OIL POLLUTION AND MICROBIAL REGULATION

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Summary

Petroleum contamination of marine and terrestrial environments remains a major problem in today's world. The devastating effects of large oil spills from shipping accidents are well known. Fewer people, however, may be aware that the major sources of petroleum contamination in the sea are natural oil seepages from the sea floor and chronic discharges of hydrocarbons from land-based human activities. Petroleum is a complex mix of many thousands of different compounds of varying toxicity to life, ranging from the relatively benign *n*-alkanes that are readily degraded by microorganisms to toxic polycyclic aromatic hydrocarbons that can persist for years in sediments. The compositions of crude oils are highly variable and the compounds present can be identified using capillary gas chromatography-mass spectrometry and other analytical techniques. Fingerprinting techniques can readily distinguish between oils and can be used in legal proceedings to establish the source of an oil spill. The shoreline biota is most vulnerable to oil pollution either through smothering or by acute and chronic exposure to toxicants: particularly sensitive areas include salt marshes, mangroves, coral reefs, seagrass meadows, intertidal reefs and sandy beaches. Contamination of soils and groundwater aquifers with BTEX compounds (benzene, toluene, ethylbenzene and xylenes), which are major constituents of gasoline, is a major problem near industrial and urban areas. The need to remediate soils has become more

acute as industrial land is converted to housing and other purposes. Spilled oil will degrade over time and the sequence of compositional changes is now well described. However the rates can be slow, and degraded oils have even been found in some sediments more than 30 years after the original spill. A variety of remediation techniques, including addition of nutrients to shoreline sediments as done following the *Exxon Valdez* spill, have been developed that can speed up degradation processes many fold.

1. Introduction

Conventional fossil fuels (coal and crude oil) provide nearly 90 percent of the world demand for commercial primary energy. Despite increasing contributions from alternative sources such as hydro- and wind power, we can expect that oil production will continue to play a major role in meeting energy demands worldwide. Exploration and associated activities of production, sea transport, refining and distribution has attendant risks of contamination of the atmosphere, freshwater, groundwater, marine waters and sediments with petroleum-derived organic compounds. Hydrocarbons also enter the environment from a variety of urban activities such as accidental spillages, road transport, industrial activities, shipping, combustion etc.

Crude oils are composed primarily of hydrocarbons with variable, but usually small, amounts of sulfur-containing compounds such as thiophenes and thiolanes, and more polar compounds containing oxygen or nitrogen such as porphyrins and other functionalized compounds (collectively referred to as the NSO fraction or “resins plus asphaltenes”). Aliphatic hydrocarbons contain either no double bonds (termed alkanes or saturates) or one or more double bonds (alkenes). Alkanes may be straight-chain (e.g. *n*-heptadecane which is referred to by the shorthand nomenclature, n-C_{17}), branched-chain (e.g. monomethyl-branched alkanes and the isoprenoid alkanes such as pristane and phytane), or cyclic (e.g. biomarkers containing 5- or 6-membered rings such as steranes and hopanes). Aromatic hydrocarbons contain one or more carbocyclic rings with alternating double and single bonds (i.e. conjugated double bonds). The simplest aromatic hydrocarbon is benzene (C_6H_6). Alkyl substituents are often present on one or more of the rings (e.g. toluene: CH_3-C_6H_5). Compounds with three or more fused aromatic rings are termed polycyclic aromatic hydrocarbons (PAHs), and these may also have alkyl substituents. PAHs are considerably more toxic than aliphatic hydrocarbons and thus environmental studies often focus on these compounds (see *Chemistry of Organic Pollutants, Ecological Chemistry*).

The composition of crude oils is highly variable and depends on the type of source rock and thermal maturity at which the oil was produced and whether its composition has been affected by water washing and biodegradation in the petroleum reservoir. A "typical" oil might consist of about 50–60 percent aliphatic hydrocarbons, 20–30 percent aromatic hydrocarbons and 10–15 percent asphaltenes and NSO compounds, but of course compositions range from highly volatile condensates to viscous heavy crude oils. The asphaltene fraction is composed mainly of cross-linked aromatic structures. The distributions of aliphatic hydrocarbons, particularly the isoprenoid, sterane and hopane biomarkers, are often very useful for ascertaining the source of the oil pollution and can be used to “fingerprint” the oil.

A number of indicators have been proposed for identifying petroleum hydrocarbon contamination in environmental samples. These include (i) high concentrations (>100 ppm) of total hydrocarbons in sediments, (ii) C_{21}-C_{35} *n*-alkanes having no predominance of odd over even chain-lengths, (iii) complex distributions of isomers, (iv) an unresolved complex mixture ("hump" or "UCM") in the gas chromatogram of the hydrocarbon fraction, (v) thermally-mature stereoisomers of pristane and phytane and (vi) characteristic distributions of biomarkers such as steranes and hopanes. Such measures are needed because there are many sources of natural hydrocarbons in the environment as well as hydrocarbons from combustion and naturally occurring oil seepage.

2. Measuring the Amount of Oil

Total hydrocarbons are readily determined by weighing the hydrocarbon fraction obtained from the sample by solvent extraction with hexane or more polar solvent and subsequent fractionation by adsorption chromatography. However, accurate data can be difficult to obtain where the sample size is small. If sediments are extracted, then elemental sulfur must be removed from the extract, usually by treatment with activated copper, otherwise erroneously high values will be measured. When more detailed information on the distributions is required, the analyst usually turns to high resolution capillary gas chromatography with a flame ionization detector (GC-FID) or mass spectrometer (GC-MS) as the detector. Examples of the GC-FID analysis of a whole oil from the Middle East and hydrocarbons extracted from a polluted sediment are shown in Figure 1. Aromatic hydrocarbons are sometimes measured by high performance liquid chromatography (HPLC) with UV or fluorescence detection. IR spectroscopy can also be used for semi-quantitative estimation of total HC concentrations in seawater.

3. Oil Pollution of the Sea

Oil spills at sea have focused the attention of the public and Governments on the problem of petroleum contamination with a succession of major shipping accidents in recent decades. See Table 1 for some recent examples. Oil spills from the sinking or grounding of tankers such as *Torrey Canyon*, *Exxon Valdez* and *Braer* have received much publicity. As dramatic as these events are, they must be put into context. The global discharge of petroleum into the sea has been estimated by the National Academy of Sciences to be about 1.3×10^6 tonnes per annum.

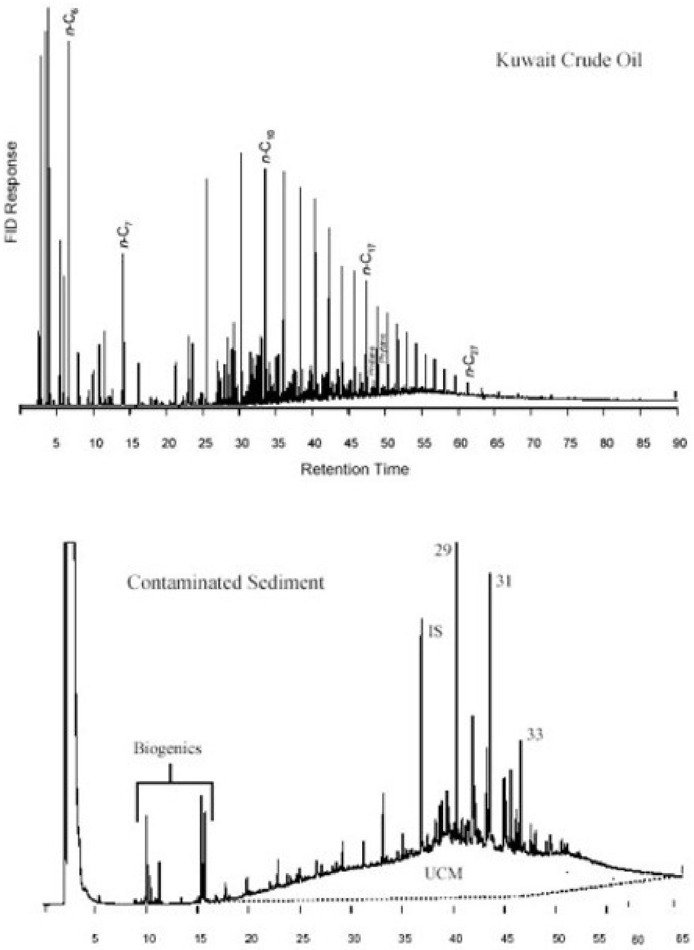

Figure 1: GC-FID chromatogram of the total hydrocarbons in a crude oil from Kuwait. *n*-C_x is *N*-alkane with x carbon atoms. Note the lack of odd-even preference in *n*-alkanes and the presence of pristane and phytane. Volatile hydrocarbons C_1 to C_7 are also present but are rapidly lost once oil is spilled into the environment. Also shown is a GC-FID chromatogram of hydrocarbons present in polluted intertidal sediment. Oil pollution is clearly evident from the predominant unresolved complex mixture (UCM) and presence of biomarkers in the high molecular end of the chromatogram. Also present are shorter-chain biogenic hydrocarbons from microalgae and bacteria as well as long-chain *n*-alkanes (*n*-C_{27}–*n*-C_{35}) from plant waxes with a characteristic predominance of odd-chain lengths.

This results from four main sources: natural oil seeps (600 000 tonnes per annum), petroleum consumption (car and boat use, road runoff etc; 480 000 tonnes per annum), petroleum transport (150 000 tonnes per annum) and petroleum extraction (38 000 tonnes per annum). The largest amount is thought to be from natural seeps, but it should be noted that there is considerable uncertainty about this figure. Oil seeps tend to be individually small and sporadic. Their low rate of production is such that the surrounding environment is usually able to adapt. Slow chronic releases from human activities represent the most significant source of petroleum entering the marine environment and have the potential to cause greatest harm. Atmospheric inputs of polycyclic aromatic hydrocarbons derived from the combustion of petroleum, coal, wood and other products are also important because of the carcinogenicity or systemic toxicity of these compounds.

Vessel Name or Source of Oil Spill	**Spill Date (day/month/year)**	**Location**	**Tonnes**
Nova	6/12/85	Middle East	72 626
Petroleum refinery	27/04/86	Bahia las Minas, Panama	34 286
Abkutan 91 Well	23/10/86	Gulf of Mexico	35 286
Athenian Venture	22/04/88	NW Atlantic	36 060
Odyssey	10/11/88	NW Atlantic	146 600
Exxon Valdez	24/03/89	Alaska	37 415
Khark 5	19/12/89	N Africa	70 068
Mega Borg	9/06/90	SE USA	17 000
Berge Broker	13/11/90	W Atlantic	13 605
Persian Gulf War	x/01/91	Middle East	1770 000
Haven	11/04/91	Mediterranean	144 000
ABT Summer	28/05/91	SE Atlantic	51 020
Kirki	21/07/91	W Australia	17 687
Katina P.	26/04/92	S Mozambique	54 421
Nagasaki Spirit	20/09/92	Straits of Malacca, SE Asia	13 000
Agean Sea	3/12/92	N Spain	74 490
Braer	5/01/93	Shetland, UK	85 034
Maersk Navigator	21/01/93	Andaman Sea, SE Asia	24 830
Cosmas A.	24/01/94	South China Sea	24 085
Nassia	13/03/94	E Mediterranean	38 500
Seki	30/03/94	Middle East	16 000
Thanassis A.	21/10/94	South China Sea	37 075
Sea Empress	15/02/96	Pembrokeshire, UK	72 361
Da Qing 243	4/06/97	China	17 000
Evoikos	15/10/97	Straits of Malacca, SE Asia	28 570

Table 1: Some major oil spills into marine environments from vessels and oil installations since 1985

4. Hydrocarbon Contamination of Coastal and Estuarine Waters and Sediments

The shoreline is most vulnerable to the effects of oil pollution. Particularly sensitive areas include salt marshes, mangroves, coral reefs, seagrass meadows, intertidal reefs and sandy beaches. All of these can be severely impacted simply by being smothered with oil, as well as by acute and chronic exposure to toxicants. On exposed rocky coasts the effects are likely to be less severe, although the visual pollution can still be considerable. The choice of oil spill remediation must be tailored to match the characteristics of the shoreline being affected. For this purpose, maps of the sensitivity of shorelines and their biological populations to oil spills are produced by many counties and typically presented in Geographical Information System (GIS) form. In the USA, Environmental Sensitivity Index (ESI) maps have been available for much of the coastline since 1990.

Hydrocarbon concentrations in unpolluted intertidal and estuarine sediments may range from below detection limit to about 10 ppm (μg g^{-1} dry weight). Total hydrocarbon concentrations greater than 500 ppm are indicative of significant pollution. Values up to 1800 ppm have been reported in petroleum-contaminated surface sediments from the New York Bight. *n*-Alkanes with a chain-length range of C_{15} to C_{35} are present in nearly all sediments. These are often a mixture of *n*-alkanes from petroleum that show no predominance of odd or even carbon numbers plus plant wax alkanes with odd-chain alkanes about 8–10 times more abundant than even-chain *n*-alkanes. Shorter-chain alkanes and alkenes from sedimentary micro-organisms and microalgae can also be abundant (see Figure 1).

Scientific evidence indicates that an oiled area will recover, although the time taken may range from a few days to decades. For example, relatively fresh oil is still being found at depth at many coastal sites oiled after the grounding of the *Exxon Valdez* in March, 1989. Weathered oil is still present in saltmarsh sediments in West Falmouth that were contaminated over 30 years ago with No. 2 fuel oil from the barge *Florida*.

There are debates as to what constitutes recovery. Some authors suggest that "recovery" should be viewed as the re-establishment of a healthy biological community in which plants and animals characteristic of that community are present and functioning normally. This definition does not require that the community have the same composition or age structure as that before the oil spill. A "clean" system is one in which the level of petroleum hydrocarbons is so reduced that it has no detectable impact on the function of an ecosystem. This is a subjective definition which does not require a return to some pre-existing background level or the complete removal of hydrocarbons.

5. Petroleum Contamination of Soils, Freshwater and Groundwater

Contamination of soils and groundwater aquifers is a major problem in areas near industrial and urban developments. The need to remediate soils has become more acute as previous industrial land is converted to other purposes such as housing. However, knowledge of the extent of contaminated sites has been poor, with more than 100 000 contaminated sites existing in Britain. Hydrocarbon contamination is often in the form of coal tars, although BTEX (benzene, toluene, ethylbenzene and xylenes) and other petroleum hydrocarbons and phenols are also important. Excavation and off-site disposal is still widely practiced, especially where housing is to be built, but this simply moves the problem. (see *Bioremediation for Soil Reclamation*)

Petroleum can enter aquifers from leakage of fuel storage tanks (e.g. jet fuel from some air bases; gasoline (petrol) leaks from petrol station tanks), industrial inputs and stormwater discharges. This has led to water quality problems in parts of the USA, Europe and elsewhere, particularly in countries where groundwater is a major source of freshwater for domestic purposes (e.g. 30 percent of public water in England and Wales comes from groundwater). The main concern here is usually the low molecular-weight aromatic compounds (BTEX) which have a relatively high solubility in freshwater and thus can be readily transported through groundwater systems. These compounds are known carcinogens, but they can be degraded by microorganisms. Gasoline contains high contents of BTEX.

Field observations indicate that microbially mediated BTEX degradation is achieved by multiple terminal electron-accepting processes including aerobic respiration, denitrification, Fe(III) reduction, sulfate reduction, and methanogenesis involving a range of electron accepters such as O_2, NO_3-, Fe^{3+} and SO_4^{2-}. In most sand and gravel aquifers Fe(III) as iron oxides is abundant although its spatial distribution can be patchy. The spatial distribution of other electron acceptors will also vary, so that these reactions must be viewed on small spatial scales. Many aquifers show considerable heterogeneity which can lead to steep concentration gradients away from the contaminant source due to redox boundaries, and the effects of advection and dispersion. Microbial and geochemical reactions in turn are very sensitive to these distributions of concentrations. ^{14}C-labeled compounds can be used to follow the fate of petroleum hydrocarbons in aquifers and sediments. For example, in methanogenic sediments from a petroleum-contaminated aquifer, ^{14}C-benzene was converted to methane and carbon dioxide without an apparent lag. Phenol, acetate, and propionate were intermediates in benzene mineralization (see *Chemistry of Organic Pollutants).*

Multiple lines of evidence can be used to determine the role of microbial degradation in changing the composition of a contaminant plume. Redox-sensitive parameters in the vicinity of the plume show reductions in dissolved oxygen and SO_4^{2-} and increases in Fe^{2+}, Mn^{2+} and methane contents. Further evidence is the accumulation of aromatic acids within the plume. Microbial biomass can be measured by direct counts or by measurements of phospholipid fatty acids (PLFA) as a proxy for the presence of lipid-containing membranes in intact, living cells. Changes to PLFA patterns often reveal metabolically more diverse microbial communities in contaminated aquifers than those in uncontaminated aerobic zones. Recent work suggests that the PLFA composition of the hydrocarbon-degrading bacteria is modified as hydrocarbons are assimilated and converted to fatty acids by β-oxidation.

Despite this increasing understanding of the biological processes responsible for hydrocarbon degradation, active remediation of groundwaters is still not widely practised. Indeed, it is likely that contaminated groundwaters are still under-reported and under-investigated. Cost is still a problem, as is the long time-frame (in some cases years to decades) needed for biological clean-up. Many of the early attempts in the US to treat large volumes of groundwater by pump-and-treat were expensive and only partly successful. Bioreactors have been used for more than a century to treat wastewater and these are being successfully modified for treatment of groundwater where contaminant loads are typically one to two orders of magnitude lower and the requirements for the final water quality are much stricter. The types being used include trickling filters, upflow fixed film reactors and fluidized bed reactors. A common feature is the retention of a bacterial biofilm on a solid support over which the water is passed. Fluidized-bed reactors with granular-activated carbon as the support material are often used since these have rapid microbial growth due to sorption of contaminants onto the carbon.

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Bibliography

Atlas R.M. (1995). Petroleum biodegradation and oil spill bioremediation. *Marine Pollution Bulletin* **31**, 178-182. [Provides a good general discussion of oil degradation; this journal is a good source of information on studies of specific oil spills.]

Clark R.B. (editor) (1982). *The Long-Term Effects of Oil Pollution on Marine Populations*, *Communities and Ecosystems*, pp. 57-71, The Royal Society, London. [Provides a variety of articles describing early studies of the effects of oil spills.]

Fingas M. (2002). *The basics of oil spill cleanup*. Lewis Publishers, CRC Press, 233 pp. [An excellent primer of the methods used to clean up oil spill, includes a list of major oil spills, response and disposal options and effects on the environment.]

Gin K.Y.H., Huda M.K., Lim W.K., and Tkalich P. (2001). An oil spill-food chain interaction model for coastal waters. *Marine Pollution Bulletin* **42**, 590-597. [Discusses the effects of oil spills on marine organisms in the framework of modeling.]

Langwaldt J.H. and Puhakka J.A. (2000). On-site biological remediation of contaminated groundwater: a review. *Environmental Pollution* **107**, 187-197. [Discusses reactor types, operational costs and fates of contaminants in the subsurface.]

Lee K. and Merlin F.X. (1999). Bioremediation of oil on shoreline environments: Development of techniques and guidelines. *Pure and Applied Chemistry* **71**, 161-171. [A fairly up-to-date review summarizing methods for shoreline clean-up.]

National Research Council (2003). *Oil in the Sea III: Inputs, Fates, and Effects*. National Academy Press, Washington. [A thorough account of the sources of oil in the sea, and their biological effects; includes a summary of major oil spills. This is major update of the previous report issued in 1985.]

Peters K.E. and Moldowan J.M. (1993). *The Biomarker Guide. Interpreting Molecular Fossils in Petroleum and Ancient Sediments*. Prentice Hall, Englewood Cliffs, New Jersey, USA. [A comprehensive guide to biomarkers used to fingerprint petroleum hydrocarbons.]

Rice S.D., Spies R.B., Wolfe D.A., and Wright B.A. (editors) (1996). *Proceedings of the Exxon Valdez Oil Spill Symposium. American Fisheries Society Symposium 18*. American Fisheries Society, Bethesda, Maryland. [A wide ranging compilation of research papers presented at the conference in 1993.]

Volkman J.K., Revill A.T., and Murray A.P. (1997). Applications of biomarkers for identifying sources of natural and pollutant hydrocarbons in aquatic environments. In Molecular Markers in Environmental Geochemistry. (Ed R.P. Eganhouse) ACS Symposium Series 671, pp. 110-132. [Review of biomarker techniques for identifying hydrocarbon sources in the marine environment; other articles in this book are also relevant.]

Wang, Z.D., Fingas M., and Page D.S., (1999). Oil spill identification. *Journal of Chromatography A* **843**, 369-411. [Reviews advances in chemical fingerprinting and data interpretation techniques.]

Biographical Sketches

John Volkman gained his Ph.D. from the Department of Organic Chemistry of the University of Melbourne in 1978 and then carried out postdoctoral research at the University of Bristol (England), Woods Hole Oceanographic Institution, Massachusetts (USA) and in 1981 he took up an appointment as a petroleum geochemist with the Western Australian Institute of Technology in Perth, Western Australia. He joined CSIRO in 1982 to establish a new research area in marine organic chemistry in the Division of Oceanography. This research expanded to include environmental studies of organic pollutants, applications of organic geochemistry, marine lipids, aquaculture nutrition and microalgal biotechnology. He is now a Chief Research Scientist in CSIRO's Marine Research Division in Hobart. Dr Volkman has published over 150 scientific papers, over 120 conference abstracts and over 60 reports for clients including consultancies on oil spill fingerprinting. He is well known internationally for his biomarker research and often invited to give plenary lectures. He is an Honorary Research Fellow and CRC Fellow at the University of Tasmania, a Fellow of the Royal Australian Chemical Institute and Leader of the Environment program in Aquafin CRC. With his colleague Dr Revill, he has published several reviews on the applications of biomarkers for characterising oil pollution in the marine environment. He and Dr Revill and colleagues authored the chapter on oil spills in the book *Environmental Implications of Offshore Oil and Gas Development in Australia - The Findings of an Independent Scientific Review* commissioned by the Australian Petroleum Exploration Association. He is thus particularly well versed on petroleum research relating both to exploration and environment.

Dr Andrew Revill graduated in 1986 with first class honours in Environmental Science, and subsequently spent three years carrying out hydrocarbon surveys around North Sea oil and gas installations in sediment, water and atmospheric samples. In 1992 he completed a Ph.D. at Plymouth University, UK, looking at the "Characterisation of Unresolved Complex Mixtures of Hydrocarbons" and was appointed as a Postdoctoral Fellow and then Research Scientist in the CSIRO Division of Oceanography to investigate the organic geochemistry of the tasmanite oil shale and a wider assessment of the petroleum prospectivity of Tasmania. He has since worked on fingerprinting hydrocarbons in Port Phillip Bay and sulfur geochemistry in Antarctic Lakes. Currently he is a Senior Research Scientist in CSIRO Marine Research. His present research interests include the fingerprinting of hydrocarbon mixtures found in the environment, the origins of certain "biomarker" compounds in oils and the use of stable isotopes in environmental research. Between 1994 and 1998 he was the leader of a program looking at the persistence of oil from the *Iron Baron* oil spill in Northern Tasmania. Recently he has been using stable isotopes of carbon and nitrogen to assess inputs of organic matter and nutrients to coastal environments. Dr Revill has experience in bulk and compound-specific isotope analyses of a wide variety of marine samples, including sediments, algae, invertebrates and higher organisms.