Using Systematic and Comparative GC/MS and GC/FID 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/FID data is described. The characterizations of the unknown oil were not only through a variety of analyses including individual aliphatic, aromatic, and biomarker hydrocarbons, but also through target source-specific-marker analysis, diagnostic ratio analysis, and "pattern recognition" plot 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 finally concluded that (1) the residual oil on the birds was most probably the old Bunker type oil, and definitely not from the suspected barge oil; (2) the heavy Bunker type oil has been relatively highly weathered; (3) some biodegradation of oil had occurred.

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 occured 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. In most cases, the oil, after accidentally being released to the environment, is immediately subject 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 make complex spilled oil samples become even more complicated. Therefore, the ability to unambiguously identify and differentiate 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 extremely important and critical in settling questions of environmental impact and legal responsibility or liability.

Environmental Protection Agency (EPA) methods such as 602, 610, and 624 [3] have been used for identifying and 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, thus lacking the ability to identify the source and to track the fate of spilled oil. In recent years, analytical techniques have made major advances [4-13]. 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 [14-21]. The characterizations not only are through a variety of analyses including individual aliphatic, aromatic, and biomarker hydrocarbons, but also through target source-specific-marker analysis, diagnostic ratio analysis, and "pattern recognition" plot 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, we report the detailed tiered and integrated analytical approach by which unknown oil-contaminated bird samples are accurately identified and how their chemical compositions are characterized.

EXPERIMENTAL

Capillary gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS)

Analyses for n-alkane distribution and total petroleum hydrocarbons (TPH) were performed on a Hewlett-Packard (HP) 5890 gas chromatograph equipped with a flame-ionization detector (FID) and an HP 7673 autosampler. A 30-m x 0.32-mm id. (0.25-µm 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 °C and 300 °C, respectively. The following temperature program was used: 2-min hold at 50 °C; ramp to 300 °C at 6 °C/min; and 16-min hold at 300 °C. A 1-µL aliquot was injected in the 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 (MSD). System control and data acquisition were achieved with an HP G1034C MS ChemStation (DOS series). The MSD was operated in the scan and selected ion monitoring (SIM) modes for identification of components, and in the SIM mode for quantitation of target compounds. An HP-5 fused-silica column with dimensions of 30-m x 0.25-mm id. (0.25-µm film) was used. The chromatographic conditions were as follows: carrier gas, helium (1.0 mL/min); injection mode, splitless; injector and detector temperature, 290 °C 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.

The oil-contaminated bird samples

Four oiled birds covered with an unknown oil were found 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 weathering and degradation extent of the spilled oil, and changes in oil character since occurrence of any possible spill, and then to determine whether the oil was related or not with leakage from a barge which grounded on Anticosti Island (approximately 100 miles away from the National Park) in the early December of 1995 or from any other known sources.

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) collected from the barge was actually 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 have been obtained from various sources during the period of 1985-1994 and are currently stored in a cold room of the ESD laboratory.

Analytical approach

Tier 1: Determination of oil residues. The bird samples contaminated by the unknown spilled oil were weighed, mixed with approximately 20-g of anhydrous sodium sulphate and spiked with an appropriate amount of PAH surrogates, and then serially extracted three times with 150 mL 1:1 hexane/dichloromethane (DCM) for 5 minutes each time using sonication (note: different from sediment extraction method[14], milder solvent mixture and shorter extraction time was used in order to avoid extraction of any excessive unwanted components from the bird itself). The extracts were combined, filtered through anhydrous sodium sulphate, concentrated to appropriate volume using rotary evaporation, and then solvent-exchanged to hexane. An aliquot of the concentrated extract was blown down with N_2 to residue and weighed on a microbalance to obtain a total solvent-extractable material weight (TSEM, expressed in mg/g of sample).

Tier 2: Determination of TPH by GC/FID and product screen. The microcolumn fractionation technique [15] was employed for sample cleanup and fractionation of extracted oil. Appropriate aliquots of the concentrated extracts (containing TSEM ~ 20 mg) were applied to the 3-gram silica gel column which had been preconditioned with 12 mL of hexane. Half of the hexane fraction (F1) was used for analysis of saturates and biomarker compounds; half of the 50% benzene fraction (F2) was used for analysis of target PAHs and alkylated PAH homologues. The remaining half of F1 and F2 were combined (F3) and used for the determination of total hydrocarbons (TPH). These three fractions were concentrated under a stream of nitrogen to appropriate volumes, spiked with internal standards, and then adjusted to accurate pre-injection volumes for GC analysis.

Fraction 3 was analysed by GC/FID to determine GC-detectable total petroleum hydrocarbons (GC-TPH) and to clarify the products in the samples as characteristic of gasoline, diesel, or crude product. Individual n-alkanes from C_8 to C_{40} including important isoprenoids, pristane and phytane, are quantified at this level to obtain a description of the aliphatic hydrocarbon distribution in the samples.

Tier 3: GC/MS measurement of target PAHs and biomarkers and analysis of their distribution patterns. High-performance capillary GC/MS is used to obtain comprehensive quantitative pictures of PAHs (which are probably the most important analytes in oil-spill natural resource damage assessment) and biomarkers (these highly degradation-resistant hydrocarbons are specially useful for characterization of highly weathered crudes and oil residuals) in the samples.

Fraction 2 is analysed by GC/MS in SIM mode for over 25 individual PAH compounds, and for 5 target alkylated PAH homologues which are valuable for oil differentiation and monitoring of the oil weathering process. Under certain circumstances, analysis of BTEX and alkyl-substituted benzenes is performed [19] to obtain distribution pattern of alkylated benzenes. If there are any major unknown chromatographic peaks in both analyses, they are identified for purposes of potential product-source identification .

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

Tier 4: Determination and comparison of diagnostic ratios of the "source-specific-marker" compounds with the suspected source oil. Source-specific marker compounds (such as methyldibenzothiophene isomers, alkylated PAH homologous compounds, paired biomarker compounds C_{23} and C_{24}-terpane, C_{29} and C_{30} $\alpha\beta$-hopane, C_{32} and C_{33} 22S/22R hopanes) 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 [13,14,17].

Comparison of all diagnostic ratios and the "source-specific marker" compounds from the unknown oil with the suspected source oils can then be done (a database containing detailed chemical composition of oils and petroleum products is required to conduct such comparison and analysis), in order to confirm identification of the contamination source.

Tier 5: Determination of weathered percentages of residual oil. Calculation based on hopane analysis to estimate weathered percentages of oil can be more accurately performed than the traditional method [7,17], 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.

RESULTS AND DISCUSSION

Determination of oil residues

Table 1 presents the hydrocarbon analysis results for the bird samples by gravimetric and GC/FID methods. In addition to the TESM and GC-determined TPH, ratios of saturates/TSEM, aromatics/TSEM, resolved peaks/TPH and UCM/TPH, and TPH/TSEM are also listed in Table 1. 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 1 can be summarized as follows: (1) the birds were badly contaminated by oil, indicated by high values of TPH from 9449 to 37735 ppm; (2) the values of saturates in TPH and the ratios of UCM/TPH are around 50% and 8% (many fresh oils often show much higher values, around 70% and 20% respectively) for all 4 bird samples respectively, which is often one of the characteristics of weathered oil; (3) extremely high-abundant oil-unrelated peaks which eluted out between 36.78 and 44.74 min (approximate 70% of resolved peaks) were detected in the GC/FID chromatogram of F2 of the sample D-4. They were identified to be fatty alcohols and fatty acid monoesters. However, these interferencing surfactant-type compounds can be completely removed by successively passing the fraction 2 eluted from the first column, through the second silica gel column.

Note that all results of the sample chemical composition discussed below are expressed on the TSEM basis. TSEM gives a reasonably equal basis for the determination of the relative composition changes of hydrocarbon groups in samples. It is only by this way that the quantitation results are comparable. It should be understood, however, that TSEM would measure petrogenic material as well as other non-petrogenic extractable material, and so in some cases could give values greater than the true petrogenic hydrocarbon values, as some of what we are measuring does not come from the residual oil. In order to lessen the interference of non-petrogenic extractable material on analysis results, careful design of extraction and correction of analytical data is needed in some cases.

Product type screen and analysis of aliphatic hydrocarbons

Figure 1 presents the GC/FID chromatograms for aliphatic hydrocarbon analysis. Table 2 summarizes the concentration values of n-alkanes including pristane and phytane. Figure 2 depicts graphically the n-alkane distribution.

Analysis of aliphatic hydrocarbons clearly demonstrates that (1) the value of the carbon preference index (CPI, defined as the sum of odd over even-carbon-number n-alkanes) near 1.0 is a clear indication of petroleum. Therefore, the contaminated product on the bird definitely is crude petroleum, not any other type of petroleum product; (2) the fact that the total of n-alkanes was determined to be only from 10 to 14 mg/g of TSEM, and that the n-alkane homologous series before n-C_{14} were completely lost and the abundances of n-alkanes after n-C_{14} were dramatically decreased, indicates that the oil on the birds has undergone relatively heavy degradation and weathering process and the chemical composition has been further altered since the birds were oil-contaminated; (3) the profiles of GC traces for the 4 birds are almost identical, demonstrating that they were contaminated by the same oil; (4) the very close ratio values of C_{17}/pristane, C_{18}/phytane and pristane/phytane for 4 birds may illustrate that these 4 birds were contaminated by oil at the same time.

The GC traces, the hump profiles and Figure 2 demonstrate that the oil is primarily composed of heavy residual oil, showing the maxima of homologous series of n-alkanes around n-C_{31}. This kind of GC traces is often the characteristic GC traces of weathered older type Bunker C oil. Bunker C fuel oil 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. However, Bunker type marine fuels are currently produced by blending residuum (material remaining unevaporated from refining processes) and lighter distillate stocks in various ratios, to yield fuel of a specified viscosity for different purposes. Therefore, depending on production oilfields, production years, and blending ratios, Bunker C and marine fuels can have widely different physical properties and chemical compositions [22].

Several different Bunker C type oils were analyzed to obtain their fingerprints.

Table 1 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	29329	293	54	8.0	92
D-2	Oil-contaminated duck 2	113.9	13.99	37735	307	51	8.0	92
D-3	Oil-contaminated duck 3	179.4	6.70	9449	253	53	7.9	92
D-4	Oil-contaminated duck 4	28.0	1.90	18729	276	49	7.8	92

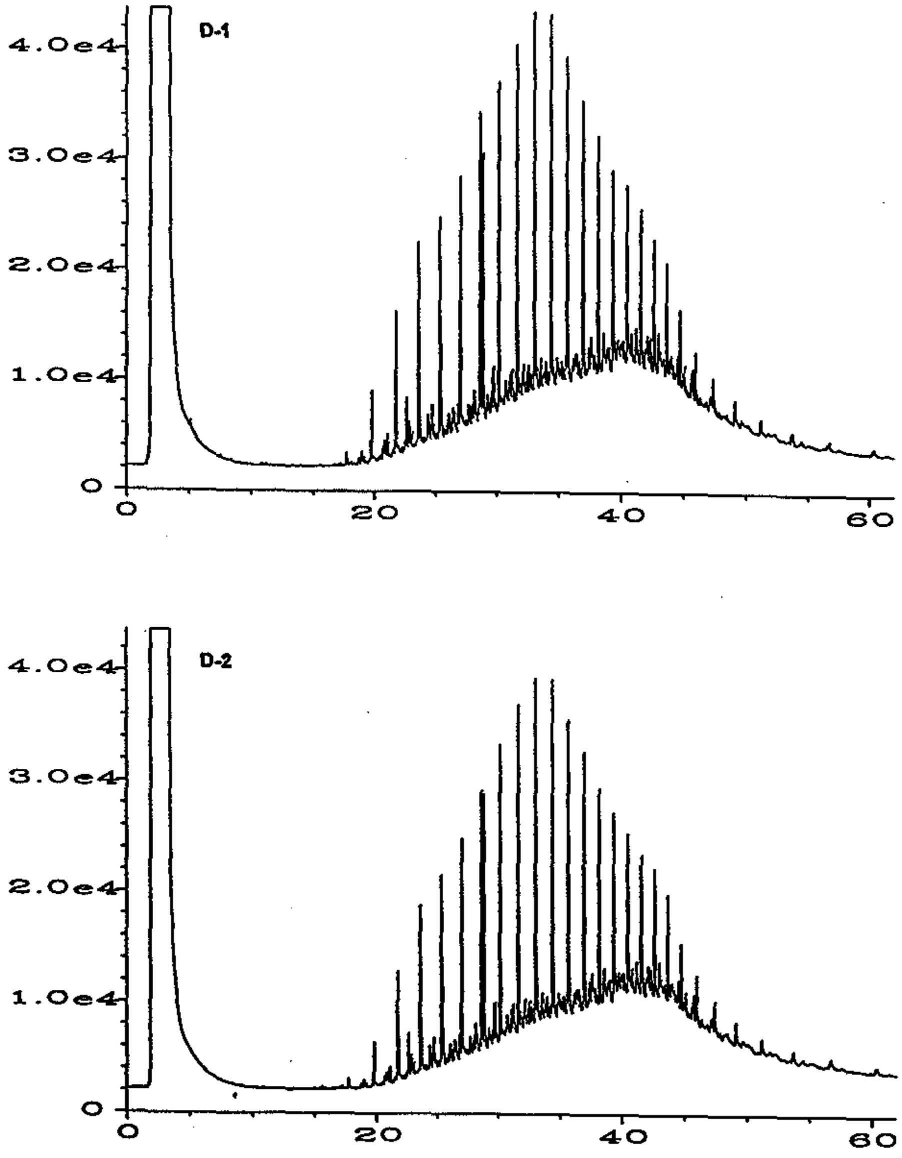
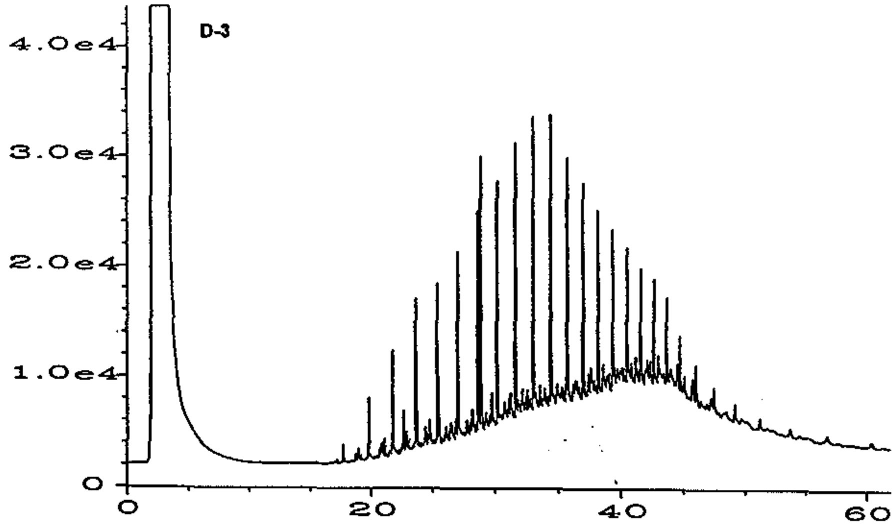


Figure 1 GC/FID chromatograms for aliphatic hydrocarbon analysis of oil-contaminated bird samples D-1 to D-4, and the suspected source oil Bunker C and Barge oil.


D-3
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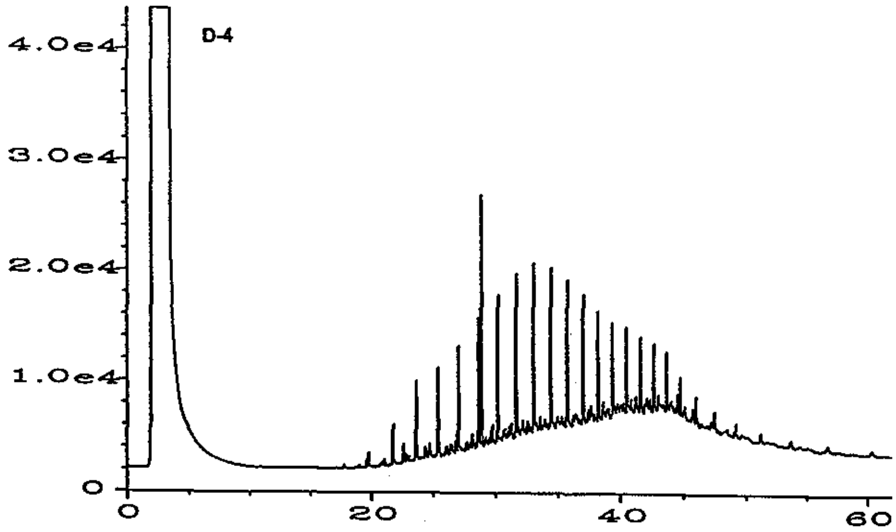
3.0e4
2.0e4
1.0e4
0
0
20
40
60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1.0e4
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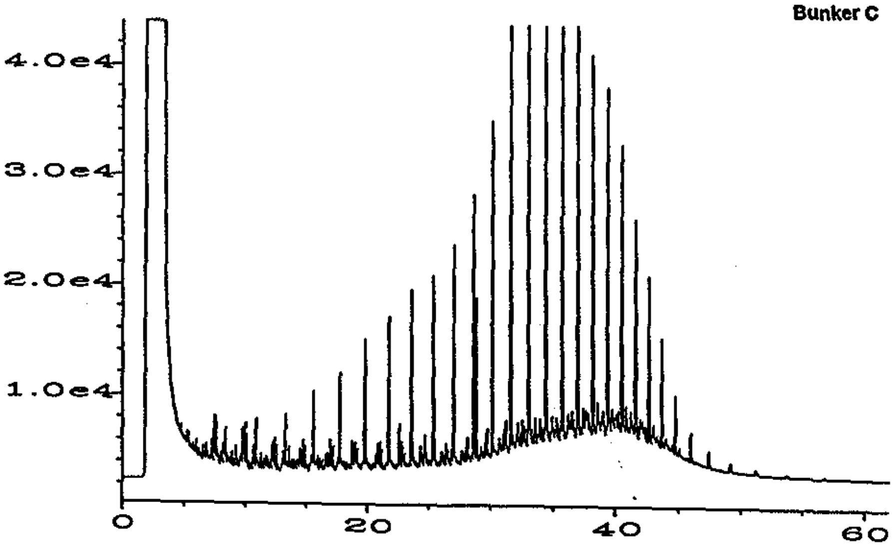
0
20
40
60


Bunker C
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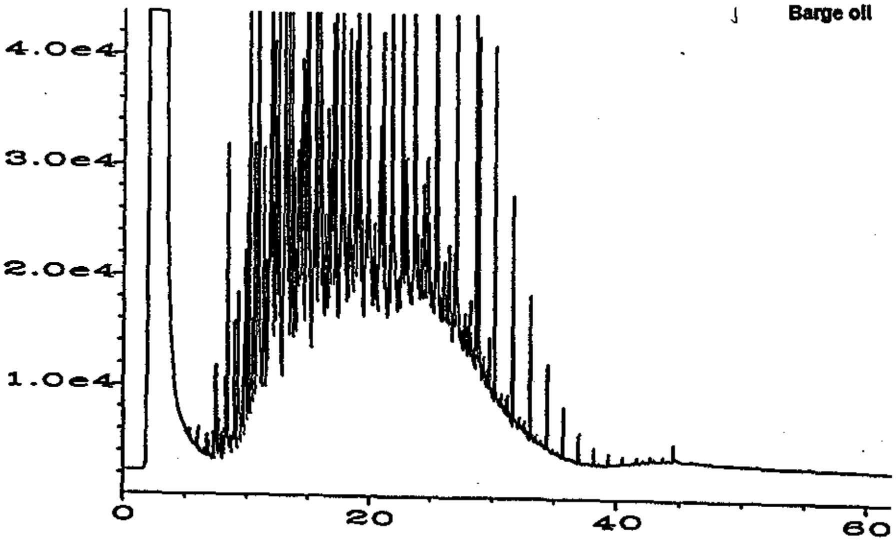
3.0e4
2.0e4
1.0e4
0
20
40
60


Barge oil
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3.0e4
2.0e4
1.0e4
0
20
40
60

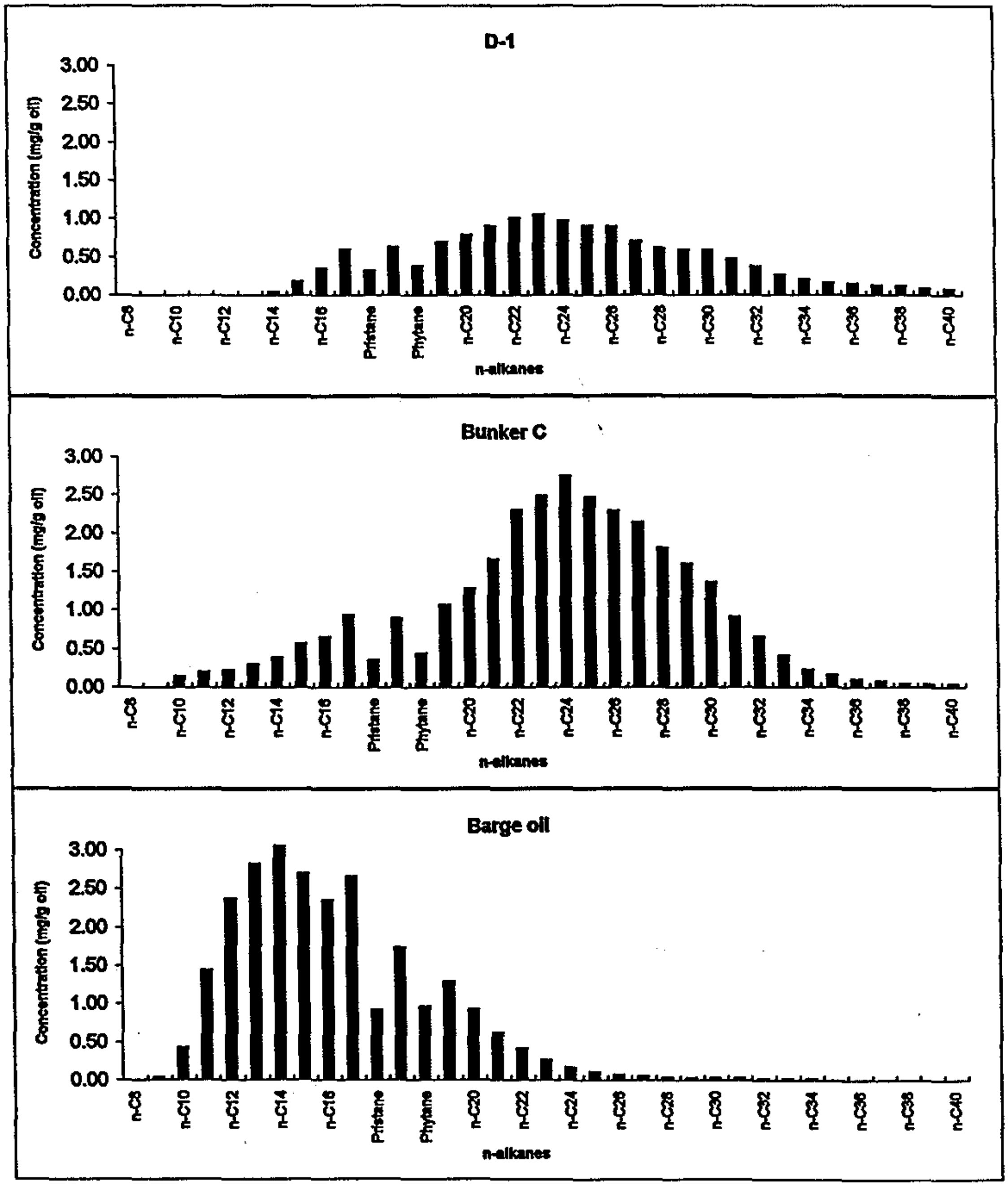

Figure 2 n-alkane distribution patterns for the sample D-1, Bunker C, and Barge oil.

Table 2 n-Alkane Analysis Results

Sample Type n-Alkanes	D-1 (mg/g TSEM)	D-2 (mg/g TSEM)	D-3 (mg/g TSEM)	D-4 (mg/g TSEM)	Bunker C (mg/g oil)	Barge oil (mg/g oil)
n-C8						
n-C9						0.03
n-C10					0.13	0.41
n-C11					0.19	1.43
n-C12					0.21	2.36
n-C13					0.29	2.82
n-C14	0.04	0.03	0.06	0.02	0.38	3.04
n-C15	0.17	0.12	0.16	0.06	0.55	2.69
n-C16	0.34	0.28	0.28	0.18	0.64	2.34
n-C17	0.59	0.51	0.46	0.38	0.92	2.65
Pristane	0.31	0.28	0.25	0.22	0.34	0.91
n-C18	0.61	0.55	0.47	0.42	0.88	1.73
Phytane	0.37	0.35	0.29	0.27	0.43	0.96
n-C19	0.68	0.62	0.53	0.49	1.06	1.28
n-C20	0.77	0.71	0.61	0.58	1.27	0.92
n-C21	0.89	0.84	0.70	0.67	1.64	0.62
n-C22	0.99	0.97	0.80	0.77	2.29	0.41
n-C23	1.04	1.00	0.84	0.77	2.47	0.25
n-C24	0.96	1.02	0.87	0.74	2.73	0.16
n-C25	0.89	0.87	0.73	0.67	2.46	0.09
n-C26	0.88	0.79	0.73	0.67	2.28	0.06
n-C27	0.69	0.70	0.59	0.56	2.13	0.04
n-C28	0.60	0.58	0.48	0.46	1.80	0.02
n-C29	0.58	0.54	0.46	0.40	1.59	0.02
n-C30	0.57	0.54	0.44	0.38	1.35	0.02
n-C31	0.46	0.40	0.33	0.35	0.90	0.01
n-C32	0.36	0.36	0.29	0.30	0.64	0.01
n-C33	0.25	0.24	0.21	0.24	0.40	0.01
n-C34	0.19	0.16	0.12	0.17	0.22	0.01
n-C35	0.15	0.16	0.12	0.15	0.15	
n-C36	0.14	0.15	0.10	0.12	0.09	
n-C37	0.12	0.11	0.09	0.11	0.06	
n-C38	0.11	0.11	0.09	0.10	0.04	
n-C39	0.08	0.10	0.07	0.08	0.03	
n-C40	0.08	0.09	0.07	0.08	0.03	
TOTAL	14.0	13.2	11.2	10.4	30.6	25.3
C17/Pristane	1.90	1.85	1.83	1.72	2.72	2.91
C18/Phytane	1.64	1.57	1.61	1.56	2.05	1.80
Pristane/Phytane	0.83	0.80	0.86	0.82	0.79	0.95
CPI	0.99	0.99	0.99	0.99	0.99	1.04

For comparison purposes, the n-alkane data obtained from the analysis of one of our lab's Bunker C oil samples and the barge oil are also listed in Table 2. Analysis results demonstrate that the bird samples show very similar profiles of GC traces to our lab's old-type Bunker C oil except with much larger UCMs and smaller total n-alkanes.

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 [10,12,14,17].

Figure 3 shows the GC/MS total ion chromatograms in the SIM mode for the alkylated PAHs. Table 3 summarizes analysis results of the 5 target alkylated PAH series and other target PAHs. Figure 4 depicts graphically the distribution of alkylated PAHs (please note Y-axis scale, 0-20,000, applied to the barge oil is different from D-1 and Bunker C).

The sum of the 5 target alkylated PAHs was determined to be 14285, 13239, 12641, and 6243 μg/g of TSEM for samples D-1, D-2, D-3, and D-4, respectively. Major compositional changes of PAHs observed are summarized as follows: (1) BTEX (the collective name of benzene, toluene, ethylbenzene, and the xylene isomers) and lighter alkylbenzene compounds were completely lost, evidenced by the fact that no alkylbenzene peaks were detected before the retention time of 17 minutes. This feature often indicates that the weathered percentages of the residual oil are greater than 10-15%; (2) the distribution pattern with the alkyl naphthalene series being the most abundant among the 5 target alkylated PAH series is observed for many fresh oils. Only very few "fresh" oils (such as Bunker C) show higher alkyl phenanthrenes than alkyl naphthalenes in abundance. The samples show the most pronounced decrease in the abundances of the alkyl naphthalenes, especially the C_0-N, C_1-N, and C_2-N series, indicating the residual oil on the birds has been relatively heavily weathered; (3) the highest abundances of alkyl phenanthrenes then followed by the alkyl chrysenes, are clearly demonstrated; (4) 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; (5) among other PAHs, the loss of lower molecular weight PAHs such as biphenyl, acenaphthalene, and acenaphthene is obvious in comparison to Bunker C.

For comparison purposes, Figure 5 depicts the relative distribution of alkylated PAHs (normalized to C_2-P = 1.0) of the oil-contaminated bird sample D-1 and our lab's Bunker C. It can be clearly seen from Figures 4 and 5 that (1) the relative distribution patterns of alkylated PAHs are almost identical between bird samples (distribution patterns of the other 3 bird samples are not shown here) and between bird samples and Bunker C, but totally different from the barge oil; (2) the unusual high abundances of alkyl phenanthrene and chrysene series relative to other alkylated PAH homologous series in bird samples and Bunker C oil are pronounced; (3) compared to Bunker C, accumulation of C_2-P, C_3-P, C_4-P, and the whole alkylated chrysene group (C_0- to C_3-), is pronounced for bird samples. For example, the total of alkyl phenanthrenes and alkyl chrysenes were determined to be 8814 and 2647 μg/g of TSEM for D-1 respectively, obviously higher than the corresponding values (6990 and 2168 μg/g of oil) for Bunker C; (4) the effect of weathering on the composition changes of bird samples is apparent, that is, the loss of alkyl naphthalene, dibenzothiophene, and fluorene series relative to C_2-P is obvious.

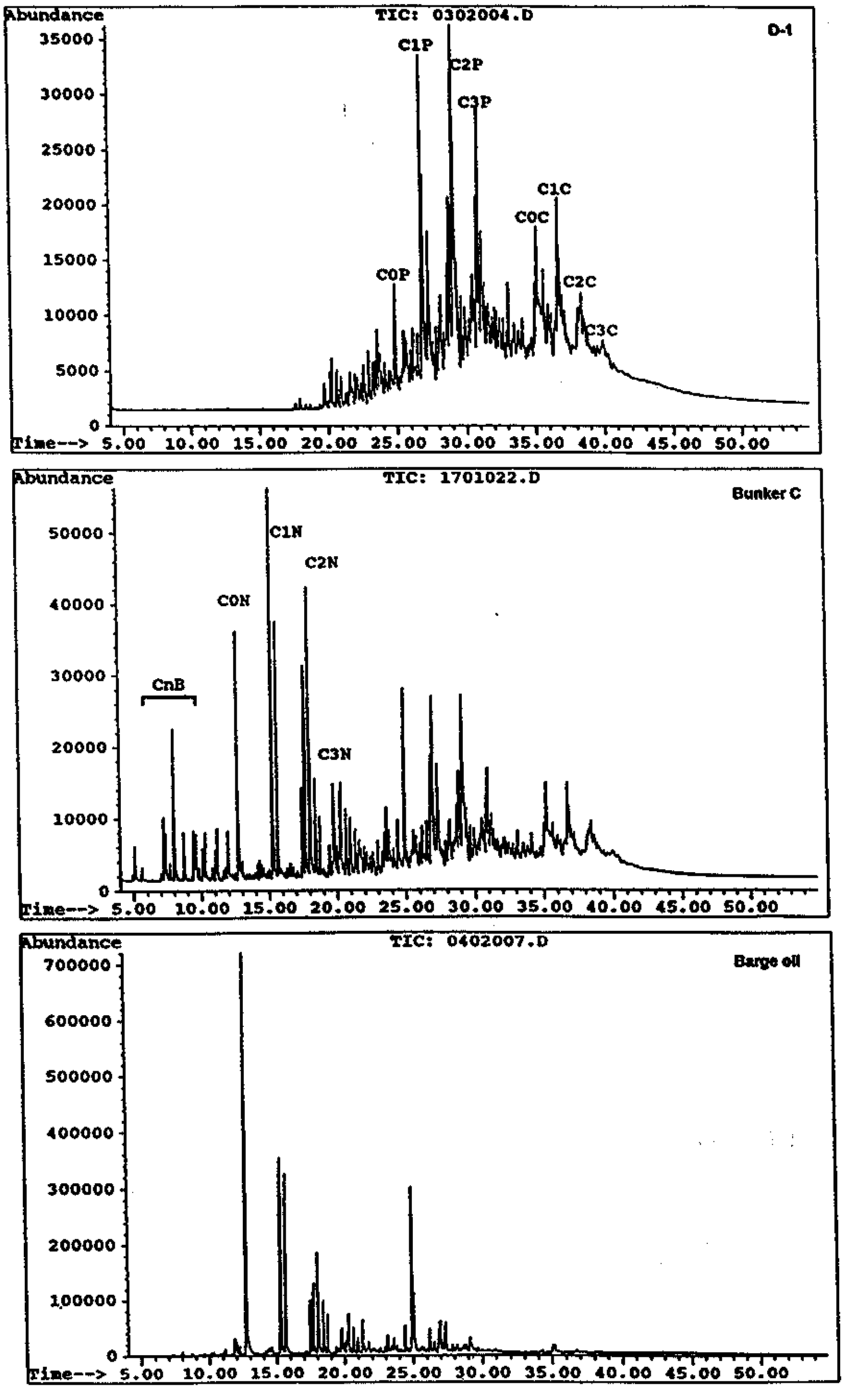

Figure 3 GC/MS total ion chromatograms (in SIM mode) for the alkylated PAHs analysis. B, N, P, and C represent benzene, naphthalene, phenanthrene, and chrysene, respectively; n, 0, 1, 2, 3, and 4 represent carbon numbers of alkyl groups in alkylbenzenes and alkylated PAH homologues.

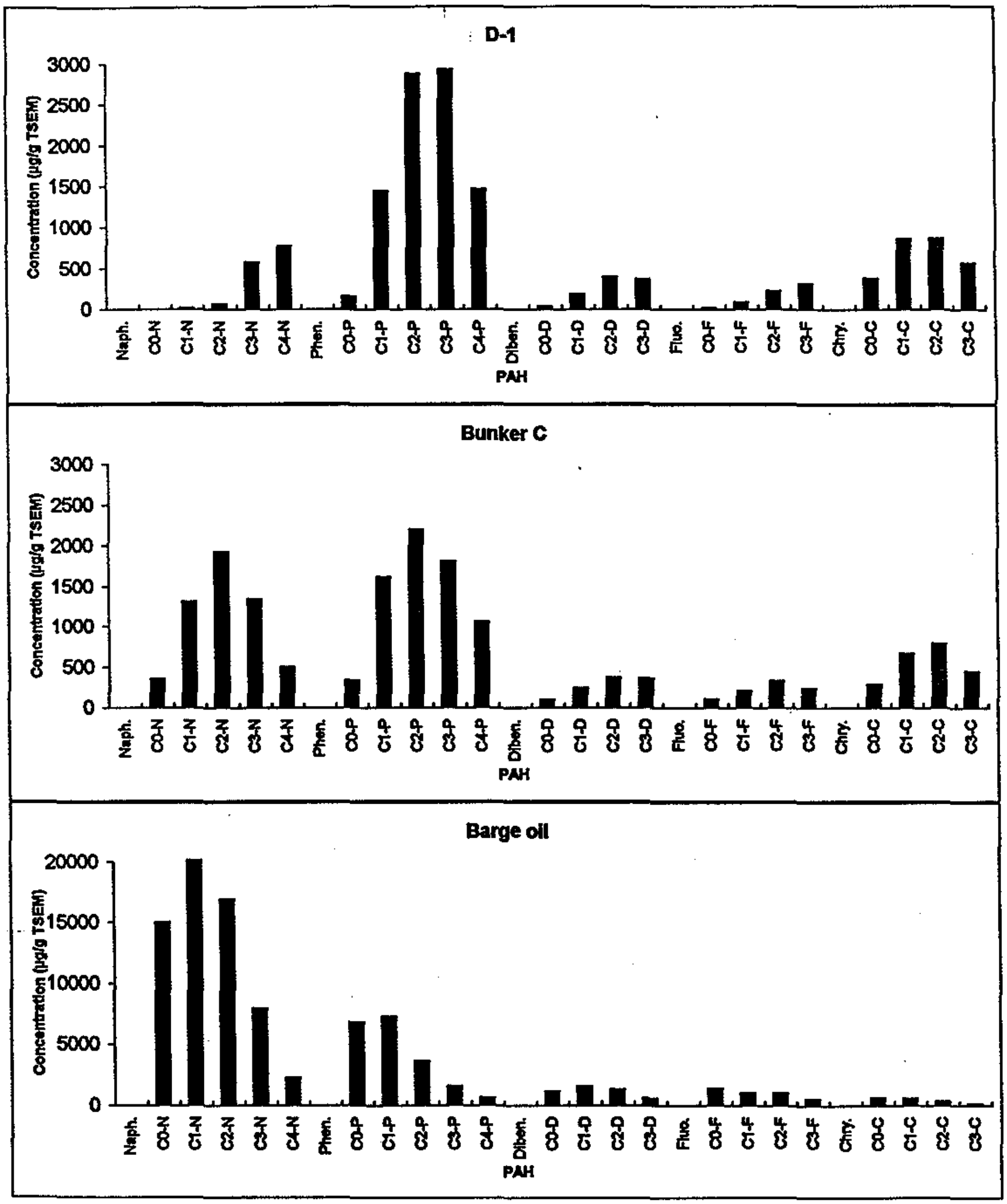

Figure 4 Alkylated PAH fingerprints of the sample D-1, Bunker C, and Barge oil, illustrating similarity and difference of petrogenic PAH compositions between oils. N, P, D, F, and C represent naphthalene, phenanthrene, dibenzothiophene, fluorene, and chrysene, respectively; 0, 1, 2, 3, and 4 represent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

Table 3 Analysis Results of Alkylated PAHs and Other PAHs

Sample Type PAHs	D-1	D-2	D-3	D-4	Bunker C	Barge oil
		(µg/g TSEM)			(µg/g oil)	
Naphthalene						
C0-N	1.3	1.5	1.7	5.3	347.7	14939.9
C1-N	4.6	21.7	13.7	15.5	1294.7	20087.0
C2-N	56.8	169.2	208.6	38.6	1914.9	16853.9
C3-N	566.7	562.2	799.2	114.3	1335.2	7892.3
C4-N	762.4	675.4	803.9	190.4	490.9	2192.4
Sum	**1391.8**	**1430.1**	**1827.0**	**364.0**	**5383.3**	**61965.6**
Phenanthrene						
C0-P	149.6	174.7	171.5	67.1	330.4	6759.6
C1-P	1431.9	1354.4	1304.9	590.4	1609.5	7209.8
C2-P	2865.7	2583.1	2349.5	1183.5	2196.1	3560.0
C3-P	2918.1	2609.2	2398.6	1294.9	1803.1	1549.8
C4-P	1448.4	1266.6	1156.0	765.5	1050.9	589.5
Sum	**8813.7**	**7988.0**	**7380.6**	**3901.3**	**6990.1**	**19668.7**
Dibenzothiophene						
C0-D	31.5	34.4	33.5	12.7	93.4	1117.7
C1-D	172.4	168.0	161.9	65.9	232.4	1514.3
C2-D	386.2	340.3	321.8	166.4	365.8	1230.7
C3-D	354.5	337.2	315.9	166.5	354.7	639.6
Sum	**944.6**	**879.9**	**833.2**	**411.5**	**1046.4**	**4402.3**
Fluorene						
C0-F	7.7	13.2	14.6	2.9	98.5	1351.1
C1-F	76.0	84.6	89.7	24.6	200.3	972.6
C2-F	220.8	225.8	222.6	93.4	328.7	944.4
C3-F	308.0	273.7	258.7	129.7	238.3	486.9
Sum	**612.5**	**597.2**	**585.6**	**250.6**	**865.8**	**3754.9**
Chrysene						
C0-C	370.9	263.2	237.1	138.3	282.7	576.7
C1-C	856.0	781.2	683.7	400.9	660.5	548.3
C2-C	858.7	786.4	657.4	449.4	784.7	298.5
C3-C	561.8	512.6	436.9	327.0	440.0	77.9
Sum	**2647.4**	**2343.5**	**2015.2**	**1315.6**	**2168.0**	**1501.4**
TOTAL	14410	13239	12641	6243	16454	91293
			Other PAHs			
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	7.3	26.5	892.5
Pyrene	143.0	133.4	113.3	43.9	128.1	1303.7
Benz(a)anthracene	111.7	110.4	98.5	58.5	92.8	579.4
Benzo(b + k)fluoranthene	98.3	96.6	83.7	43.9	53.0	247.7
Benzo(e)pyrene	49.2	59.8	44.3	21.9	53.0	130.8
Benzo(a)pyrene	71.5	69.0	29.6	14.6	48.6	271.0
Perylene	13.4	13.8	9.9	7.3	8.8	18.7
Indeno(1,2,3cd)pyrene	0.0	0.0	0.0	0.0	4.4	121.5
Dibnez(a,h)anthracene	8.9	13.8	9.9	7.3	17.7	14.0
Benzo(ghi)perylene	13.4	13.8	9.9	7.3	26.5	65.4
TOTAL	541	543	433	212	574	9131

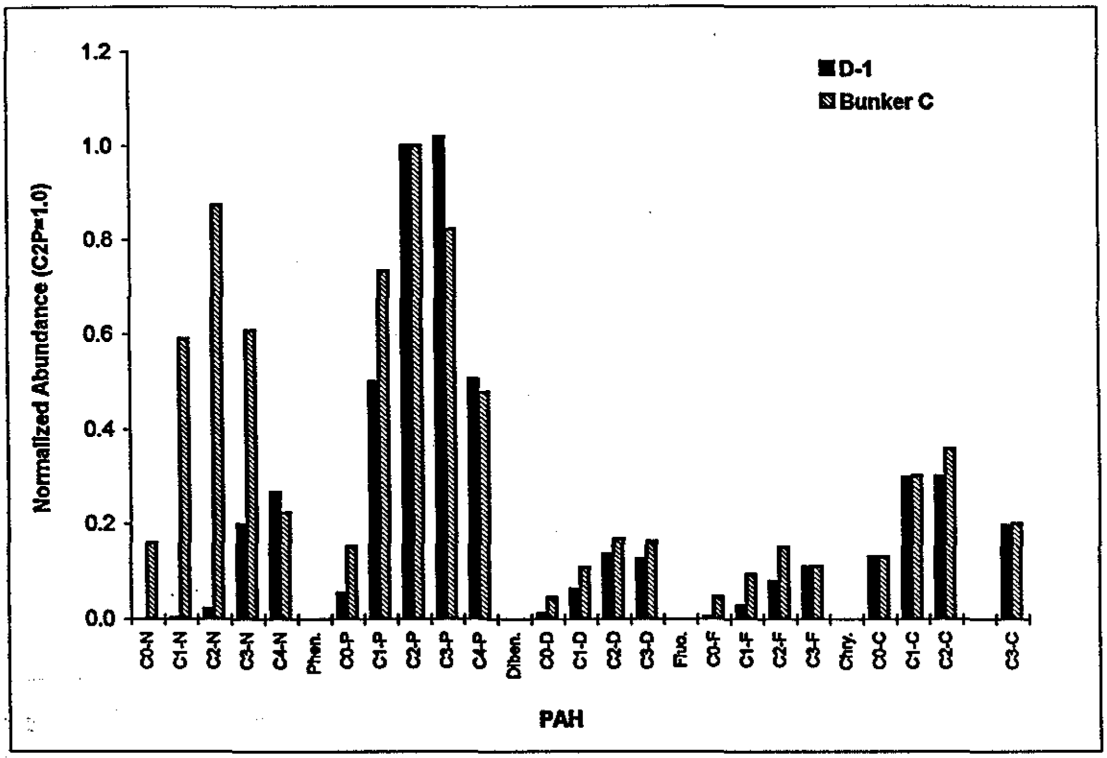

Figure 5 Comparison of relative distribution (C_2-P=1.0) of alkylated PAHs for the sample D-1 and Bunker C. Weathering effects on alkylated PAHs can be illustrated by abundance changes relative to C_2-P.

Analysis of distribution of biomarkers

Figure 6 presents distribution chromatograms of biomarker terpane compounds at m/z 191 for the sample D-1 (other 3 samples show almost identical distribution pattern and profile as D-1), Bunker C oil, and barge oil. Figure 7 presents distribution chromatograms of steranes at m/z 217 for the sample D-4, Bunker C oil, and barge oil. Figures 6 and 7clearly demonstrates the typical and characteristic features and composition of petroleum biomarker compounds of the birds and Bunker C. Only crude oils show such distribution pattern and profile of biomarkers. Terpanes distribute in a wide range from C_{19} to C_{35} with C_{30} and C_{29} $\alpha\beta$-hopanes (peaks 23 and 22) being the most. From Figure 7 the dominance of C_{27}, C_{28}, and C_{29} steranes (with the epimers of $\alpha\beta\beta$-steranes being much higher abundant) is apparent. There is also a significant contribution from the diasteranes. Figures 6 and 7 clearly demonstrate similarity of distribution profiles of the bird samples to the Bunker C oil. In addition, the absolute concentrations of target biomarker terpanes (expressed in µg/g of TSEM) are in the same level with the values (expressed in µg/g of oil) determined for the Bunker C oil (Table 4).

As for the barge oil, its terpane and sterane chromatograms at m/z 191 and 217 demonstrates the product nature is diesel-dominant petroleum.

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

For comparison purposes, the GC chromatograms and analysis data for our lab's Bunker C oil and the barge oil are presented in the corresponding figures and tables together with the bird samples.

Table 5 summarized the diagnostic ratios of the "source-specific-marker " compounds of the unknown oil and the suspected source oils.

Careful analysis of all chromatograms and analysis data demonstrate the following:

(1) The bird samples show very similar profiles of GC traces and n-alkane distribution to Bunker C. The only difference observed is that the bird samples show larger UCM hump areas, smaller 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 the residual oil on the birds was further weathered and degraded.

(2) The bird samples also show similar distribution patterns and concentration levels of both target alkylated PAHs and biomarkers to the old "Bunker C"-type oil.

(3) The highest abundances of the alkyl phenanthrenes among 5 target alkyl PAH homologous series and higher abundances of alkyl chrysenes series than alkyl dibenzothiophene and fluorene series are well demonstrated, which is another characteristic feature of the Bunker C-type oil.

(4) The values of relative ratios of biomarker terpanes C_{23}/C_{24}, C_{29}/C_{30}, $C_{32}(S)/C_{32}(R)$ and $C_{33}(S)/C_{33}(R)$ for the birds and Bunker C are close to each other, but the birds show lower ratios of C_{23}/C_{30} and C_{24}/C_{30} than Bunker C, most probably due to faster degradation of lighter C_{23} and C_{24} than C_{30} [17] after the birds came in contact with the oil.

(5) Among the 5 target alkylated PAH series, the chrysenes are the most highly degradation-resistant series. Therefore, as the weathering process is undergoing, the

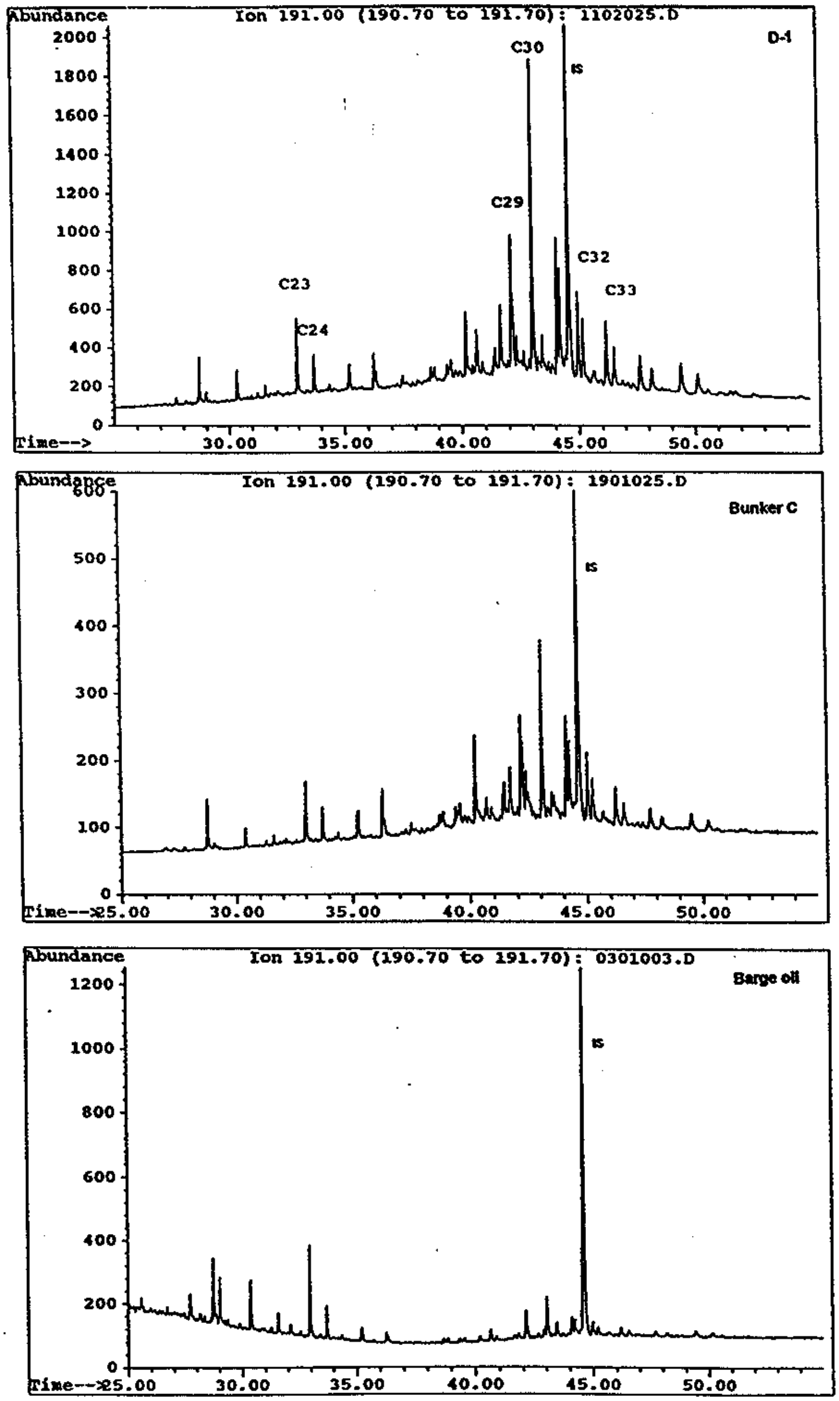

Figure 6 Distribution of biomarker terpanes (m/z 191) in the sample D-1, Bunker C, and Barge oil. IS represents the internal standard. C_{23}, C_{24}, C_{29}, C_{30}, C_{32}, and C_{33} represent C_{23} and C_{24} terpane, C_{29} and C_{30} αβ-hopane, and 20S/20R of C_{32} and C_{33} hopane isomer pairs, respectively.

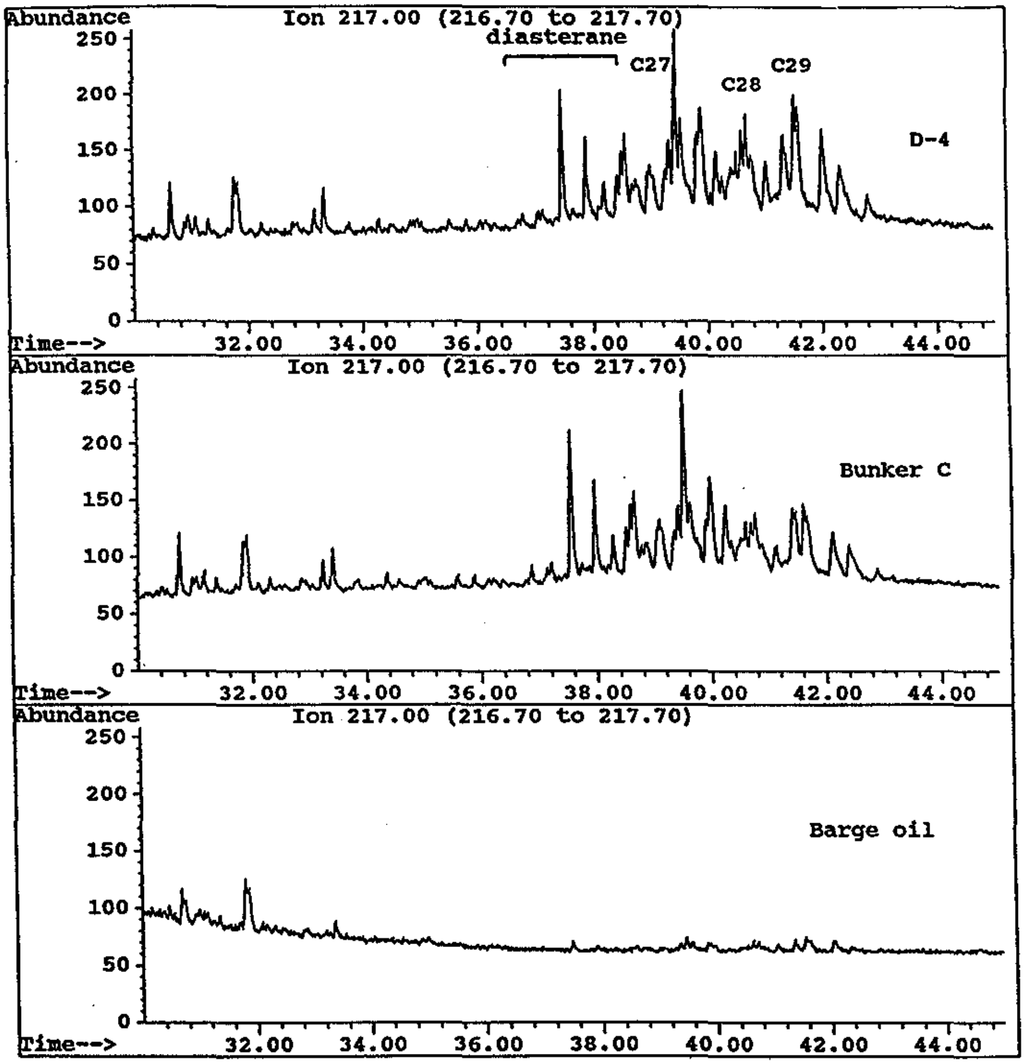

Figure 7 Distribution of steranes (m/z 217) in the sample D-4, Bunker C, and Barge oil, illustrating similarity and difference of sterane distribution profiles and patterns between oils. C_{27}, C_{28}, and C_{29} represent C_{27}, C_{28}, and C_{29} steranes.

Table 4 Concentrations and Ratios of Target Biomarker Compounds

	Concentrations (µg/g TSEM) Terpanes									Ratios Terpanes					
Samples	C23	C24	C29	C30	C32(S)	C32(R)	C33(S)	C33(R)	Total	C23/C24	C29/C30	C32(S)/C32(R)	C33(S)/C33(R)	C23/C30	C24/C30
D-1	27.3	13.5	48.5	129.0	39.3	26.3	36.5	22.3	343	2.03	0.38	1.50	1.64	0.21	0.09
D-2	24.3	11.7	44.3	121.8	38.3	24.6	35.6	21.7	322	2.08	0.36	1.56	1.64	0.20	0.09
D-3	20.6	10.0	40.9	101.5	33.5	23.7	30.5	18.8	280	2.06	0.40	1.41	1.62	0.19	0.08
D-4	22.0	11.4	43.2	109.9	34.8	22.8	33.8	20.8	298	1.93	0.39	1.53	1.63	0.19	0.10
Bunker C	24.6	12.4	37.3	87.9	34.5	24.0	25.9	17.1	261	1.98	0.42	1.44	1.52	0.28	0.14
Barge oil	22.3	8.6	6.5	14.2	5.0	5.2	3.4	2.0	65	2.59	0.46	1.57	1.65	1.57	0.61

Table 5 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 n-alkanes (mg/g TSEM or oil)	13.9	13.1	11.2	10.3	30.5	25.3
Total alkyl PAHs (µg/g TSEM or oil)	14285	13239	12641	6243	16433	91293
Total of target terpanes (µg/g TSEM or oil)	343	322	280	298	261	65
Conc. of 3 isomers of M-DBT (µg/g TSEM or oil)	172	168	162	66	247	1514
DIAGNOSTIC RATIOS						
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 : 1.01 :0.32	1 : 1.12 : 0.29	1 : 0.65 : 0.23
Naphs/Chrys	0.54	0.61	0.90	0.28	2.48	41.3
Phens/Chrys	3.40	3.41	3.66	2.97	3.22	13.1
Dibens/Chrys	0.37	0.38	0.41	0.36	0.48	2.9
Fluos/Chrys	0.24	0.25	0.29	0.19	0.40	2.5
pristane/phytane	0.84	0.80	0.86	0.81	0.79	0.75
C2D/C2P : C3D/C3P	0.13 : 0.12	0.13 : 0.13	0.14 : 0.13	0.14 : 0.13	0.16 : 0.19	0.35 : 0.35
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(S)/C32(R)	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 and P represent methyldibenzothiophene, dibenzothiophene and phenanthrene, respectively.

relative ratios of less degradation-resistant alkyl fluorene and dibenzothiophene series to alkyl should be expected to decrease. The bird samples demonstrated such trends when compared to Bunker C oil: the relative ratios of alkyl fluorene and dibenzothiophene series decreased from 0.48 and 0.40 for Bunker C to 0.31-0.41 and 0.19-0.29 for the bird samples, respectively.

(6) The isomeric distributions of 4-, 2-/3-, and 1-methyldibenzothiophene were determined to be around 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, respectively. This very characteristic and valuable ratio has been used as a marker for differentiation and source-identification of crude and weathered oils [18].

Searching for the database on the ratios of 3 isomers of methyldibenzothiophenes for over 30 oils, only the Bunker C oil shows higher absolute abundance of 2-/3- over 4-methyldibenzothiophene and unusually high relative ratio of 2-/3- to 4-methyldibenzothiophene, 1.0:1.12:0.30. Extensive studies conducted in this lab [18] have demonstrated that in most cases 2-/3-methyldibenzothiophene is most preferentially biodegraded within the isomeric series and 1-methyldibenzothiophene biodegrades at slower rate than 4-methyldibenzothiophene, resulting in decrease of the relative ratios of 2-/3- to 4-methyldibenzothiophene and slight increase of 1- to 4-methyldibenzothiophene. Therefore, as weathering and biodegradation continues, the relative ratios of 2-/3- and 1- to 4-methyldibenzothiophene should be expected to be gradually lower than 1.1 and slightly higher than 0.29. The weathered residual oil on the birds show exactly such trends in the relative ratios of 3 isomers of methyldibenzothiophenes.

This important finding , combined with other analysis data, implies that the oil on the birds was mostly probably from spilled Bunker C oil which has similar chemical composition to the Bunker C stored in our lab. The oil on the birds was definitely not from the barge oil which has significantly different ratios of 3 isomers of methyldibenzothiophenes.

(7) The values of the double ratio of C_2D/C_2P to C_3D/C_3P [10] are almost the same for the birds but lower than Bunker C, further indicating that 4 birds were contaminated by the same oil and that the residual oil on the birds has been weathered if compared to our lab's Bunker C.

(8) Analysis results clearly demonstrate the residual oil on the birds is completely different from the barge oil. The barge oil shows completely different distribution patterns of GC traces, n-alkanes, PAHs and biomarkers, and diagnostic ratios of "source-specific-markers". Extremely high concentrations of target alkylated PAHs (91293 µg/g oil, almost 6 times higher than Bunker C) with the alkyl naphthalenes being the most abundant were detected, indicating the barge oil is highly toxic from the environmental point of view. All of the chromatographic features lead to the conclusion that the barge oil is of typical diesel-containing petroleum product and that the residual oil on the birds was absolutely not from the barge oil.

Estimation of weathered percentages of residual oil on the birds

A method using the highly biodegradation-resistant hopane as an internal oil reference to estimate the depletion of crude oil in oiled sediments has been developed and successfully applied to quantify weathered percentages of residual oil in samples [7,17]. Using this method, the average weathered percentages of the residual oil on the birds, based on the concentrations of C_{29} and C_{30} $\alpha\beta$-hopane in our lab's Bunker C oil, were estimated to be around 20%. For light and medium crudes, the weathered percentages caused by evaporation and other factors can be up to 70 and 40% of their volume, respectively [23]. But, for heavy or residual oils such like Bunker C type oil, the weathered percentages caused by various weathering processes would correspondingly be smaller. The estimated weathered percentage quantitatively implies that the oil on birds has been highly weathered.

CONCLUSION

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

All evidence in this work indicates that (1) the residual oil on the birds was most probably Bunker type oil, and definitely not from the suspected barge oil; (2) the heavy Bunker type oil has been relatively highly weathered, the average weathered percentage was estimated to be around 20%; (3) some biodegradation of oil had occurred, as evidenced by decreases in the ratios of C_{17}/pristane and C_{18}/phytane, preferential loss of some target PAHs, and loss of low molecular weight biomarker compounds such as C_{23} and C_{24} terpanes; (4) the rate of degradation and weathering of oil on the birds should be logically expected to be faster than the sediment samples due to the relatively higher temperature of the birds' body and due to air exposure. The age of the weathered oil on the birds is estimated to be at least several weeks.

It should be understood that there is no single chromatogram or ratio which can be used along for positive source-identification of an unknown spilled oil, especially for those 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, comprehensive, systematic and tiered analytical approach should be designed and conducted. In addition, complete sets of analytical data, especially the data about those unique "source-specific-marker" compounds, should be accurately determined and carefully analysed, and compared with the suspected source oils, if they are available.

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