

- Stanier, R. Y., Palleroni, N. J. & Doudoroff, M. (1966). The aerobic Pseudomonads: a taxonomic study. *J. gen. Microbiol.*, **43**, 159–271.
- Walker, J. D. & Colwell, R. R. (1974). Microbial petroleum degradation: use of mixed hydrocarbon substrates. *Appl. Microbiol.*, **27**, 1053–1060.

Walker, J. D. & Colwell, R. R. (1976). Measuring the potential activity of hydrocarbon-degrading bacteria. *Appl. envir. Microbiol.*, **31**, 189–197.

Walker, J. D., Colwell, R. R. & Petrakis, L. (1976). Biodegradation of petroleum by Chesapeake Bay sediment bacteria. *Can. J. Microbiol.*, **22**, 423–428.

Marine Pollution Bulletin, Vol. 13, No. 6, pp. 202–205, 1982.
Printed in Great Britain.

0025-326X/82/060202-04 \$03.00/0
© 1982 Pergamon Press Ltd.

Characterization of Floating and Sinking Tar Balls in the Marine Environment

T. I. BALKAS,* I. SALIHOĞLU,* A. F. GAINES,† M. SUNAY* and J. MATTHEWS†

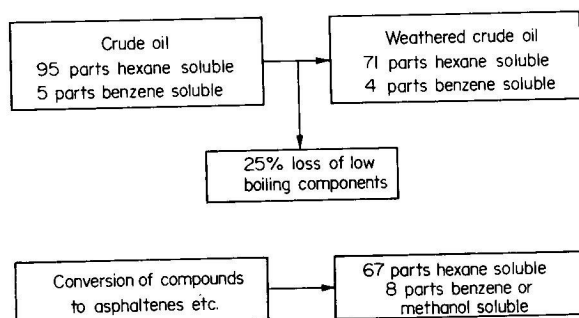
*Middle East Technical University, Marine Science Department, P.K. 28, Erdemli, İçel, Turkey

†The University of Aston in Birmingham, Solid Fuel Research Group, Department of Chemistry, Gosta Green, Birmingham B4 7ET, England, UK

Tar balls, according to their densities, the prevailing temperature and their degree of weathering, are observed as floating, sinking, or are washed onto the beaches in the marine environment. To characterize the differences between floating and sunken tar balls, samples were analysed by IR, H-nmr, GC and GC/MS techniques. Hexane, benzene and methanol solubilities as well as densities were measured. The degree of weathering of the tar balls was investigated in terms of the losses of *n*-paraffinic/aromatic hydrocarbons. The ratio of paraffinic/aromatic hydrocarbon concentrations was calculated by IR and H-nmr whereas pristane/phytane ratios were estimated from GC measurements. The comparison of some high molecular weight, but minor, components between the major *n*-paraffins was obtained from GC/MS measurements.

Hydrocarbon pollution of the north-eastern Mediterranean, while not yet as calamitous as in the western Mediterranean (Zsolnay, 1979), is nevertheless significant. The area contains an oil terminal, an oil refinery and two busy ports. Figure 1 summarizes the dissolved/dispersed petroleum hydrocarbon levels in seawater in this region. Hydrocarbons such as crude oils released into the marine environment lose low-boiling material by evaporation and dissolution (Erhardt & Blumer, 1972); at the same time additional high-boiling material is formed by oxidative polymerization of hexane-soluble material to give asphaltenes.

The process has been represented (Balkas, 1980) as below.



Obviously, such a process raises the softening point of the oil and when this exceeds the temperature of the surroundings, the oil congeals and a tar ball forms. Generally, of course, tar balls are observed after they have been washed up on beaches or when they are seen floating on the sea. The necessity for their investigation has become more piquant since our observations during the last two years of tar balls resting on the sea bed or in the interface layers. Such observations have been made by chance when trawling for fish and when diving.

Sunken tar balls have been found in a variety of locations but it is obviously difficult to know how common they are or to determine their effect on the environment. Naturally their location near a spawning ground would be disastrous. The difficulty of surveying the sea bed emphasizes the importance of determining the origin of sunken tar balls whenever they are found. Furthermore, analyses of sunken and floating tar balls can give us information about whether the weathering on the sea bed is the same as the weathering near the surface; there is also the question of what causes oil to sink.

In order to see the differences between floating and sunken tar balls, a series of instrumental analyses covering IR, H-nmr, GC and GC/MS techniques were applied. The fractionation recoveries by various solvents and densities of the tar balls were also estimated.

The sunken tar balls were collected from Girne (S9 TAR), Tartar (S4 TAR), Atas (S5 TAR), Tarsus (S6 TAR) and Seyhan (S13 TAR). The floating tar balls were collected from Campus Beach (CABT), Campus offshore (FLOAT), Cyprus-Girne (CYP), Seyhan (SEYT) and Malta-Anchor Bay (MALTA).

Experimental

The tar balls, both floating and sunken, were all fractionated in a Soxhlet apparatus by hexane, benzene and methanol, consecutively. The extracts were cleaned and further fractionated by eluting through an activated Silica Gel Alumina (10:1) column, then concentrated and analysed instrumentally.

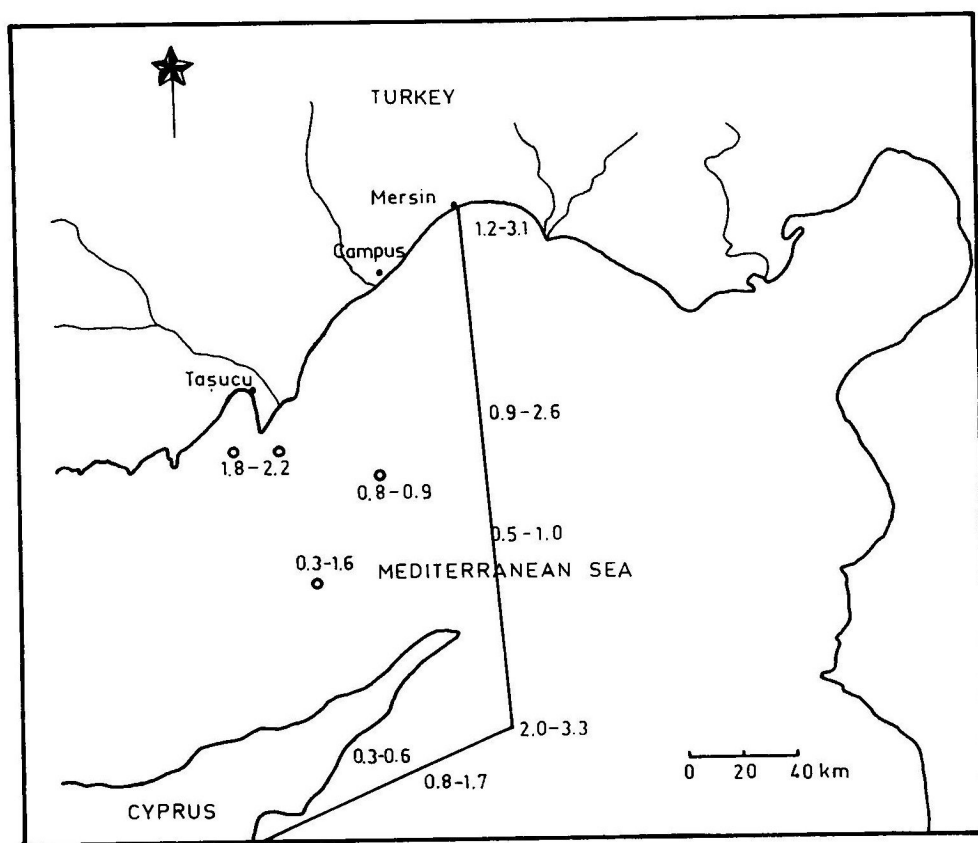


Fig. 1 Dissolved/dispersed petroleum hydrocarbons in seawater, in NE Mediterranean ($\mu\text{g g}^{-1}$).

IR spectra were obtained by the KBr disc technique using a Beckmann Acculab 2. Routine H-nmr spectra of the benzene fractions were obtained in CCl_4 using a Varian EM 300. TMS was used as an internal reference.

Gas chromatograms were generally obtained using a Packard Becker Model 421. Eight foot columns coated with 3% SE-30 were heated at a rate of 4°C min^{-1} from 100 to 260°C with isothermal heating at initial and final temperatures.

GC/MS involved a Pye Unicam 304 Gas Chromatograph having a capillary column coated with OV-1 and heated at the same rate given above for GC. The Gas Chromatography was coupled directly to a VG Micromass 12/12 quadrupole Mass Spectrometer. Ionization with 70 eV electrons was carried out at about 10^{-6} mm Hg with scans at every 2 s.

Results and Discussion

Throughout this work it has been very difficult to distinguish floating from sunken tar balls. In Table 1, some of the physical characteristics, i.e. density, extraction recoveries and *n*-paraffin ranges, of tar balls are given. The *n*-paraffin ranges do not help to differentiate the two types of tar balls but do indicate their degree of weathering. None of the physical characteristics tabulated in Table 1, not even density, can be used with certainty to separate those that floated from those that sank. Generally, but not always, the densities of sunken tar balls were higher than those of floating tar balls but none of the densities was much greater than that of Mediterranean seawater.

One is inclined to the opinion that hydrocarbon liquid in the marine environment floats or sinks according to the circumstances of its discharge and density. The latter may well be modified by sands and similar matter which may become attached to the oil. The removal of low boiling material by weathering is likely to increase the chance of sinking. However, the difference in density between a tar ball and the sea is often small and whether a ball floats or sinks may depend on the prevailing temperature. A well understood example is the tar ball collected off Kadiköy harbour, in Istanbul. This originated from a tanker carrying Libyan crude oil which caught fire in the Bosphorus. Unburnt oil was discharged below the surface of the water and there was remarkably little visible pollution of the nearby harbour. Some three weeks after the fire and partial sinking of the tanker, the weather grew colder and the water temperature dropped and its density increased accordingly. Within a matter of hours tar balls defaced the harbour walls. The samples collected and analysed did not show

TABLE 1
Physical characteristics of tar balls.

Sample	Density	Extraction recovery			<i>n</i> -paraffin range
		Hexane	Benzene	Methanol	
S4 TAR	0.98	80	14	5	$\text{C}_{12}\text{--C}_{31}$
S5 TAR	0.89	71	16	6	$\text{C}_{14}\text{--C}_{32}$
S6 TAR	1.23	31	29	21	$\text{C}_{18}\text{--C}_{23}$
S9 TAR	1.25	77	14	6	$\text{C}_{16}\text{--C}_{26}$
S13 TAR	0.12	73	24	3	$\text{C}_{14}\text{--C}_{32}$
FLOAT	0.80	86	8	3	$\text{C}_{13}\text{--C}_{25}$
CABT	0.86	92	8	2	$\text{C}_{14}\text{--C}_{29}$
CYP	0.94	87	8	3	$\text{C}_{15}\text{--C}_{25}$
MALTA	—	86	10	4	$\text{C}_{14}\text{--C}_{26}$

TABLE 2
Pristane/phytane measurements.

Sample	Pristane/phytane
S4 TAR	1.59
S5 TAR	1.50
S6 TAR	Pristane is not well resolved
S9 TAR	0.70
S13 TAR	1.45
FLOAT	1.17
CABT	0.76
CYP	Pristane is not well resolved
MALTA	1.37

exceptional loss of low boiling material and seemed perfectly typical in every way. The range of *n*-paraffins observed by gas chromatography, shown in Table 1, is a measure of the amount of low boiling material lost by weathering. The table indicates that S4 had been least weathered and tar balls S6 and S9 had been most affected by weathering. The pristane ratios given in Table 2 are normally used to identify the source material of tar balls but they may also give information about the state of weathering. Pristane is probably derived from phytane (or phytol) by loss of a carbon atom and the weathering (or loss) of low boiling material lowers the pristane/phytane ratio. This has clearly happened to tar ball S9 and probably also to tar balls S6, CABT and CYP. In Table 3, the ratio of some certain frequencies obtained by IR spectra and H-nmr spectra are given. It was impossible to separate the two types of tar balls on the basis of IR spectra, but it will be seen from Table 3 that H-nmr spectra revealed some interesting differences. Generally there were some gross exceptions; sunken tar balls had similar CH₃/aromatic hydrogen to crude oils (Balkas, 1980), but floating tar balls possessed higher ratios. Again the CH₂/CH₃ ratios of floating tar balls were generally lower than those of sunken tar balls. One needs to analyse a large number of samples to establish such observations which suggest that the mechanism of weathering may have differed in the two cases.

There was no distinctive difference in the chemical compositions of tar balls as revealed by GC/MS. All the tar balls were mainly paraffinic, though they contained more asphaltenes and methanol-soluble polar material than crude oils. None of the tar balls contained large quantities of oxygenated compounds, as revealed by GC/MS. Since pristane/phytane ratios may be changed by weathering, other 'marker' characteristics of crude oils should be sought. Obviously those should preferably be substances of higher molecular weight than phytane and not be susceptible to oxidation during weathering. Mass spectra of the

TABLE 3

IR and H-nmr spectroscopy measurements.

Sample	IR			H-nmr		
	1600/1450	1450/1375	810/1375	CH ₂ /CH ₃	CH ₂ /Aro	CH ₃ /Aro
S4 TAR	0.16	2.74	2.58	9.80	3.80	0.22
S5 TAR	0.74	2.28	6.18	34.00	5.50	—
S6 TAR	—	—	4.36	0.74	0.17	—
S9 TAR	0.16	1.68	5.33	4.00	0.75	0.24
S13 TAR	0.44	2.17	4.36	32.00	7.33	0.17
FLOAT	—	—	3.09	15.11	4.89	—
CABT	0.21	2.00	5.07	19.00	3.75	0.07
CYP	0.10	2.22	4.67	19.60	4.20	0.14
MALTA	0.16	2.29	1.96	45.00	23.00	—

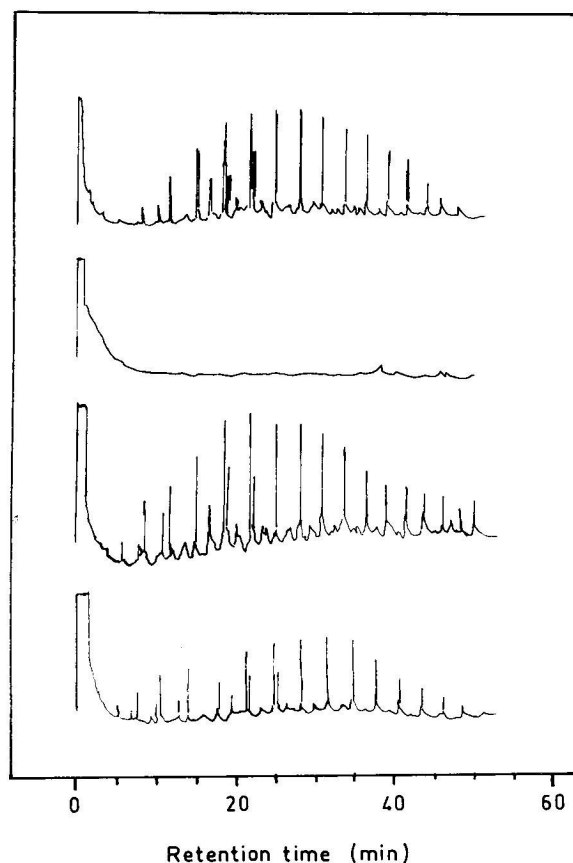


Fig. 2 TIC chromatograms of tar balls. (i) S5 TAR, (ii) S6 TAR, (iii) S13 TAR, (iv) S4 TAR.

compounds that gave the most significant GC peaks between the *n*-paraffins are shown in Table 4. In addition, mass fragmentograms for an *m/e* of 191 were compared. Some results are shown in Fig. 3. A mass number of 191 indicates either substituted anthracene or phenanthrenes of branched or cyclic aliphatics such as 'hopanes' with a molecular ion of C₁₄H₂₃⁺ (Albaiges, 1980). Both the mass

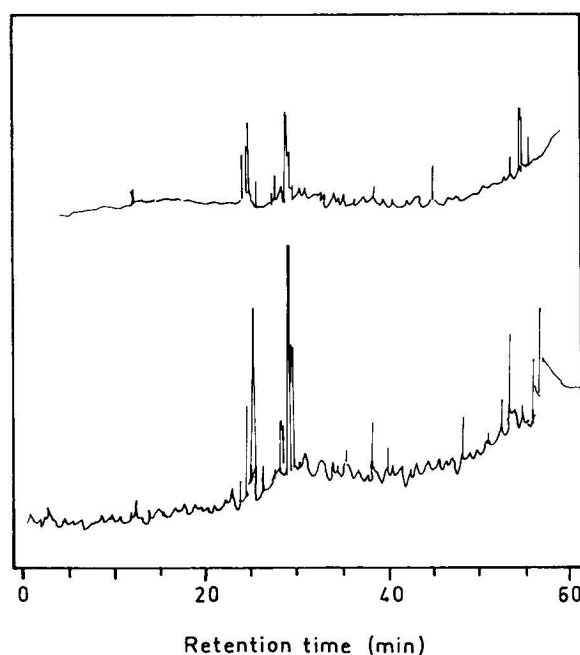


Fig. 3 *m/e* 191 fragmentograms of tar balls. (i) CABT, (ii) S13 TAR.

TABLE 4

GC/MS measurements (the main fragments of the minor components between major *n*-paraffin hydrocarbons).

Sample	C ₁₄ -C ₁₅	C ₁₆ -C ₁₇	C ₁₇ -C ₁₈	C ₁₈ -C ₁₉	C ₁₉ -C ₂₀	<i>m/e</i> 191*
S5 TAR	57, 43, 71, 41, 55, 85, 141	43, 57, 41, 71, 85, 99, 127	57, 43, 41, 55, 71, 83, 82, 111	55, 83, 82, 41, 71, 43, 69, 111	55, 83, 82, 41, 43, 69, 206, 191, 189	1.4/ - / -
S13 TAR	71, 57, 43, 41, 85, 55, 69, 155	57, 43, 71, 41, 55, 85, 105	57, 43, 41, 55, 71, 69, 83, 82, 85, 97, 111	43, 83, 41, 69, 68, 57, 82, 105	83, 55, 82, 41, 191, 189, 206	1.2/2.6/2.0
FLOAT	57, 43, 71, 41, 85, 55, 113	57, 43, 71, 41, 85, 99, 113	57, 43, 42, 56, 71, 69, 85, 83	57, 71, 77, 43, 85, 96,	43, 71, 69, 57, 42, 56, 85, 97	1.0/ - / -
CABT	43, 57, 71, 41, 55, 85, 97, 141	57, 43, 71, 41, 55, 85, 105, 133, 165	57, 43, 41, 71, 55, 83, 82, 97, 111, 175	57, 43, 71, 41, 55, 85, 69, 112, 192, 189, 165	-	1.2/1.8/1.5

*In specific ion fragmentograms with an *m/e* value of 191, generally three major peaks are present. The values given in this column are the ratios of these peaks to each other.

spectra in Table 4 and the ratios of the heights of the three major peaks on the 191 fragmentograms could be used as 'markers' to aid recognition of the source material of the tar balls. It should be noted, however, that mass spectrometry is showing that crude oils possessing similar patterns of retention times nevertheless contain different compounds and the problem of determining the origin of tar balls remains difficult.

Conclusions

(1). It is very difficult to distinguish between floating and sunken tar balls in terms of their densities, extraction properties and *n*-paraffin hydrocarbon ranges.

(2) In practise, the most important factor which governs whether a tar ball floats or sinks is the temperature of the surroundings. An increase in seawater temperature decreases the density of seawater and may cause the tar ball

to sink. A sudden decrease in temperature may bring a sunken tar ball to the surface.

(3) H-nmr spectra suggests that floating and sunken tar balls may weather in different ways.

(4) Previous work (Balkas, 1982) showing the use of UV, IR and GC to identify the source material of tar balls has been usefully extended by consideration of the minor components revealed by GC/MS.

Albaiges, J. (1979). Fingerprinting marine pollutant hydrocarbons by computerized GC/MS. *Int. J. env. Analyt. Chem.*, **6**, 171-190.

Balkas, T. I. *et al.* (1980). Analysis of crude oils and tar balls. *v^{es} Journées Etud Pollutions*, pp. 25-32. Cagliari, C.I.E.S.M.

Balkas, T. I. (1982). *The Identification of Sources of Tar Balls*. DOĞA, Turkish Scientific and Technologic Research Council Publications (in press).

Erhardt, M. & Blumer, M. (1972). The source identification of marine hydrocarbons by GC. *Envir. Pollut.*, **3**, 179-194.

Zsolnay, A. (1979). Hydrocarbons in the Mediterranean sea. *Mar. Chem.*, **7**, 343-352.

Copper, Cadmium and Zinc in Seaweeds from the South Coast of Ireland

J. P. CULLINANE and P. M. WHELAN

Botany Department, University College, Cork, Ireland

Copper, cadmium and zinc levels were determined by atomic absorption spectrophotometry in five seaweed species from the south coast of Ireland. The levels from eight sites on the south coast were compared with those from five sites in Cork harbour. Whereas the levels of copper and cadmium from Cork harbour compared favourably with those of the other sites, the levels of zinc at some of the Cork harbour sites were appreciably higher than those of the other sites.