

# 6 Application of Stable Isotope Ratios in Spilled Oil Identification

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## 6.1 Introduction

The characterization of spilled oil, slicks, and tars, and their correlation with potential sources, can often be readily accomplished by chemical techniques, as discussed in several chapters. Gas chromatographic techniques such as GC/FID and GC/MS have been used to produce hydrocarbon and other molecular distributions or fingerprints for comparison with the distributions in the sources. Stable isotope ratio measurements provide an additional technique for comparison of spilled petroleum with sources. In a correlation study, this is a useful, nonchemical technique that can complement chemical techniques to support the interpretation.

Stable isotope ratios assume an increased importance where weathering has altered the molecular composition of the spilled oil. Evaporation removes the more volatile chemicals at a rate that depends on the spill conditions and the initial composition of the spilled material. Thus, surface spills in an area where the material can quickly spread, such as from oil tankers at sea, can result in a rapid loss of light hydrocarbons from spilled oil. For a light crude oil, containing a large percentage of volatile hydrocarbons, this can produce a dramatic change in the molecular composition. For lubricating oils and heavy fuel oils, such as Bunker C, which contain much lower proportions of volatile hydrocarbons, evaporation may result in only minor changes in the molecular compositions. Oil spilled into water can lose the more water-soluble chemicals by dissolution into the underlying water.

Aromatic hydrocarbons, particularly benzene, toluene, ethylbenzene, and the xylenes, are more soluble than other compounds in oil (McAuliffe, 1966) and can be rapidly lost from relatively thin layers of product. Microbes in the environment can utilize petroleum hydrocarbons as a carbon source. Because they preferentially degrade certain hydrocarbons, such as *n*-alkanes (Chapelle, 2001), microbial biodegradation results in a change in the composition of the original petroleum. Chapters 3, 11, and 13 provide more detailed discussion of these processes.

Later in this chapter it will be shown that, under most circumstances, stable isotope ratios are changed much less by environmental alteration than are molecular compositions. In some cases, environmental alteration changes the molecular composition so severely that any meaningful correlation between a spilled oil and a possible source is not possible. In these circumstances, stable isotope ratio measurements may provide the only reliable way to correlate the spilled oil with the source. The importance of stable isotope ratio data in a correlation study increases with the degree of weathering. Most of the studies discussed in this chapter concern oil spilled in the sea, which is a particularly harsh environment. The application of stable isotope ratios in the wider area of environmental forensics has been reviewed by Philp (2002).

## 6.2 Isotope Ratios and Their Measurement

The major elements in petroleum are carbon and hydrogen, and the stable isotope ratios of

these elements have been the most intensively studied. Petroleum may also contain sulfur, nitrogen, and oxygen, and stable isotope ratios of these elements may provide additional information to correlate spilled material with sources.

The basis of the stable isotope ratio method is that each of these elements exists in nature in more than one isotopic form. Carbon exists in three isotopic forms:  $^{12}\text{C}$ , with 6 protons and 6 neutrons, accounts for about 99% of carbon;  $^{13}\text{C}$ , with 6 protons and 7 neutrons, accounts for about 1% of carbon; and  $^{14}\text{C}$ , with 6 protons and 8 neutrons, is unstable and decomposes. The hydrogen isotopes of interest are  $^1\text{H}$  (99.98%) and  $^2\text{H}$  (deuterium, 0.02%). The isotopes of the other elements in petroleum that find use as stable isotope ratios are nitrogen ( $^{14}\text{N}$ , 99.6%, and  $^{15}\text{N}$ , 0.36%), oxygen ( $^{16}\text{O}$ , 99.8%, and  $^{18}\text{O}$ , 0.02%), and sulfur ( $^{32}\text{S}$ , 95%, and  $^{34}\text{S}$ , 4%). The ratio of the isotopes of an element is not the same in all naturally occurring compounds. There are small variations caused by the different atomic weights of the isotopes. Thus,  $^{13}\text{C}$  reacts the same as  $^{12}\text{C}$  in chemical transformations, but the heavier  $^{13}\text{C}$  can be discriminated against in processes where weight is important, such as evaporation and diffusion. The heavier isotope also forms slightly stronger bonds with other atoms, and when this bond is broken in chemical or enzymatic reactions, slightly less of the  $^{13}\text{C}$ —X bonds are broken. If all the C—X bonds are broken, the ratio of  $^{12}\text{C}/^{13}\text{C}$  in the starting material is the same as in the product. But if the reaction does not go to completion, the product may be enriched in  $^{12}\text{C}$ , and the starting material enriched in  $^{13}\text{C}$ . This accounts for many of the differences in the stable isotope ratios in natural materials. A more detailed discussion of stable isotope geochemistry is given in Hoefs (1997).

Stable isotope ratios are conventionally referenced to an internationally recognized standard, and are expressed in the  $\delta$  notation, where, for carbon,

$$\delta^{13}\text{C} = (\text{R}_{\text{sample}}/\text{R}_{\text{standard}} - 1) \times 1000, \text{ and} \\ \text{R} = {}^{13}\text{C}/{}^{12}\text{C}.$$

Units are per mil (‰). The standard, by definition, has a  $\delta$  value of 0, and samples may have positive or negative  $\delta$  values depending on whether the sample is enriched or depleted in the heavier isotope. More positive  $\delta$  values are commonly referred to as being isotopically heavier, and more negative  $\delta$  values are referred to as isotopically lighter. The international standard for carbon is Pee Dee Belemnite (PDB) (Craig, 1957). This sedimentary carbonate lies at the heavy end of the naturally occurring carbon range, so most materials have negative  $\delta$  values. The range of  $\delta^{13}\text{C}$  values in naturally occurring materials (Figure 6-1) is almost 100‰, but in petroleum it is only about 15‰. The international standard for hydrogen is Standard Mean Ocean Water (SMOW) (Hagemann et al., 1970).

The elements important in the stable isotope analysis of oils and tars (H, C, N, O, and S) have to be isolated from the material and converted to a gas that can be introduced into an isotope ratio mass spectrometer (IRMS). The gaseous forms of these elements that are commonly used are  $\text{H}_2$  (H),  $\text{CO}_2$  (C, O),  $\text{CO}$  (O),  $\text{N}_2$  (N), and  $\text{SO}_2$  (S). For example, the stable isotopic forms of  $\text{CO}_2$ ,  $^{13}\text{CO}_2$  and  $^{12}\text{CO}_2$ , are converted in the ion source (Figure 6-2) to the positively charged ions  $m/e$  45 and 44, respectively, which are separated in a stable magnetic field, and the intensities of the ions measured to provide the  $^{13}\text{C}/^{12}\text{C}$  ratio.

Where a sample consists of a mixture of compounds, e.g., *n*-alkanes in an oil sample, bulk stable isotope ratios can be measured on the total element content in a sample. For example, the  $\delta^{13}\text{C}$  value of an oil sample is measured by combusting the entire sample in an oxidizing furnace to convert the bulk carbon to  $\text{CO}_2$ . Alternatively, the  $\delta^{13}\text{C}$  value of an individual *n*-alkane in the oil is measured in compound specific isotope analysis (CSIA), by isolating and combusting each of the individual *n*-alkanes in the oil. A schematic of the CSIA equipment is shown in Figure 6-2.

The first stable isotope ratio instruments were equipped with dual inlets, one for the sample gas, the other for the standard. The sample and standard were analyzed sequentially multiple

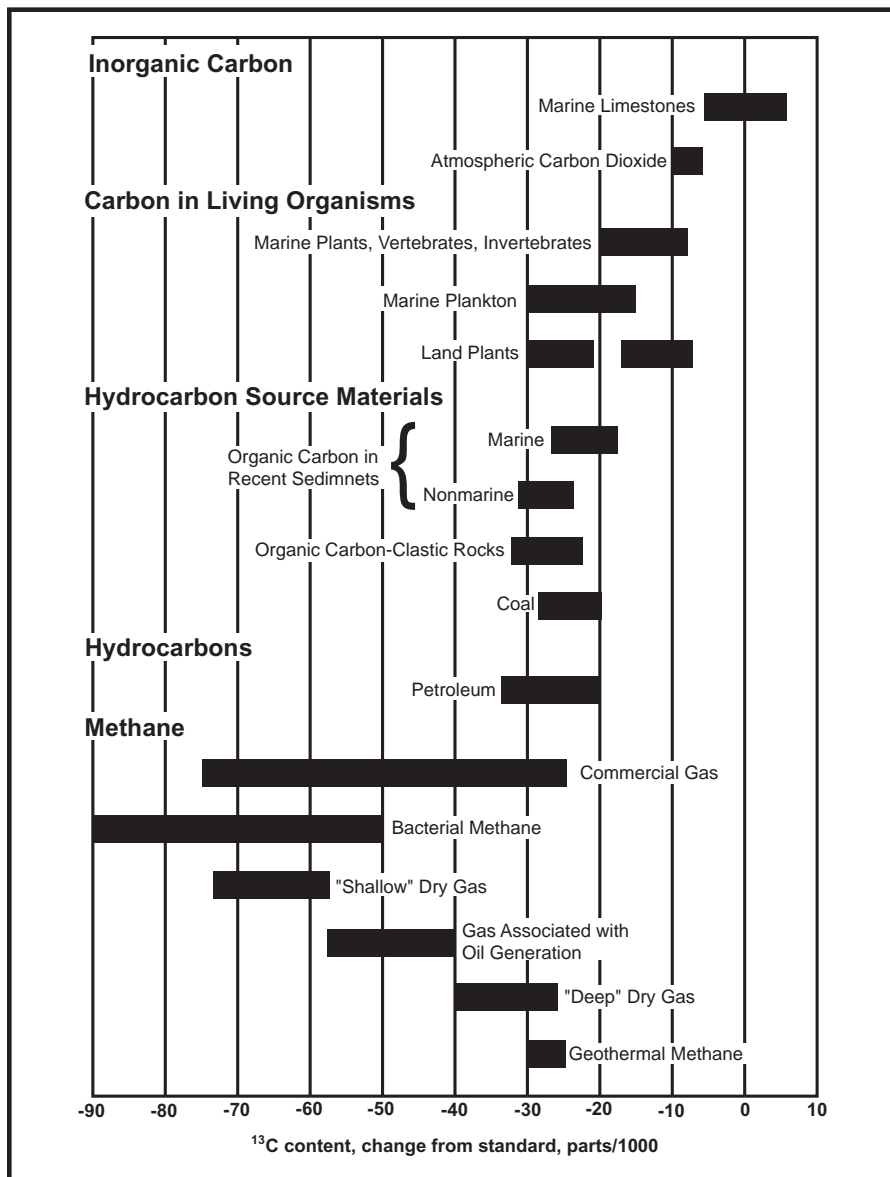


Figure 6-1 Carbon isotope ratios in a variety of geochemical materials. (Reprinted with permission from Fuex (1977). Copyright (1977) Elsevier Science.)

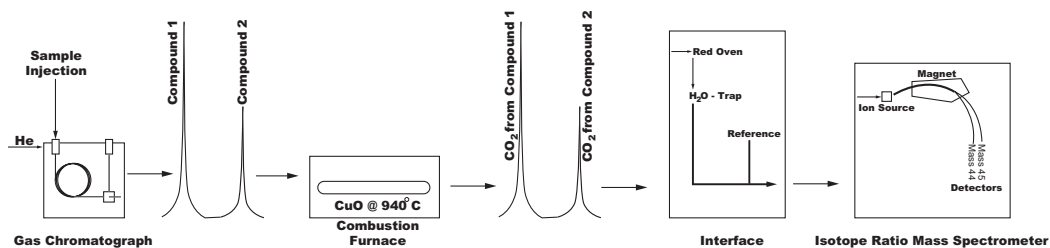


Figure 6-2 Schematic diagram of an instrument set-up to perform compound specific carbon isotope analysis.

times. The sample was converted, in a separate high vacuum line, to an inlet gas, which was then transferred to the inlet system of the IRMS. Analysis of the isotope ratios of an individual compound in a mixture would require isolation of the compounds by preparative GC, conversion of the minute amounts of each compound to the inlet gas, and transfer of the equally minute amounts of gas to the IRMS inlet system. This operation is laborious for a multicomponent mixture, and fraught with many opportunities for isotopic fractionation. The development of continuous-flow isotope ratio instruments greatly enhanced the ability to measure the isotope ratios of individual compounds in a mixture.

In continuous-flow instruments, a carrier gas, helium, continuously passes through the mass spectrometer, similar to analytical GC/MS instruments. This allows the conversion to inlet gases and other preparative techniques to be performed online and in tandem with the isotope ratio measurement. The application of continuous-flow isotope ratio techniques has been reviewed by Lichtfouse (2000). Preparative instruments such as oxidative and reductive element analyzers enable bulk isotope ratio measurements to be performed rapidly online. A GC placed in front of a combustion/pyrolysis furnace, as in Figure 6-2, allows an individual compound in a mixture to be separated, converted to the appropriate inlet gas, and their isotope ratios measured in one operation. The chromatographic process may introduce isotopic fractionation within the peak that represents a particular compound. So the entire peak has to be converted to the inlet gas. Coelution with another compound will obviously complicate the measurement. These experimental difficulties will be discussed in the section on CSIA. Reference gases cannot be introduced in a separate inlet, as in dual inlet instruments, and are added after the conversion stage and analyzed as sample peaks.

### 6.3 Bulk Isotope Ratios

One of the first reviews of the application of carbon isotope ratios in geochemistry and oil

exploration was provided by Fuex (1977), who compiled the bulk carbon isotope ratios of a wide range of geochemical materials in the classic graph shown in Figure 6-1. The factors that controlled the carbon isotope ratio of petroleum hydrocarbons were identified as the ratio of the source organic matter, the isotopic fractionation involved in the formation of the hydrocarbon, and the isotopic fractionation after formation. Fuex considered both fractionations to be unimportant to high-molecular-weight petroleum hydrocarbons, which were influenced primarily by the source material. He stressed that stable isotope ratios be used in conjunction with other geochemical information.

Several studies of bulk isotope ratios in oils have identified some general isotopic relationships that have application to oil spill identification. Silverman and Epstein (1958) distinguished several Tertiary oils from marine and nonmarine source rocks based on carbon isotope ratios. Sofer (1984) extended this by measuring the carbon isotope ratios of the saturate and aromatic fractions of 339 oils. When these values were plotted against each other, oils sourced from terrigenous organic matter could be distinguished from oils sourced from marine organic matter.

There is a general trend toward heavier carbon isotope ratios with decreasing age of petroleum (Stahl, 1977). More recently, Andrusevich et al. (1998) measured the carbon isotope ratios of 514 crude oils spanning a wide age range from Precambrian to Tertiary. Again there was a general trend toward heavier values in younger oils with abrupt isotopic changes at certain discrete time intervals. The trend was attributed to the diversity of phytoplankton preserved in the source rock of the oils.

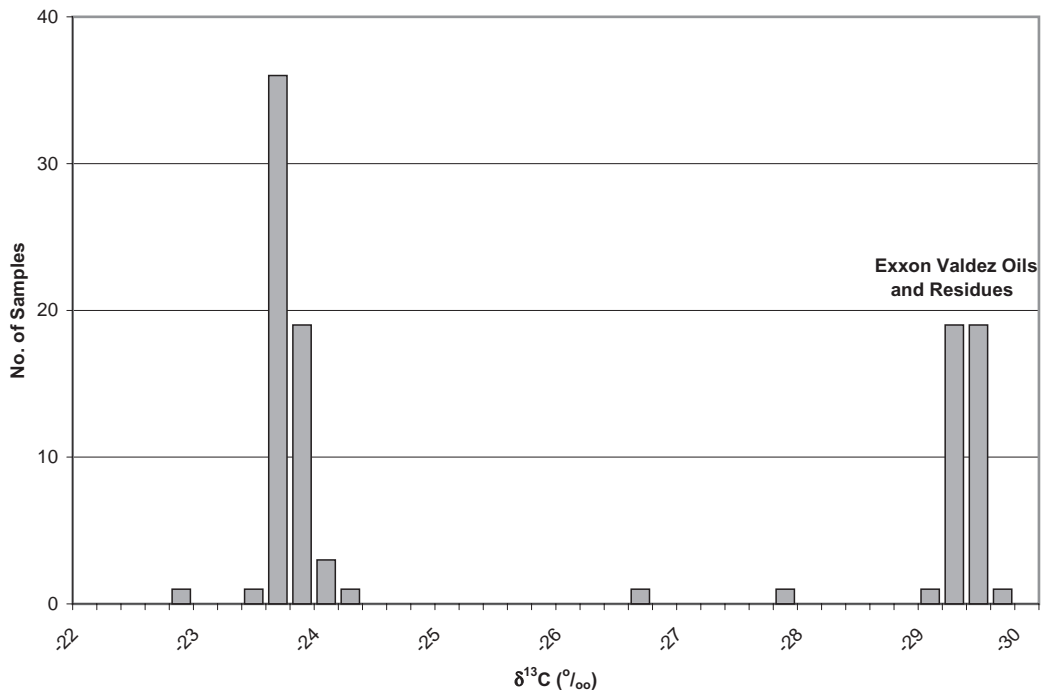
One of the first uses of stable isotope ratios to identify petroleum-derived pollution was reported by Calder and Parker (1968) in industrial areas in east Texas. At a petrochemical plant, they measured the carbon isotope ratios of dissolved and particulate carbon in the intake and outflow waters. In the outflow ponds, the  $\delta^{13}\text{C}$  values of dissolved organic carbon was much lighter ( $-26$  to  $-39\text{‰}$ ) than the intake water ( $-17$  to  $-19\text{‰}$ ), which was

attributed to contributions from isotopically light petroleum-derived chemicals. In the Houston Ship Channel, which receives contributions from refineries and other industrial activities, light isotope ratios were measured at many locations, reflecting pollution from petroleum-derived chemicals.

Hartman and Hammond (1981) measured the carbon and sulfur isotope ratios and sulfur concentrations of the asphaltene fractions of beach tars deposited near Los Angeles. The asphaltene fraction was used because it was considered to be least affected by weathering. The carbon and sulfur isotope ratios fell within the range of the ratios in local oil seeps ( $\delta^{13}\text{C} = -22.5$  to  $-23.2\text{‰}$ ;  $\delta^{34}\text{S} = +8$  to  $+15\text{‰}$ ) and oil from offshore oil wells and were different from crude oils transported into the area by tankers. The source of the majority of the tars was found to be natural seepage from Coal Oil Point, 150 km to the northwest. During the spring, summer, and fall, seeps are transported on ocean currents toward Los Angeles. A

smaller number of the beach tars were derived from natural oil seeps in Santa Monica Bay. The authors considered the isotope ratios to be useful in identifying the sources of the beach tars even after 2–4 weeks of weathering.

Macko and Parker (1983) measured carbon and nitrogen isotope ratios of aliphatic, aromatic, and polar fractions of two large oil spills in the northwestern Gulf of Mexico and a number of beach tars along the south Texas coast. Isotopic signatures similar to the oil spills were identified, along with 16 other distinct isotopic signatures in the beach tars. The authors concluded that the multiple signatures reflected multiple sources of spilled oil from oil exploration and transport. The *Exxon Valdez* tanker spill in 1989 deposited a large amount of oil and tarballs along the shore of Prince William Sound in Alaska. Kvenvolden et al. (1993, 1995) analyzed many oil residues that had similar carbon isotope ratios and molecular distributions to the *Exxon Valdez* oil. However, as shown in Figure 6-3, other

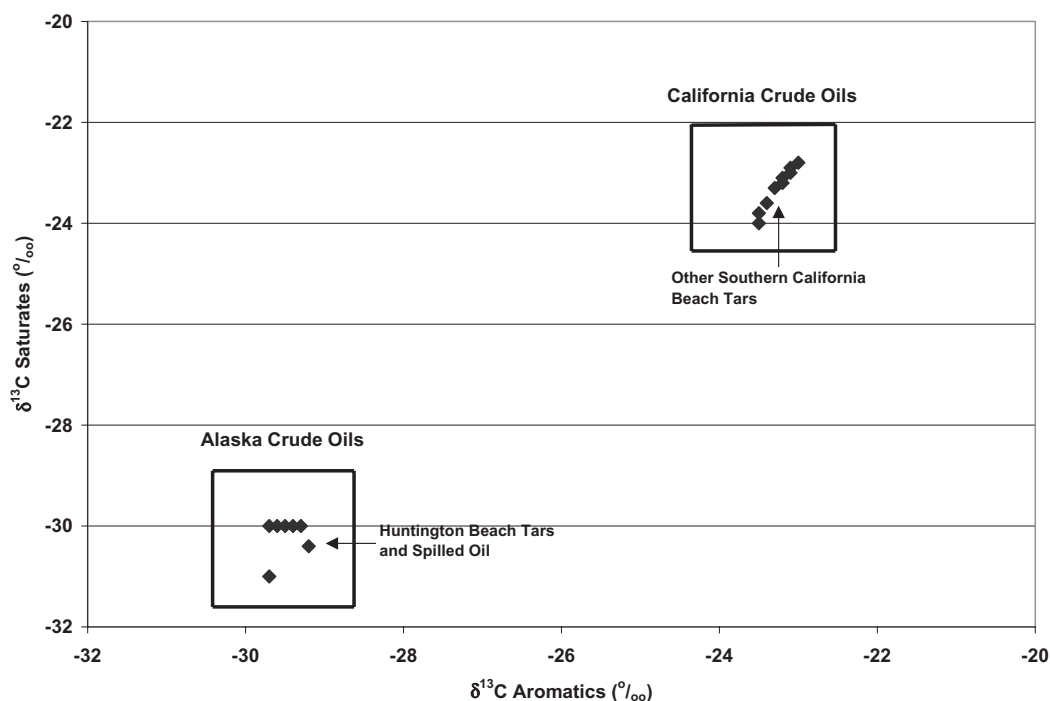


**Figure 6-3** Carbon isotope ratios of Exxon Valdez oil and residues, and other tar balls collected in Prince William Sound, Alaska and analyzed by Kvenvolden et al. (1997).

tarballs from the area showed very different characteristics, including  $\delta^{13}\text{C}$  values averaging  $-23.7 \pm 0.2\text{‰}$ , as compared to the *Exxon Valdez* oil ( $-29.4 \pm 0.1\text{‰}$ ). The carbon isotope ratios and biomarker distributions identified the source of the isotopically heavy oil residues as the Southern California Monterey Formation. Kvenvolden et al. (1998) documented the wide use in Alaska of California Monterey oil products, including fuel oils, asphalt, lubricants, and tars, prior to 1970 and the availability of North Slope Alaska oil. The source of the isotopically heavy tarballs was attributed to fuel oil and asphalt stored in the town of Valdez, and spilled during the 1964 Alaskan earthquake. North Slope Alaska oil was the most likely source of lighter carbon ( $-29.3 \pm 0.2\text{‰}$ ) in modern pavement and runways.

The opposite situation was demonstrated in a study of an oil spill from the tanker *BP American Trader* in 1990 off Huntington Beach

in Southern California (Global Geochemistry Corp., 1990). The oil spilled from the tanker formed a slick that was carried onshore and formed tarballs that could be linked visually with the slick. Tarballs are common along Southern California beaches, and the U.S. Coast Guard, which investigated the spill, was concerned that the extent of the contamination from the spill be delineated scientifically. Biomarker and carbon isotope analyses were performed on oil from the tanker, the oil slick off Huntington Beach, tarballs linked to the slick, and tarballs north and south of the contamination. The carbon isotope ratios of the saturate and aromatic fractions of the samples are compared in Figure 6-4. Tarballs away from the contamination had carbon isotope ratios similar to those measured by Hartman and Hammond (1981), in the range of Southern California Monterey oils. Tanker oil, slick oil, and tarballs associated with the spill had carbon isotope ratios similar to North Slope



**Figure 6-4** Carbon isotope ratios of saturate and aromatic fractions of oil and tar balls associated with the *BP American Trader* spill offshore Huntington Beach, California. These are compared with ratios of tar balls north and south of the spill site.

Alaska oil, which was the cargo carried by the *BP American Trader*.

Wang et al. (1998) analyzed tarballs along the west coast of North America, from northern California to Vancouver Island, British Columbia, by GC/MS and bulk carbon isotope ratios. The  $\delta^{13}\text{C}$  values of tarballs from northern California and Oregon had a very narrow isotopic range ( $-26.8 \pm 0.1\text{‰}$ ), which is different from the two largest sources of oil on the West Coast, Southern California Monterey and North Slope Alaska. The source of these tarballs was believed to be bunker C fuel oil from a passing tanker.

Carbon and sulfur isotope ratios were used to help identify the sources of crude oil spilled near a tank farm in the western U.S. (Zymax Forensics, 2003). Oil was discovered in several monitoring wells near the tank farm, which had stored crude oil from a number of oil fields in the western U.S. and Canada. Table 6-1 shows the isotope ratios of product samples from four monitoring wells and two seeps, and a sludge sample from an excavation pit. These were compared with six potential source oils. Six of the seven spill samples had very similar  $\delta^{13}\text{C}$  values ( $-29.0$  to  $-29.2\text{‰}$ ), which correlated with oils 3 and 4. The sulfur isotope ratios of three of the products were

similar to oils 3 and 4; the two seep samples were isotopically heavier. Stable isotope ratios could not distinguish between oils 3 and 4. However, nickel and vanadium concentrations allowed oil 3 to be identified as the most likely source of six of the spill samples. Product sample 3 had different carbon and sulfur isotope ratios from the other spill samples, and these ratios correlated with oils 2 and 6. Nickel and vanadium concentrations again allowed oil 6 to be identified as the source of this product. This illustrates the importance of having other geochemical data available when the stable isotope ratios by themselves are inconclusive.

Bulk isotope ratios of oil fractions (saturate, aromatic, polar, and asphaltene) found early use in oil exploration (Stahl, 1977). Northam (1985) increased the number of fractions for isotopic measurement by distilling oil into 11 fractions based on boiling point. Chung et al. (1994) separated 69 oils into 12 fractions, again based on boiling point. When  $\delta^{13}\text{C}$  was plotted against fraction number, the slope of the  $\delta^{13}\text{C}$ -fraction line varied in oils from different source environments. The CSIA approach, which is discussed in the next section, has replaced these time-consuming techniques.

**Table 6-1** Stable Isotope and Metal Concentrations in Spilled Oils Near a Tank Farm in Western U.S. Compared with Potential Crude Oil Sources

<i>Sample ID Spill Products</i>	<i>Carbon Isotope Ratio ‰</i>	<i>Sulfur Isotope Ratio ‰</i>	<i>Vanadium mg/kg</i>	<i>Nickel mg/kg</i>
Product 1	-29.0	6.3	4.1	1.5
Product 2	-29.0	9.4	4.5	1.7
Product 3	-29.9	5.7	7.6	2.8
Product 4	-29.0	9.4	4.9	1.5
Pit sample	-29.2	9.5	6.4	3.0
Seep 1	-29.1	11.0	4.8	1.6
Seep 2	-29.0	10.0	5.4	1.7
<b>Oils</b>				
Oil 1	-28.1	7.1	1.2	<1
Oil 2	-30.1	6.4	22.0	5.5
Oil 3	-28.8	9.1	6.9	2.8
Oil 4	-29.2	9.4	17.0	6.9
Condensate 5	-24.1	24.7	<1	<1
Oil 6	-30.1	6.9	8.3	3.5

## 6.4 Compound-Specific Isotope Analysis (CSIA)

The first paper discussing the CSIA method was published by Mathews and Hayes (1978), who separated and measured the carbon isotope ratios of methyl esters of  $C_7$ – $C_{13}$  acids and amino acid esters. More recently, CSIA principles and technical aspects have been reviewed by Meier-Augustine (1999), who described its applications to areas such as the authenticity of flavors, fragrances, wine, fruit juice, honey, vegetable oils, and drugs. Applications of CSIA in the environmental sciences have been reviewed by Schmidt et al. (2004). A schematic illustration of a CSIA setup is shown in Figure 6-2. Up to the present time, CSIA has been applied to four elements, H, C, N, and O, as shown in Table 6-2. Sulfur isotope ratios have been measured by continuous-flow techniques, but there has been no CSIA application as yet due to the low abundance of sulfur in most organic compounds. In oils, the most obvious applications of CSIA are to C and H isotope ratios. N- and O-containing compounds constitute a very minor component of most oils. S-containing compounds are more important constituents of some oils and are often concentrated by environmental alteration of spilled oil. When CSIA methods are developed for S-containing compounds, they may be very useful in characterizing certain oil spills.

As with other areas of petroleum geochemistry, CSIA of crude oils was developed as a tool in petroleum exploration. This provided a way to isotopically compare individual

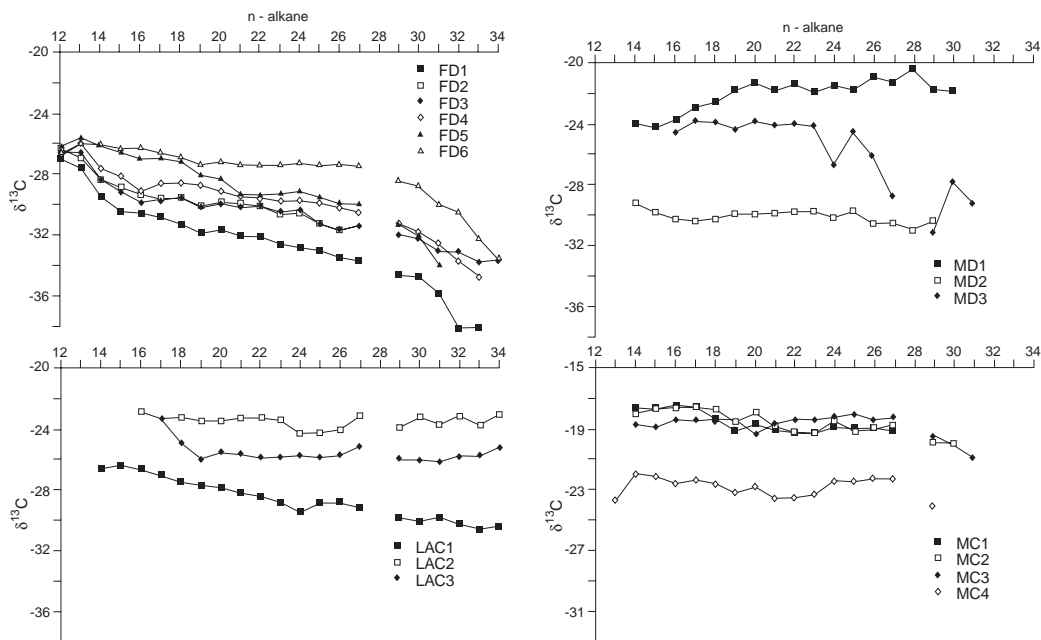
hydrocarbons in oils with those in potential source rocks. It could also be used to compare hydrocarbons in oils from different locations to determine if they were genetically related.

The carbon isotope ratio of bulk petroleum is inherited from the source organic matter, but is influenced by maturity and by physical and chemical alteration after generation. Isotope ratios of individual hydrocarbons in petroleum are subject to the same controls. But, in addition, they may reflect heterogeneity within the source organic matter itself. Thus, low-molecular-weight *n*-alkanes may reflect algal or bacterial contributions; high-molecular *n*-alkanes may originate in plant waxes or from degradation of complex algal and plant molecules. These contributions influence both the  $\delta^{13}C$  values of the *n*-alkanes and the slope of the  $\delta^{13}C$ -alkane relationship.

An illustration of the carbon isotope distribution of *n*-alkanes in a number of Tertiary oils formed in different depositional environments is shown in Figure 6-5 (Murray et al., 1994). The lightest oils included fluvio-deltaic oils in the range  $-25$  to  $-37\text{‰}$ ; the heaviest were marine carbonate oils in the range  $-17$  to  $-22\text{‰}$ . An interesting feature was the difference in the slope of the  $\delta^{13}C$ -alkane line with increasing *n*-alkane size. In fluvio-deltaic oils, the slope was steep, with *n*-alkanes larger and the  $C_{30}$  being much lighter isotopically; in most lacustrine, marine deltaic, and marine carbonate oils, the slope was much shallower or flat. This provides a way of screening possible sources for spilled oil or beach tars of unknown origin, especially in areas of heavy

**Table 6-2** Sensitivity and Precision for Elements Reported to Date of Compound-Specific Isotope Analysis (CSIA). Data from Schmidt et al. (2004)

<i>Stable Isotopes</i>	<i>Detection Limits (Nmole Element on Column)</i>	<i>Precision (‰)</i>	<i>CSIA Commercially Available Since</i>
D/H	8–10	5	1998
$^{13}C/^{12}C$	1	0.2	1988
$^{15}N/^{14}N$	0.8–1.5	0.5	1989
$^{18}O/^{16}O$	5	0.8	1998
$^{34}S/^{32}S$	n.a.	n.a.	—



**Figure 6-5** Carbon isotope ratios of individual *n*-alkanes in a number of oils sourced from fluvio-deltaic (FD), lacustrine (LAC), marine deltaic (MD), and marine carbonate (MC) depositional environments. (Reprinted with permission from Murray et al. (1994). Copyright (1994) Elsevier Science.)

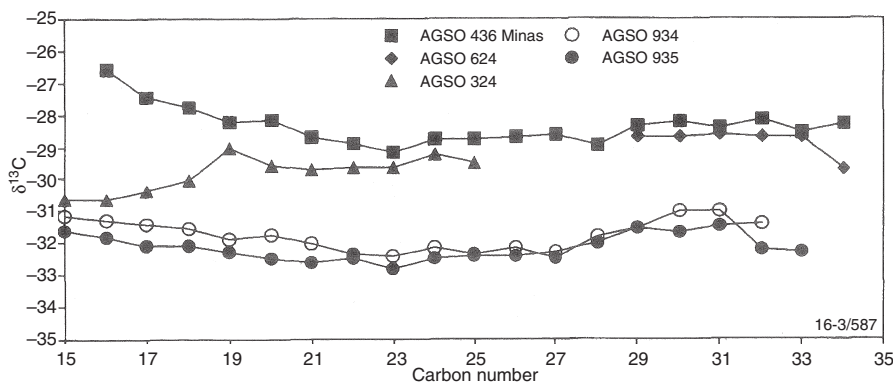
tanker traffic carrying oils from different geographical regions.

In one of the first studies to utilize CSIA to identify oil pollution, Ishiwatari et al. (1994) and Uzaki et al. (1993) measured the carbon isotope ratios of  $C_{27}$ – $C_{33}$  *n*-alkanes in a sediment core from Tokyo Bay. In the deeper section of the core, odd- and even-numbered *n*-alkanes had similar  $\delta^{13}C$  values, in the range  $-29.6$  to  $-32.9\text{‰}$ , which were attributed to higher plant waxes. In the shallower section, dated as post-1965, the isotopic range was shifted toward heavier values ( $-28.2$  to  $-31.5\text{‰}$ ). More significant was that even-numbered *n*-alkanes were heavier than odd-numbered *n*-alkanes. The heavier even-numbered *n*-alkanes were attributed to oil pollution, and the authors noted that pollution in Tokyo Bay increased significantly in the period 1960–1965.

In a comprehensive study of bitumens or beach tars along the northern and southern coasts of Australia, Dowling et al. (1995) used carbon isotope ratios of *n*-alkanes and other

hydrocarbons to help identify the source of the bitumens. The bitumens could be divided into two physical types: a soft, waxy paraffinic type and a hard asphaltic type.

The waxy bitumens occurred along both coasts and had *n*-alkane carbon isotope ratios that fell within the range of the Minas and Duri oils of the Central Sumatra Basin in Indonesia. Molecular biomarkers in the bitumens also corresponded to those in the Minas and Duri oils, particularly the presence of botryococane, a marker for oils from lacustrine source rocks. Botryococane was also markedly isotopically heavier ( $\delta^{13}C = -10$  to  $-15\text{‰}$ ) than the *n*-alkanes ( $-25$  to  $-30\text{‰}$ ). Carbon isotope ratios of the isoprenoids, pristane and phytane, were slightly heavier than the *n*-alkanes. Bjorøy et al. (1991) also reported that isoprenoids were generally heavier than *n*-alkanes in the same oil. The origin of the waxy bitumens in Australia was attributed to natural seeps in Sumatra, coming either from offshore reservoirs, or from onshore reservoirs by river or fault migration to the ocean. The oil then



**Figure 6-6** Carbon isotope ratios of individual *n*-alkanes in asphaltic bitumens from S. Australia (AGSO 934, 935), in a bitumen from N. Australia (AGSO 624), and in oils from Seram, Indonesia (AGSO 324) and Minas, Indonesia (AGSO 436). (Reprinted with permission from Dowling et al. (1995). Copyright (1995) Elsevier Science.)

would have been transported south and east on oceanic currents.

The asphaltic bitumens, which were restricted to the southern coast, were isotopically lighter, with  $\delta^{13}\text{C}$  values in the range  $-31$  to  $-33\text{‰}$  (Figure 6-6). Biomarker evidence suggests an origin for these bitumens in clastic marine source rocks. They are believed to arise from natural seeps, but the location was not established.

A bitumen from northern Australia (AGSO 624) exhibited *n*-alkane carbon isotope ratios very similar to the Minas oil, as shown in Figure 6-6. However, biomarker data identified the bitumen as being derived from a marine carbonate source, which eliminated the lacustrine Minas oil as the source. This illustrates the need to combine stable isotope evidence with molecular evidence to identify the source of spilled oil. In this case, Minas oil, although plausible on a geographical and isotopic basis, was ruled out by the lack of a molecular match. Middle Eastern oil spilled from tankers is one possible source.

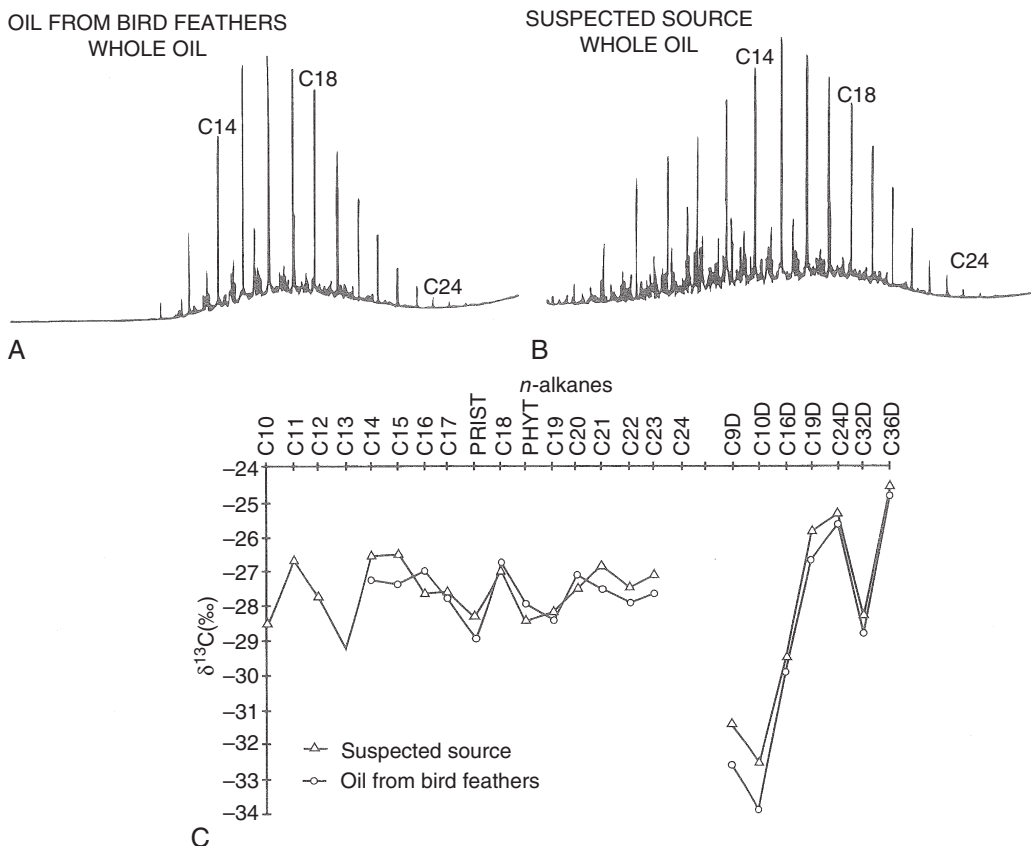
Experimental difficulties affecting the CSIA data are illustrated by anomalous results for the carbon isotope ratios of  $n\text{C}_{28}$  reported by Murray et al. (1994) and omitted from Figure 6-5. This *n*-alkane was lighter than neighboring alkanes and showed poor reproducibility. This was found to be caused by a non-normal alkane eluting very close to  $n\text{C}_{28}$ , illustrating

the problems that coelution can cause CSIA measurements.

Correlation of spilled light oils, both crude and refined, with potential sources is complicated by the lack of high-molecular-weight biomarkers, such as steranes and triterpanes.  $\text{C}_{15}$ – $\text{C}_{16}$  sesquiterpanes can be useful in some cases, but in my experience, weathering can alter the sesquiterpane distribution, making its correlation with an unweathered potential source inconclusive. CSIA is particularly useful in these cases. Mansuy et al. (1997) showed that three mildly weathered light fuel oils could be correlated with their unweathered source oils with a high degree of confidence, based on carbon isotope ratios of *n*-alkanes and isoprenoids.

Birds are particularly sensitive to the effects of oil spills, and fouled birds provide a very emotional illustration of the ecological costs of an oil spill. The pathetic sight of oil-soaked birds ignites public indignation and prods the authorities to find the culprit. It is not surprising, therefore, that several studies have focused on identifying the source of oil on bird feathers.

In a light fuel oil spill, which had fouled the feathers of birds, Mansuy et al. (1997) extracted the oil from the feathers to correlate it with a potential source. High-molecular-weight biomarkers were absent, so CSIA provided a unique way to correlate the oils. As



**Figure 6-7** Gas chromatograms and carbon isotope ratios of individual alkanes in oil from the feathers of birds killed in a major oil spill and from the oil that was the suspected source of the spill. (Reprinted with permission from Mansuy et al. (1997). Copyright (1997) American Chemical Society.)

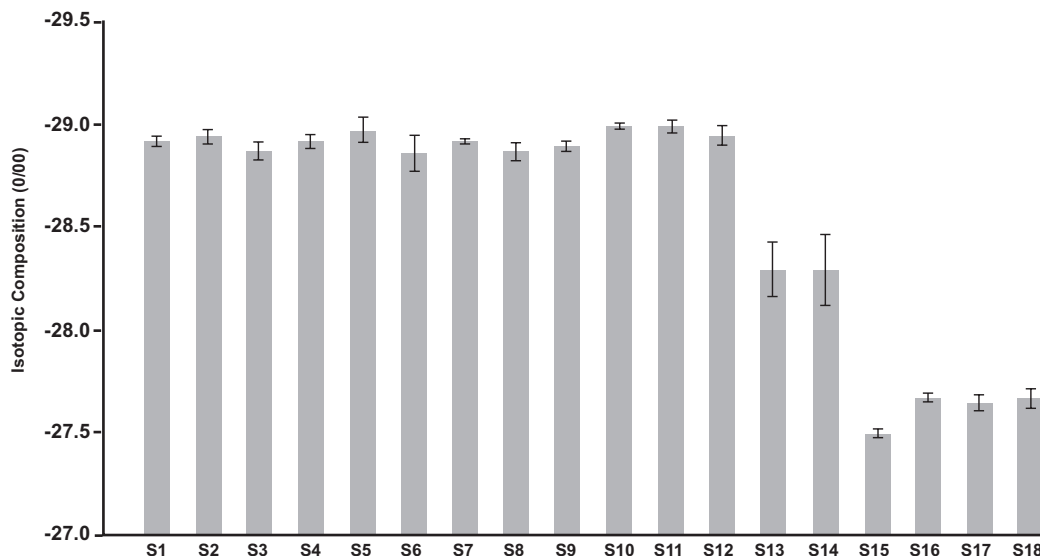
shown in Figure 6-7, the oil on the feathers was depleted in the more volatile hydrocarbons compared to the suspected source. However, the similarity between the oils was demonstrated by the carbon isotope ratios of *n*-alkanes and isoprenoids.

Mazeas and Budzinski (2002) used stable carbon isotope ratios, both bulk and compound-specific, to identify the source of oiled bird feathers and oil residues along the Atlantic coast of France after the wreck of the tanker *Erika* in 1999. The tanker was carrying heavy fuel oil, 10,000 tons of which are believed to have spilled. However, other tankers took advantage of the incident to flush their tanks, providing potentially multiple sources for beached oil along the coast. Bulk isotope ratios of oil and tarballs show a significant difference in the samples collected along the northern

coast from those collected along the southern coast (Figure 6-8). Northern samples were isotopically very similar to the *Erika* oil. The southern group contained four isotopically heavy samples and two samples with values intermediate between the two groups.

The bulk isotope ratios of oil from fouled bird feathers were found to have large uncertainties caused by interfering compounds. However, CSIA of *n*-alkanes were unaffected by the contamination, and identified the *Erika* oil as the source of the oil on the feathers. In addition, CSIA confirmed the bulk isotope source allocations in the oils and tarballs.

CSIA was also performed on phenanthrene, and methyl, dimethyl, and trimethyl phenanthrenes. This also confirmed the source allocations and showed that the intermediate bulk isotope ratios of the two southern samples in



**Figure 6-8** Bulk carbon isotope ratios of oil from the Erika tanker spill (S1) off the Atlantic coast of France, 1999, and of oil residues collected from the northern (S2–S12) and southern (S13–S18) Atlantic coast. (Reprinted with permission from Mazeas and Budzinski (2002). Copyright (2002) American Chemical Society.)

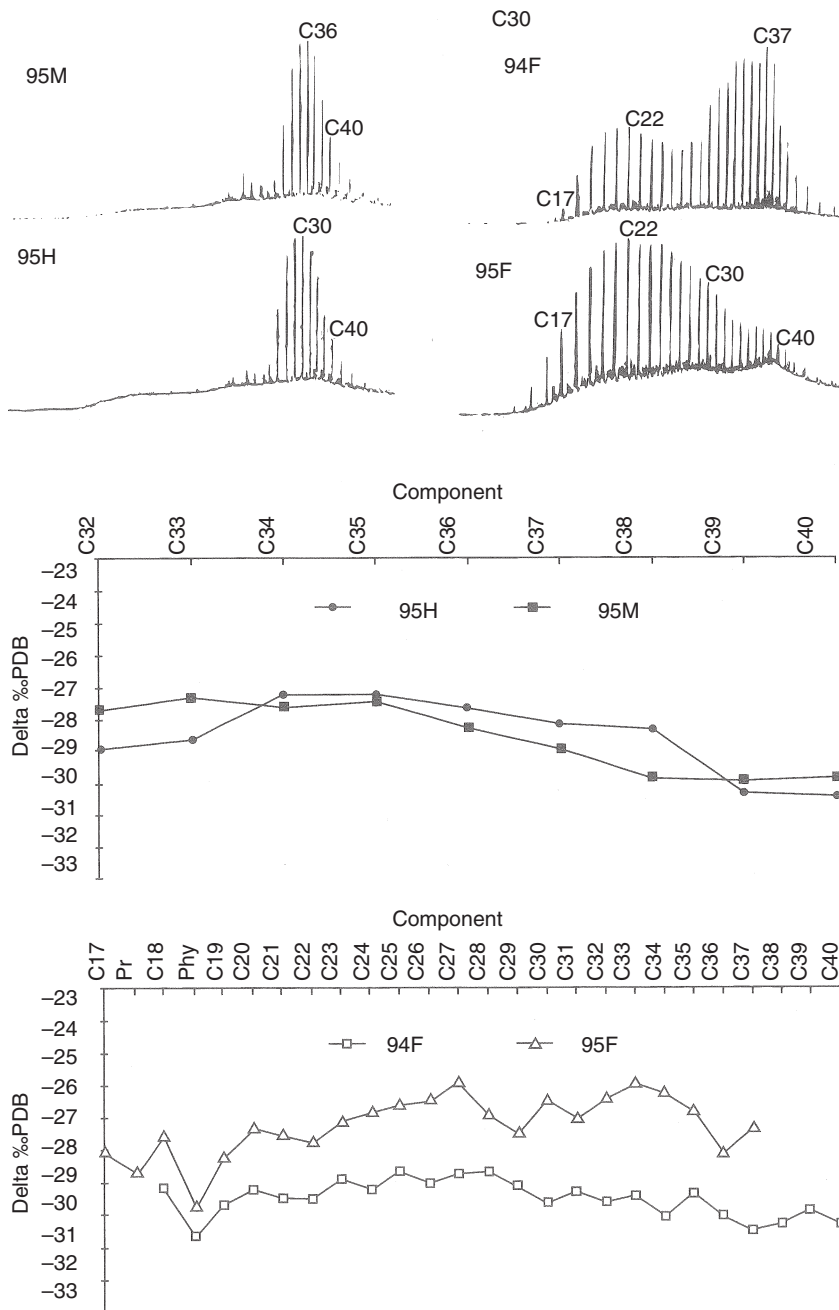
Figure 6-8 were not due to mixing of *Erika* oil and the heavier oil, but represented a third source.

The conclusion from the study was that bulk carbon isotope ratio measurement is useful as a screening tool for source identification because it is rapid and requires little sample preparation. However, as illustrated by the oiled bird feathers, contamination can alter the values.

Rogers and Savard (1999) used CSIA to help distinguish naturally occurring hydrocarbons from petroleum pollution along the St. Lawrence River near Quebec City. At sites farthest from industrial activity,  $\delta^{13}\text{C}$  values of *n*-alkanes were relatively light ( $-29$  to  $-31\text{‰}$ ), with odd and even high-molecular-weight *n*-alkanes showing isotopic differences. At sites near areas of industrial activity,  $\delta^{13}\text{C}$  values of *n*-alkanes were heavier ( $-27$  to  $-30\text{‰}$ ), with little difference between odd and even *n*-alkanes. The isotopic profiles of *n*-alkanes at some contaminated sites were similar to a bunker fuel ( $\delta^{13}\text{C} = -28$  to  $-29\text{‰}$ ) used by ships in the region. The heaviest *n*-alkanes ( $-25$  to  $-28\text{‰}$ ) were found at a contaminated site where bioremediation with fertilizers was

in progress. The authors attributed the heavy values to biodegradation.

Philp (2002) studied a number of beach tars from the Seychelles Islands to determine if the source was tanker traffic or natural seeps. Figure 6-9, which shows the gas chromatograms of four tars, indicates a strong similarity in the hydrocarbon distribution in 95M and 95H. Carbon isotope ratios of the high-molecular-weight *n*-alkanes that dominate these samples are also similar. The sterane and terpane distributions were similar and indicated that the tars had been derived from a carbonate source rock and showed similarities to oils from the Middle East. Tars 95M and 95H were, therefore, attributed to releases from passing tankers. The hydrocarbon distributions in 94F and 95F showed similarities to each other, assuming that 94F was a more weathered sample, which would have reduced the maximum around  $\text{C}_{22}$  seen in 95F. However, the alkane carbon isotope ratios were different, indicating different sources for the two tars. Other information indicated similarities to lacustrine source rocks in the Seychelles area, and the sources were determined to be natural oil seeps.



**Figure 6-9** Gas chromatograms and carbon isotope ratios of individual alkanes in beach tars from the Seychelles Islands. (Reprinted with permission from Philp (2002). Copyright (2002) Academic Press.)

### 6.4.1 Experimental Considerations

Sensitivity is a major factor in the application of CSIA, particularly for dissolved compounds such as petroleum hydrocarbons, which are often in very low concentrations in natural waters due to their low solubility. Schmidt et al. (2004) report that commercially available CSIA instruments require at least 1 nmole of carbon or 8 nmole of hydrogen for precise isotopic measurement. Schwarzbauer et al. (2005) were able to measure carbon isotope ratios of anthropogenic contaminants, including aromatic hydrocarbons, reliably on concentrations as low as 50 ng/L. Wang et al. (2004) used semipermeable membrane devices (SPMD) to accumulate petroleum hydrocarbons from river water for CSIA of carbon and hydrogen, with no isotopic fractionation. The SPMDs eliminated the need to process thousands of liters of water required for conventional liquid/liquid and solid phase extraction. CSIA of *n*-alkane and PAH concentrations as low as <1 ng/L and 3 ng/L, respectively, was performed in water from the Pawtuxet River.

Sensitivity is not a major issue for separate phase oil and tars, which provide abundant carbon and hydrogen for CSIA. The major analytical issue with these materials is coelution of target hydrocarbons with other compounds, and the change in the target compound's isotope ratio that this introduces, as discussed by Dowling et al. (1995). Elaborate cleanup procedures are often necessary to eliminate potentially coeluting compounds without introducing isotopic fractionation of target compounds.

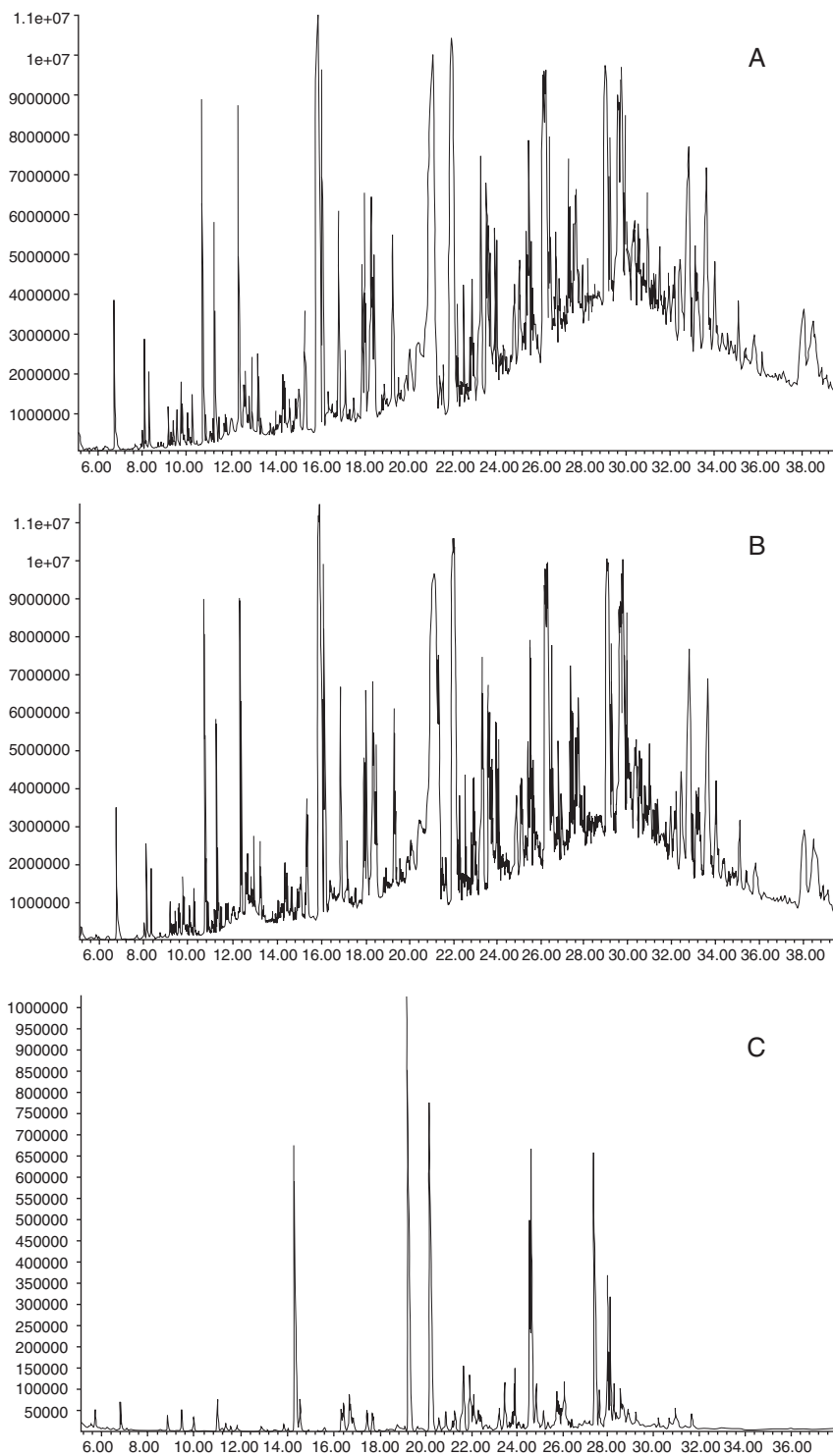
Kim et al. (2005) isolated and purified PAHs in sediments by performing column chromatography, high-performance liquid chromatography, and thin-layer chromatography sequentially on the dichloromethane extract. The increase in purity with each technique is shown in Figure 6-10. PAH recovery was 80%, and the standard deviation of the  $\delta^{13}\text{C}$  values of PAH standards purified in this way was <0.4‰. Using this purification procedure, naphthalenes and methylnaphthalenes were isolated from petroleum contaminated soils

and offshore sediments at McMurdo Station, Antarctica (Figure 6-11). Soils from the fueling station, helipad, and old oil tank showed similar isotopic distributions, which was attributed to fuel oils. The carbon isotope ratios of soil from the machine shop were more negative and were believed to reflect a wider range of petroleum products, including lubricating oils and waste oils from vehicle repair. Carbon isotope ratios in offshore sediments were even more negative and were attributed to accumulated petroleum product spills from ships and onshore operations at McMurdo Station.

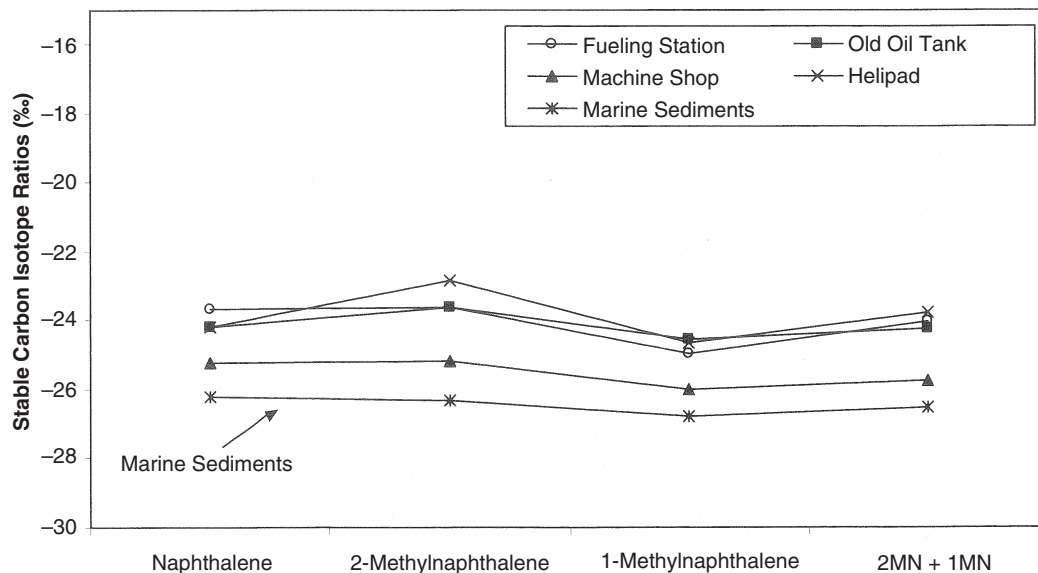
Sohxlet extraction is often the first procedure in isolating oil adsorbed onto solid material. O'Malley et al. (1994) showed this procedure did not cause carbon isotopic fractionation in PAHs.

### 6.5 Weathering

The resistance of stable isotope ratios to alteration by weathering is considered a major advantage of this technique in correlating spilled oil to possible sources. Many studies support this conclusion. Macko et al. (1981) followed the degradation of crude oil spilled in a salt marsh in Texas under weathering conditions described as harsh. Over a period of 2 years, the  $\delta^{13}\text{C}$  values of the aliphatic and aromatic fractions of the spilled oil changed by only about 0.5‰ compared to the unweathered oil. The authors considered these changes to be small enough to allow the use of carbon isotope ratios to correlate spilled oils. Several studies have shown that degradation has little effect on carbon isotope ratios of high-molecular-weight compounds (Sun et al., 2005, and references therein). O'Malley et al. (1994) reported that there was no significant isotopic fractionation in 2-5-ring parent PAHs. However, Yanik et al. (2003) reported significant isotopic changes in alkylated naphthalenes and phenanthrenes, but not dibenzothiophenes. BJORØY et al. (1994) found that evaporation did not cause significant changes in the carbon isotope ratios of  $\text{C}_4$ - $\text{C}_{20}$  hydrocarbons in oils and condensates.



**Figure 6-10** Full scan GC/MS chromatograms of a sediment extract after sequential purification procedures. (A) Al/Si column chromatography (B) High performance liquid chromatography (C) Thin-layer chromatography. (Reprinted with permission from Kim et al. (2005). Copyright (2005) American Chemical Society.)



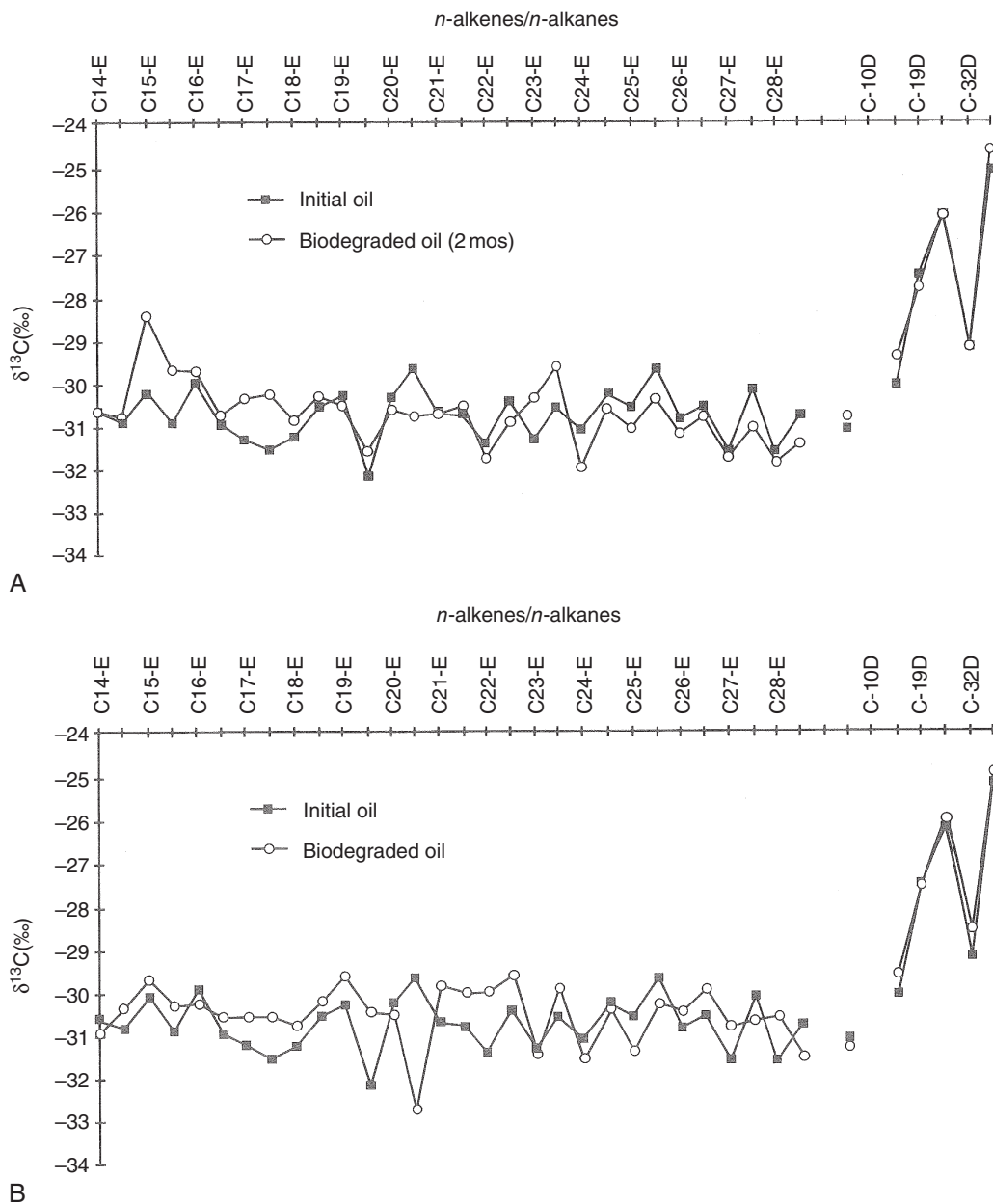
**Figure 6-11** Carbon isotope ratios of individual naphthalenes and methylnaphthalenes in soils and sediments from McMurdo Station, Antarctica. (Reprinted with permission from Kim et al. (2005). Copyright (2005) American Chemical Society.)

Neither evaporation nor partitioning into water of jet fuel hydrocarbons ( $C_{10}$ – $C_{16}$ ) leads to significant isotopic fractionation of the hydrocarbons (Harrington et al., 1999). In addition, Bugna et al. (2004) found that aqueous migration and aerobic degradation of jet fuel hydrocarbons did not result in significant isotopic fractionation. Even most light hydrocarbons in the gasoline range show little carbon isotopic change during water-washing and evaporation (Smallwood et al., 2002). Most of the hydrocarbons in gasoline that show significant isotopic changes are aromatic hydrocarbons, which are not major constituents of most oils.

Mansuy et al. (1997) artificially weathered separate samples of a crude oil by evaporation, water-washing, and biodegradation. After evaporation at room temperature for 4 years, *n*-alkanes below  $C_{14}$  were depleted; after water-washing at room temperature for 38 days, alkanes below  $C_{15}$  were depleted; and after biodegradation for 4 months, *n*-alkanes were completely degraded. The carbon isotope ratios of *n*-alkanes and isoprenoids in the evaporated and water-washed oils correlate well with the unweathered oil. In the oil bio-

degraded for 4 months, the lack of alkanes prevented isotopic comparison. After 2 months' biodegradation, however, some *n*-alkanes and isoprenoids remained, many of which showed a shift to heavier ratios compared to the unweathered oil. This was attributed to interference from the background unresolved complex mixture, which was enhanced in the biodegraded oil. This was shown by isolating *n*-alkanes in the biodegraded oil by urea adduction. The  $\delta^{13}C$  values of the purified *n*-alkanes in the biodegraded oil corresponded more closely with the values in the unweathered oil.

The complete depletion of *n*-alkanes in the oil biodegraded for 4 months highlights a major difficulty with CSIA of severely biodegraded oils. To circumvent this, Mansuy et al. (1997) pyrolyzed the asphaltene fractions of the biodegraded oils to generate *n*-alkanes. The asphaltene fraction is believed to be relatively unaffected by weathering and was used by Hartman and Hammond (1981) to correlate Southern California beach tars with probable source oils based on bulk isotope ratios. Biomarker concentrations in asphaltene pyrolysates are normally low, so correlation is



**Figure 6-12** Carbon isotope ratios of individual *n*-alkane/*n*-alkene pairs generated by pyrolysing asphaltene fractions of biodegraded oils. These are compared with ratios of individual *n*-alkanes in the undergraded (initial) oils. (Reprinted with permission from Mansuy et al. (1997). Copyright (1997) American Chemical Society.)

limited to *n*-alkane/*n*-alkene pairs. As shown in Figure 6-12, there was good carbon isotope correlation between the severely biodegraded oils and the unweathered oil. This study shows that CSIA can be used to correlate even severely biodegraded oils with potential

sources. There is evidence that some components of petroleum are changed isotopically by weathering. Palmer (1984) and Sofer (1984) reported a maximum change of 2‰ in the bulk saturate fraction of a marine oil after complete loss of *n*-alkanes. Philp (2002) also cautions

that loss of *n*-alkanes during biodegradation can result in heavier carbon isotope ratios in the saturate fraction of biodegraded oils.

The evidence to date indicates that weathering does not have a significant effect on the bulk carbon isotope ratios of oils, although biodegradation may have a disproportionate isotopic effect on saturate fractions of oil, shifting isotope ratios to heavier values. The carbon isotope ratios of individual alkanes in oil appear to be resistant to the effects of weathering. This confirms the value of stable isotope ratios, and CSIA in particular, in oil spill studies.

## 6.6 Other Isotopes

One drawback in the use of carbon isotope ratios to correlate oils, spilled or otherwise, is their relatively narrow natural range of around 15‰. Hydrogen isotope ratios, in contrast, show a much larger isotopic range of up to 160‰ (Li et al., 2001). CSIA of hydrogen isotope ratios has been developed fairly recently (Burgoyne and Hays, 1998; Hilkert et al., 1999) and was applied by Li et al. (2001) to *n*-alkanes and isoprenoids in a number of oils from western Canada. Isotope ratios of *n*-alkanes were fairly similar and differed considerably from the isoprenoids, pristane and phytane. This was attributed to the different origins of *n*-alkanes and isoprenoids during oil formation. The origin of formation water in oil source rocks was also found to have a major influence on the hydrogen isotope ratios. In a study of terrestrially sourced oils from Australia, however, Schimmelmann et al. (2004) found a progressive change in hydrogen isotope ratios with *n*-alkane size, and little difference between *n*-alkanes and isoprenoids. The reason for the differences between the two studies is unclear, but it may be related to the depositional environment of the source rocks.

Pond et al. (2002) showed that the hydrogen isotope ratios of *n*-alkanes in the range C<sub>15</sub>–C<sub>18</sub> were shifted to heavier values during biodegradation and could be used to monitor bioremediation of crude oil. High-molecular-weight

*n*-alkanes (C<sub>19</sub>–C<sub>27</sub>) were relatively unaffected during biodegradation and could be used for correlation of crude oils. This should also be a useful technique for identifying the source of spilled oil.

Sulfur isotope ratios have found use in bulk isotope studies (Hartman and Hammond, 1981). However, CSIA applications have not been reported. This would seem to be a useful approach in studies of spilled oil, as sulfur is often concentrated in weathered oils. Stable isotope ratios of oxygen and nitrogen, although used extensively in other areas of geochemistry (Brand et al., 1994), have found little use in spilled oil studies. The low concentrations of these elements in petroleum will continue to limit their use.

## 6.7 Conclusions

Stable isotope ratios have had a long history of use in the identification of the sources of spilled oil and tars. Bulk isotope analysis is relatively quick and low cost and is still useful as a screening technique for these reasons. However, bulk analysis has been largely superseded by the compound-specific isotopic analysis of individual alkanes and aromatic hydrocarbons in oil. By focusing on individual hydrocarbons, CSIA avoids the effects of sample contamination on bulk ratios. Contamination is a potential problem, particularly for carbon, with materials deposited in an environment with multiple sources of extraneous carbon. Advances in the sensitivity of CSIA techniques allow carbon isotope ratios of the low concentrations of petroleum hydrocarbons dissolved in water to be measured reliably. Coelution of target peaks remains a complication of CSIA and has to be monitored to avoid inconsistent results.

The effects of weathering on the carbon isotope ratios of alkanes appear to be minimal, and compound-specific isotope analysis of these hydrocarbons are now widely used to identify spill sources. Hydrogen isotope ratios of high-molecular-weight *n*-alkanes also appear to be minimally affected by weathering. Compound-specific hydrogen isotope analysis

of alkanes offers more resolution than carbon because of the wider  $\delta D$  range, and this technique offers promise for future studies.

Stable isotope ratios may provide the only reliable way of correlating light oils that lack conventional marker compounds like steranes and terpanes. However, many of the studies discussed in this chapter highlight the value of stable isotope ratios as one component in identifying the source of spilled oil.

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