 0.26–0.41 and 0.19–0.29 for the bird samples.

(6) Isomeric distributions of 4-, 2-/3- and 1-methyldibenzothiophene were determined to be about 1.0:1.0:0.31 for the bird samples, 1.0:1.12:0.29 for Bunker C and 1.0:0.65:0.23 for the Barge oil. This characteristic ratio has been found to be a very

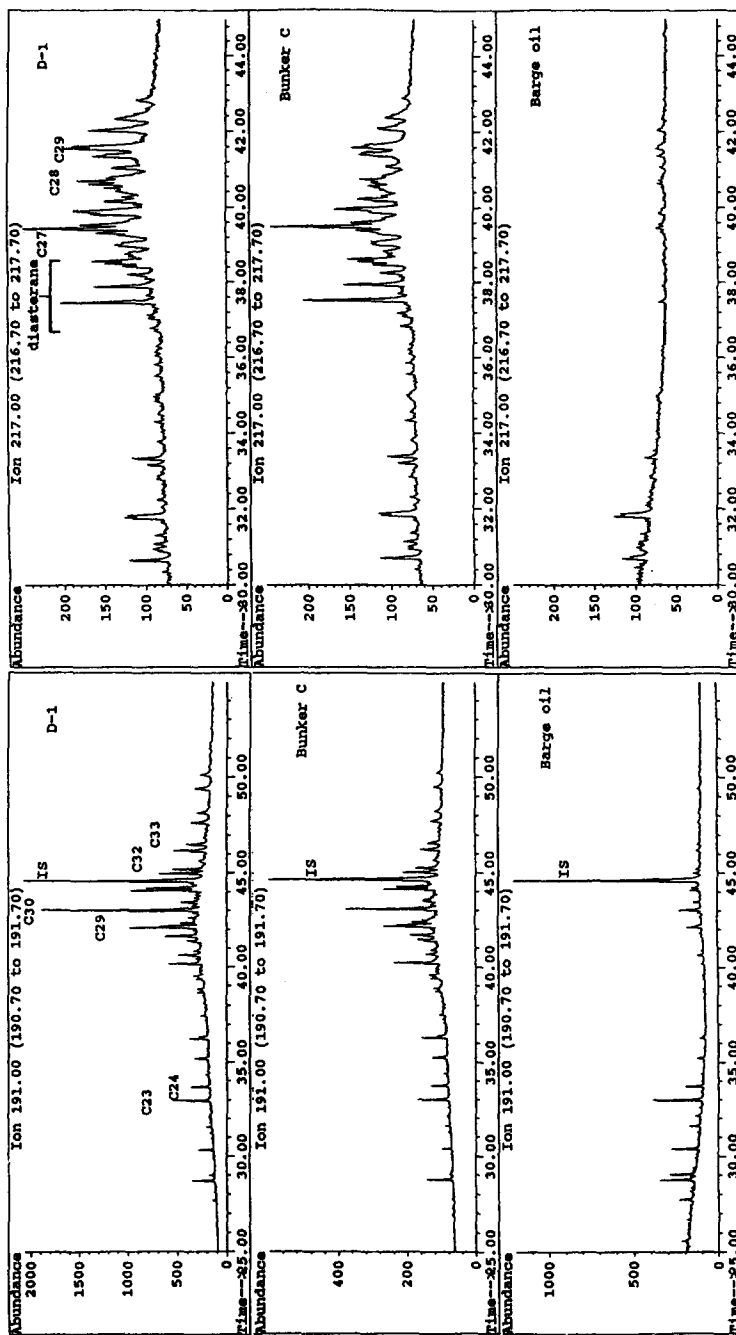


Fig. 5. Distribution of biomarker terpanes (m/z 191, left side), and steranes (m/z 217, right side) in the sample D-1, Bunker C and Barge oil. IS represents the internal standard C_{30} $\beta\beta$ -hopane. C_{23} , C_{24} , C_{29} , C_{30} , C_{32} and C_{33} represent $C_{2,3}$ and $C_{2,4}$ terpane, C_{29} and C_{30} $\alpha\beta$ -hopane and 22S/22R isomer pairs of $C_{2,2}$ and $C_{2,3}$ hopane, respectively. C_{27} , C_{28} , and C_{29} represent $C_{2,7}$, $C_{2,8}$, and $C_{2,9}$ 20S/20R ($\alpha\alpha$ and $\beta\beta$) steranes.

useful marker for differentiating crude and weathered oils [23].

Searching the ESD database for the ratios of three isomers of methyl-dibenzothiophenes in oils, only the Bunker C has both a higher absolute abundance of 2-/3- to 4-methyl-dibenzothiophene and also an unusually high relative ratio of 2-/3- to 4-methyl-dibenzothiophene. Extensive studies conducted in this laboratory [23] have demonstrated that, in most cases, 2-/3-methyl-dibenzothiophene is the most preferentially biodegraded within the isomeric series and that 1-methyl-dibenzothiophene biodegrades slower than 4-methyl-dibenzothiophene, resulting in a decrease in the ratio of 2-/3- to 4-methyl-dibenzothiophene and a slight increase in the ratio of 1- to 4-methyl-dibenzothiophene. Therefore, as weathering and biodegradation progresses, the relative ratios of 2-/3- and 1- to 4-methyl-dibenzothiophene would be expected to gradually fall below 1.1 and to rise slightly higher than 0.29, respectively. The weathered residual oil on the birds shows exactly this trend in the relative ratios of 3 isomers of methyl-dibenzothiophenes, as compared to fresh Bunker C.

This effect is mirrored by the decrease in the relative isomeric distribution of (3+2-methyl-phenanthrenes) to (4/9+1-methyl-phenanthrenes). It has been demonstrated [26] that the isomeric distributions within these alkylated PAH isomer groups exhibit consistency in relative ratios during weathering of oils. However, if biodegradation occurs, these isomeric PAH compounds exhibit unique microbial degradation patterns different from changes due to weathering, in both concentrations and relative distributions [25,33,34]. Therefore, these ratios can be used to unambiguously indicate the occurrence of biodegradation.

This evidence, in combination with other analytical data, implies that the oil on the birds was mostly probably from spilled Bunker C, which has similar chemical composition to the Bunker C used as a control in this study. The residual oil has been weathered and biodegraded to some degree as well.

(7) The values of the double ratio of C_2D/C_2P to C_3D/C_3P [10,17,18] are almost the same for the birds but lower than that for Bunker C, further indicating that 4 birds were contaminated by the same oil and that the residual oil is highly weathered compared to Bunker C.

(8) The GC traces, the distribution of the *n*-alkanes, PAHs and biomarker compounds, and the diagnostic ratios of “source-specific-markers” of the barge oil are significantly different from the residual oil. In particular, the barge oil contains high concentrations of toxic alkyl PAHs (91 293 $\mu\text{g/g}$ oil, approximately six times that occurring in Bunker C). All of these factors lead to the conclusion that the barge oil is a diesel fuel and that the residual oil on the birds is not the same as the barge oil.

3.6. Estimation of weathered percentages of residual oil on the birds

A method using the biodegradation-resistant C_{30} hopane as an internal conservative reference to estimate the depletion of crude oil in oiled sediments has been developed and successfully applied to quantify the percentages of oil lost to weathering [7,22]. Using this method, the average weathered percentage of the residual oil, based on the concentrations of C_{29} and C_{30} $\alpha\beta$ -hopane in our lab's Bunker C, was estimated to be about 20%. For light and medium crudes, the weathering losses caused by evaporation and other factors can be as much as 70 and 40% of their volume, respectively [35]. But, for heavy or residual oils such like Bunker C, the weathering losses are correspondingly smaller. The estimated weathering loss quantitatively implies that the oil on birds is highly weathered.

4. Conclusion

This paper has described a detailed systematic analytical approach by which unknown oil-related environmental samples can be accurately identified and their chemical compositions characterized. This approach, in general, can be applied for identification of the source of any other unknown oil.

All evidence in this work indicates that the residual oil on the birds was most likely a Bunker type oil, and definitely not from a nearby barge oil. Also it is clear that the residual oil was highly weathered and had experienced a loss of 20% of its mass to weathering. Finally, it was also evident that biodegradation of the oil had occurred, indicated by decreases in the ratios of C_{17} /pristane and C_{18} /

phytane, the preferential loss of specific PAHs, and the loss of low-molecular-mass biomarker compounds such as C₂₃ and C₂₄ terpanes.

It should be understood that there is no single chromatogram or ratio which can be used alone for positive source-identification of an unknown spilled oil, especially for weathered and degraded residual oils in complex environmental samples. In order to ensure accurate identification and characterization of the unknown petroleum type and quantitation of product(s) present in samples, a comprehensive, systematic and tiered analytical approach such as the one used in the present work, must be used. In addition, complete sets of analytical data, in particular the data for the unique “source-specific-marker” compounds, should be accurately determined and carefully analysed, and compared with the possible source oils, if they are available.

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