# $\delta^{13}C$ values of polycyclic aromatic hydrocarbons collected from two creosote-contaminated sites

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## Abstract

Groundwaters were sampled on two dates from several wells at each of two creosote-contaminated waste sites in Florida. Polycyclic aromatic hydrocarbons (PAHs) were extracted from the groundwaters, and their individual concentrations were measured by gas chromatography/flame ionization detection (GC/FID). The $\delta^{13}C$ values of the PAHs were then determined by gas chromatography/ion trap mass spectrometry/isotope ratio mass spectrometry (GC/ITMS/IRMS). At the American Creosote Works (ACW) in Pensacola, concentrations of PAHs were found to decrease by over four orders-of-magnitude, both with increasing depth and with increasing distance from the most contaminated area. At a wood-preserving facility in Gainesville, concentrations were also found to decrease with increasing distance from the most contaminated area. At the ACW site, $\delta^{13}C$ values of individual PAHs ranged from $-20.09‰$ to $-32.94‰$, although the majority of compounds fell in a tighter range between $-22.66‰$ and $-25.31‰$. The $\delta^{13}C$ values of over 75% of the PAHs remained constant across all wells, both with migration of the contaminant plume and over a 3-month time period. The compounds that showed the highest variability among the wells were anthracene; the heterocyclic compounds thianaphthene, dibenzothiophene, and carbazole; and the lighter PAHs naphthalene, biphenyl, and 2-methylnaphthalene. Variability of these compounds is likely the result of variations in $\delta^{13}C$ values among different creosotes added to the sites over many years. The other compounds measured were conserved across the wells and would serve as good tracers of a contaminant plume in bioremediation settings. At the Gainesville site, $\delta^{13}C$ values of individual PAHs ranged from $-18.87‰$ to $-27.05‰$, with 70% of the values falling between $-22.06‰$ and $-24.53‰$. This range is very similar to the values for PAHs at the ACW site. Comparing $\delta^{13}C$ values of specific PAHs between the two creosote-contaminated sites, 12 of 16 compounds agreed within 1.0‰. This indicates that, although there are a few compounds that may be variable across different creosotes, there may be a suite of $\delta^{13}C$ values that is conserved across PAHs of creosote origin. These characteristic PAHs could be used to

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determine whether or not creosote is contributing to the PAH contamination at a site. In addition, the compounds that are variable between different creosotes could be used as tracers of individual creosotes at polluted sites and to differentiate between possible creosote sources. © 1998 Elsevier Science B.V. All rights reserved.

*Keywords:* $\delta^{13}C$; Polycyclic aromatic hydrocarbon; Creosote-contaminated site

## 1. Introduction

Although a few isotopic investigations have been conducted on individual compounds or classes of compounds isolated from bulk samples (e.g., Parker, 1964; Des Marais et al., 1980; Macko et al., 1990; Hare et al., 1991), stable isotope studies historically involve the combustion of whole organisms or tissues. Bulk measurements may effectively mask a great deal of information contained at the molecular level. Matthews and Hayes (1978) were the first to interface a gas chromatograph to an isotope ratio mass spectrometer via a 750°C cupric oxide-packed combustion furnace. It has been only recently, however, that this instrumentation has become commercially available. The development of so-called gas chromatograph/combustion/isotope ratio mass spectrometers (GC/C/IRMS or GC/IRMS) now provides the ability for researchers to determine the isotope ratios of individual molecules within various classes of compounds (e.g., Freeman et al., 1990; Hayes et al., 1990; Rieley et al., 1991; Silfer et al., 1991; Goodman and Brenna, 1992; O'Malley et al., 1994; CoBabe and Pratt, 1995).

The capability of the GC/IRMS system permits the resolution and determination of $\delta^{13}C$ values of complex mixtures of hydrocarbon pollutants, such as polycyclic aromatic hydrocarbons (PAHs). The $\delta^{13}C$ values of individual PAHs have been measured in such samples as roadsweeps, car soot, fireplace soot, and sewage (O'Malley et al., 1994). These different PAH sources were found to have distinctive suites of $\delta^{13}C$ values which could serve as indicators of their presence in contaminated environments, thereby allowing for source apportionment. There are limitations, however, to the GC/IRMS technique. The accuracy and precision of $\delta^{13}C$ values obtained by GC/IRMS for individual compounds are affected mainly by the degree of resolution of coeluting compounds and the nature and extent of the unre-

solved complex mixture that is inherent in many environmental samples (O'Malley et al., 1994). In a recent modification, an ion trap mass spectrometer (ITMS) has been placed in-line with the GC/IRMS, creating a GC/ITMS/IRMS. Rather than having an isotope ratio mass spectrometer as the sole detector, in the new GC/ITMS/IRMS system, the column effluent is split between the ITMS, where compound identification and verification of purity are obtained, and the IRMS, where isotopic composition is measured. This configuration allows the investigator, in the same sample acquisition, to verify the identity of a specific compound and to determine if there are any coeluting compounds contributing to a peak which otherwise appears to be well-resolved. This capability is especially helpful in the analysis of complex environmental samples such as those found in contaminated areas.

The PAH contamination constitutes one of the world's most widespread environmental problems. In many cases, in situ bioremediation represents the only viable remedial solution. A challenge in the bioremediation of contaminated field sites is establishing that organisms are actually effecting the cleanup of the site (U.S. Environmental Protection Agency, 1994), because decreases in contaminant levels may only be due to transport of the pollutants to a new location or to the partitioning of the pollutants into an immobile phase. A potential tool for proving that microorganisms are indeed remediating contaminated sites is stable carbon isotope analysis. It has been well-established that consumers have close carbon isotopic similarities to their diets (Parker, 1964; DeNiro and Epstein, 1978; Fry et al., 1978; Fry and Sherr, 1984). Furthermore, the use of stable isotope analyses for tracing carbon sources into bacterial biomass has been successful in aqueous environments (Coffin et al., 1989, 1990). In bioremediation settings, if bacteria are consuming one or more pollutant compounds, their biomass

should have a carbon isotopic signal that is similar to that of the pollutant(s). Similarly, the $CO_2$ they respire should also have the same carbon isotopic signal (Trust et al., 1995). By measuring the $\delta^{13}C$ values of individual PAHs, background indigenous carbon sources, bacterial biomass, and respired $CO_2$, the flux of contaminant carbon through bacterial biomass can be traced. Also, the percentage of contaminant vs. indigenous carbon assimilated by the bacteria can be determined. Thus, the use of natural abundance stable isotopes may be a powerful tool in developing bioremediation strategies and evaluating their efficacies.

In order to determine the usefulness of stable carbon isotopes in bioremediation programs where the flow of contaminant carbon into bacterial biomass and respired $CO_2$ needs to be verified, it first needs to be established that the $\delta^{13}C$ values of a specific contaminant source are conserved over time periods relevant to bioremedial efforts (a few months to a few years) and with migration of the contaminant plume through a groundwater aquifer. Here, we report the $\delta^{13}C$ values of PAHs extracted from groundwaters collected at two creosote-contaminated sites. We provide evidence that the $\delta^{13}C$ values of a large number of PAHs are conserved over time and with migration through a groundwater aquifer. Furthermore, $\delta^{13}C$ values of individual PAHs were found to be similar between two sites, indicating that there may be a suite of $\delta^{13}C$ values, i.e., an 'isotopic fingerprint', that is characteristic of PAHs in creosote. If this isotopic fingerprint of $\delta^{13}C$ values is found to be unique to PAHs of creosote origin, then by measuring $\delta^{13}C$ values of PAHs found in plumes where the source is unknown, it may be possible to determine whether the contamination is from a creosote or closely related source.

## 2. Materials and methods

### 2.1. Sampling

Groundwater samples were obtained on June 8, 1994 and September 9, 1994 from the American Creosote Works (ACW) in Pensacola, FL. This site is an abandoned wood-preserving facility that was in operation from 1902 to 1981 and has been described

previously (Middaugh et al., 1991; Mueller et al., 1991). The sampling wells were located along a transect leading away from the most contaminated area. ACW Wells 340, 360 and 380 were situated immediately adjacent to the most contaminated area at depths of 40 ft (12.2 m), 60 ft (18.3 m) and 77 ft (23.5 m), respectively. Wells 480 and 400 were located about 125 m downgradient of the contaminated area at depths of 80 ft (24.4 m) and 100 ft (30.5 m), respectively. Well 760 was the farthest downgradient, located approximately 275 m from the most contaminated area at a depth of 64 ft (19.5 m). See site locations in Middaugh et al. (1991) for further description.

Groundwater samples also were collected from the Cabot Carbon/Koppers wood-preserving facility in Gainesville, FL on February 6, 1995 and on May 31, 1995. Like the ACW site, this site is heavily impacted by coal tar. Since the mid-1920s, the former Cabot Carbon operated a 34-acre (0.14 km$^2$) pine tar and charcoal generation facility, both of which are now discontinued. During the same time, the Kopper's Industries plant occupied approximately 90 acres (0.36 km$^2$) as a wood treatment facility. Historically, the facility used creosote, pentachlorophenol, and chromated copper arsenate (CCA) to preserve wood utility poles and timbers. The facility continues to operate, but using only CCA on site. Multiple process and storage areas associated with past facility operations have been identified as potential sources of organic wood-preserving constituents. Specifically, the former north lagoon area (which has since been filled and levelled) has been identified as a potential source of creosote constituents, with free product being detected (Fig. 1). As such, a UVB groundwater circulation system (Borchert and Sick, 1992; Herrling et al., 1993; U.S. Patent Office, 1992a,b) and 12 monitoring wells were installed in January 1995 as part of a project to determine the effectiveness of in situ bioremediation for cleaning up PAHs. Samples were collected from two depths (3.7 m and 6.1 m below ground surface) at each of the 12 monitoring wells. Wells 1, 2, 3, 10, 11, and 12 were located on a transect (Transect 1) leading away from the most contaminated area. Wells 4 through 9 were located in another transect (Transect 2) perpendicular to the first transect, crossing between Wells 3 and 10. After
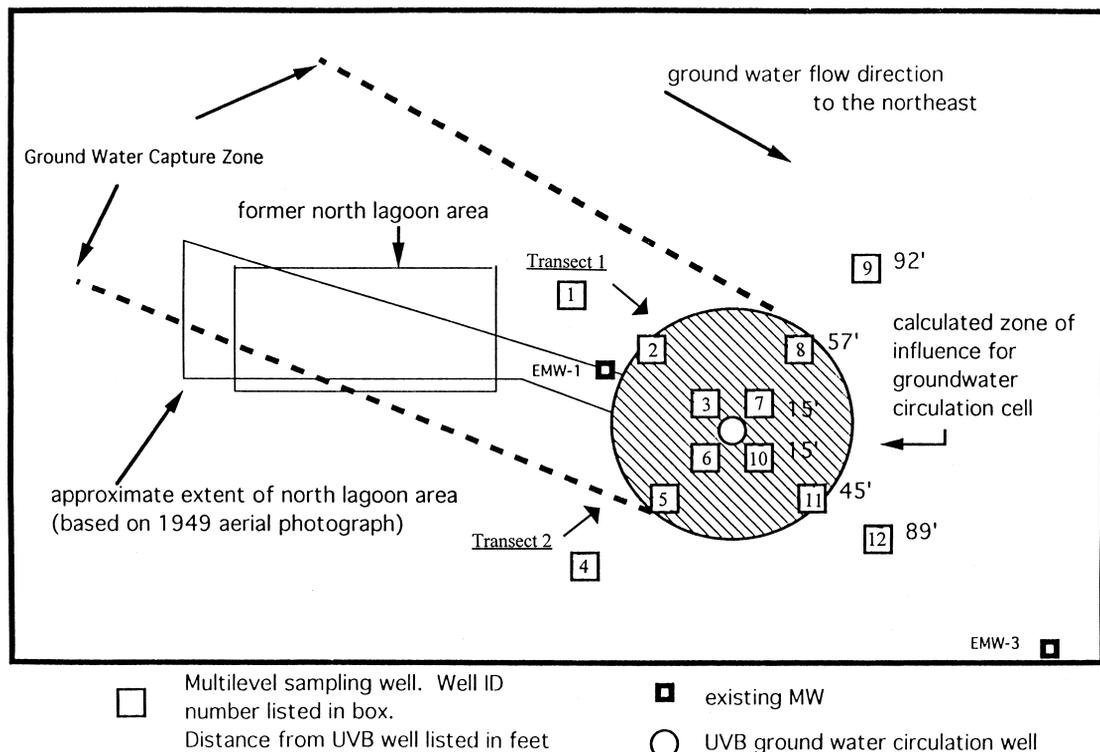
Fig. 1. Locations of sampling wells at the Cabot Carbon/Koppers facility in Gainesville, FL.

the initial groundwater sampling on February 6, 1995, the groundwater circulation system was started on February 16, 1995, so that it had run for approximately 15 weeks before the second sampling on May 31, 1995.

At the Pensacola site, groundwater samples for stable isotope analysis were pumped from the wells through 1/4 in. i.d. Teflon® tubing into glass bottles fitted with Teflon-lined caps. [4] At the Gainesville site, samples were pumped through 1/4 in. i.d. stainless steel tubing. The wells were purged by pumping a volume of water equivalent to three well-volumes before collecting approximately 500 ml for analysis. Samples were preserved with NaOH (pH = 10) and kept refrigerated at 4°C until they could be extracted for analyses of concentrations and carbon isotope ratios of PAHs.

## 2.2. Analysis

The PAHs were extracted from groundwaters according to EPA's SW-846 Method 3510A (Separatory Funnel Liquid–Liquid Extraction). The extraction solvent was methylene chloride. All extracts were dried over anhydrous sodium sulfate and concentrated to a final volume of 1 ml by gently evaporating the sample with $N_2$ gas. Concentrations of PAHs were analysed on a Hewlett-Packard 5890 Gas Chromatograph using flame ionization detection (GC/FID). The GC was equipped with an HP-5 column (25 m, 0.32 mm i.d., 0.17 μm film thickness). The carrier gas was helium-maintained at a flow rate of 1 ml/min. The injector temperature was 290°C, and the detector temperature was 315°C. The initial column oven temperature was 50°C, which was maintained for 2 min. The temperature was then ramped to 100°C at a rate of 25°C/min, and then to a final temperature of 310°C at a rate of 5°C/min, where it was held for 6 min. Concentrations of PAHs

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[4] Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

were calculated using an external calibration curve generated from standards of known concentration run under the same conditions. Analyses of random samples in triplicate revealed a precision of $\pm 0.1$ mg/l for all the PAHs measured in this study.

The $\delta^{13}C$ values of the PAHs extracted from the groundwater samples were measured using a novel GC/ITMS/IRMS system. This system consists of three parts: (1) a Varian Star 3400 CX gas chromatograph, (2) a Finnigan MAT Magnum ion trap mass spectrometer (ITMS), and (3) a Finnigan MAT Delta S isotope ratio mass spectrometer (IRMS). The GC was equipped with a Hewlett-Packard Ultra 2 column (50 m, 0.32 mm i.d., 0.52 $\mu$m film thickness). Carrier gas conditions and GC temperatures were the same as for the GC/FID. As each compound eluted from the GC column, 10% of the effluent was carried through a 290°C heated transfer line into the ion trap mass spectrometer for confirmation of compound identification and purity. The remaining 90% of the effluent passed through a 940°C combustion furnace packed with cupric oxide, platinum and nickel, where carbon constituents were converted quantitatively to $CO_2$ gas. Co-generated water vapor was removed via a Nafion® elimination trap, and interfering nitrogen oxides were reduced to $N_2$ gas via a 600°C reduction column. The purified $CO_2$ was swept into the isotope ratio mass spectrometer, where its carbon isotope composition was measured. All isotope data are reported using the conventional $\delta^{13}C$ notation:

$$\delta^{13}C_{PDB}(‰) = \left[\left(R_{sample}/R_{std}\right) - 1\right] \times 1000,$$

where $R_{sample}$ and $R_{std}$ are the $^{13}C/^{12}C$ isotope ratios corresponding to the sample and the conventional PeeDee Belemnite (PDB) carbonate standard, respectively (Craig, 1957).

In order to evaluate the precision and accuracy of the GC/ITMS/IRMS for determining $\delta^{13}C$ values of PAHs, the $\delta^{13}C$ values of 10 individual PAHs were first measured in either duplicate or triplicate using conventional isotope ratio mass spectrometry. Following the method described by Macko (1981), individual PAHs were placed into pre-combusted quartz tubes. Copper wire (5 g) and CuO (2.5 g) were added to the samples, and the tubes were evacuated and sealed. The tubes were heated to

850°C for 2 h to generate $CO_2$ gas, which was isolated cryogenically on a vacuum line. The carbon isotope content of the $CO_2$ was measured on a Finnigan MAT 252 isotope ratio mass spectrometer having a precision better than $\pm 0.1‰$ for replicate analyses of a combusted sample. The individual PAHs were then dissolved into methylene chloride to make a standard solution containing all 10 PAHs. This mixture was injected into the GC/ITMS/IRMS at varying concentrations to yield major ion beam (i.e., mass 44) signals ranging from 0.1 to 8.1 V on the IRMS. The $^{13}C$ value of each PAH was determined 54 times over a period of several months.

## 3. Results

### 3.1. Precision and accuracy of the GC / ITMS / IRMS system

The $\delta^{13}C$ values of the 10 standard PAHs as determined by GC/ITMS/IRMS are compared to those produced by the conventional IRMS method in Fig. 2. Although it had lower precision than the
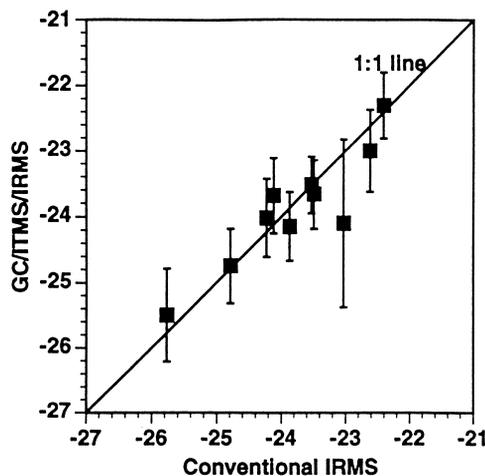


Fig. 2. Comparison of $\delta^{13}C$ values of standard PAHs measured by conventional IRMS and GC/ITMS/IRMS. Error bars represent $\pm 1$ standard deviation. Horizontal error bars for the conventional IRMS values are $< 0.1‰$; therefore, they are hidden by the symbol at each point. Compounds listed from left to right on the *x*-axis are phenanthrene, pyrene, fluoranthene, anthraquinone, anthracene, fluorene, acenaphthene, 2,6-dimethylnaphthalene, 2,3-dimethylnaphthalene, and acenaphthylene.

conventional IRMS method, as indicated by the error bars ($\pm 1$ standard deviation), the GC/ITMS/IRMS method had a high degree of accuracy. The values produced by this technique for PAHs were not significantly different from those produced by the conventional IRMS method ($p < 0.05$, Student's *t*-test). The $\delta^{13}C$ values of the PAHs, as determined by GC/ITMS/IRMS, agreed within 1.1‰ of the values produced by the conventional IRMS method. In fact, for eight out of the 10 PAHs, the two methods produced $\delta^{13}C$ values that agreed within 0.4‰ or better of each other. The good agreement of the GC/ITMS/IRMS method with the conventional IRMS method instills a high degree of confidence in this technique. This is especially exciting because the GC/ITMS/IRMS method, like regular GC/IRMS, can be used on much smaller sample sizes than the conventional IRMS method. The conventional IRMS method typically requires a sample size on the order of 10 $\mu$mol of carbon, whereas the GC/IRMS methods can be performed on nanomolar quantities of material. Furthermore, the accuracy of the GC/ITMS/IRMS method proves that there is no fractionation of carbon isotopes as the sample is split between the two mass spectrometers for analysis.

## 3.2. Concentrations and $\delta^{13}C$ values of PAHS at the ACW site

Concentrations of 21 PAHs extracted from groundwaters collected at the ACW site both on June 8, 1994 and September 9, 1994 were found to decrease with increasing distance from the known source area. Fig. 3 shows a plot of the PAH concentrations measured by GC/FID for groundwaters collected on September 9, 1994. Well 340, the well closest to the source, had the highest concentrations of PAHs, ranging from 1079 to 45,790 mg/l (1.1 to 45.8 g/l) for individual compounds. The compound found in the largest concentration was phenanthrene, followed by naphthalene. Wells 360 and 380, located immediately adjacent to Well 340 but at increasingly greater depth, had concentrations of individual PAHs that were over four orders-of magnitude less than at Well 340. Concentrations for individual PAHs at Well 360 ranged from 0.1 to 6.3 mg/l and from 0.1 to 4.9 mg/l at Well 380. Phenanthrene and naphthalene were again the most abundant PAHs in these

wells. Moving downstream from the most contaminated area, concentrations of individual PAHs again were over four orders-of-magnitude less than at the source area, measuring from undetectable levels to a maximum of 1.2 mg/l at Well 480 and a maximum of 0.7 mg/l at Well 760. Concentrations of individual PAHs at the previous June 1994 sampling were comparable to those of the September 1994 sampling. On this date, PAH concentrations at Well 400, which was not sampled in September, measured from undetectable levels to a maximum of only 31 $\mu$g/l.

Although concentrations of individual PAHs decreased dramatically across the wells, the $\delta^{13}C$ values of the majority of PAHs did not vary greatly from one well to the next on either sampling date. The $\delta^{13}C$ values of the individual compounds measured at the ACW site in both June and September 1994 are reported in Table 1. Values for the different compounds varied from $-20.09$‰ for acenaphthylene to $-32.94$‰ for dibenzothiophene. The majority of the values (100 out of 130 measurements), however, fell in a tighter range between $-22.66$‰ and $-25.31$‰.

Comparing the $\delta^{13}C$ values of any one compound across the three '300' wells, only thianaphthene, dibenzothiophene, and anthracene varied by more than 1.3‰ on both sampling dates. Of these three compounds, only anthracene was statistically different ($p < 0.05$, Student's *t*-test) for both dates. The remaining 12 compounds for which $\delta^{13}C$ values could be measured at two or more wells did not vary to a significant degree. Although four compounds (the dimethylnaphthalenes, carbazole and biphenyl) varied across the '300' wells by more than 1.3‰ on one of the two dates, each compound varied much less ($< 1.0$‰) on the other date (2,6-dimethylnaphthalene varied the greatest on the September 1994 sampling date, whereas the other three compounds varied the greatest on the June 1994 sampling date). These results indicate that $\delta^{13}C$ values of the majority of PAHs are conserved with depth, i.e., vertical migration through an aquifer.

Results also indicate that $\delta^{13}C$ values of PAHs are conserved as they move horizontally through the aquifer via groundwater flow. In a comparison of the wells moving away from the source area, biphenyl and 1-methylnaphthalene had $\delta^{13}C$ values that dif-
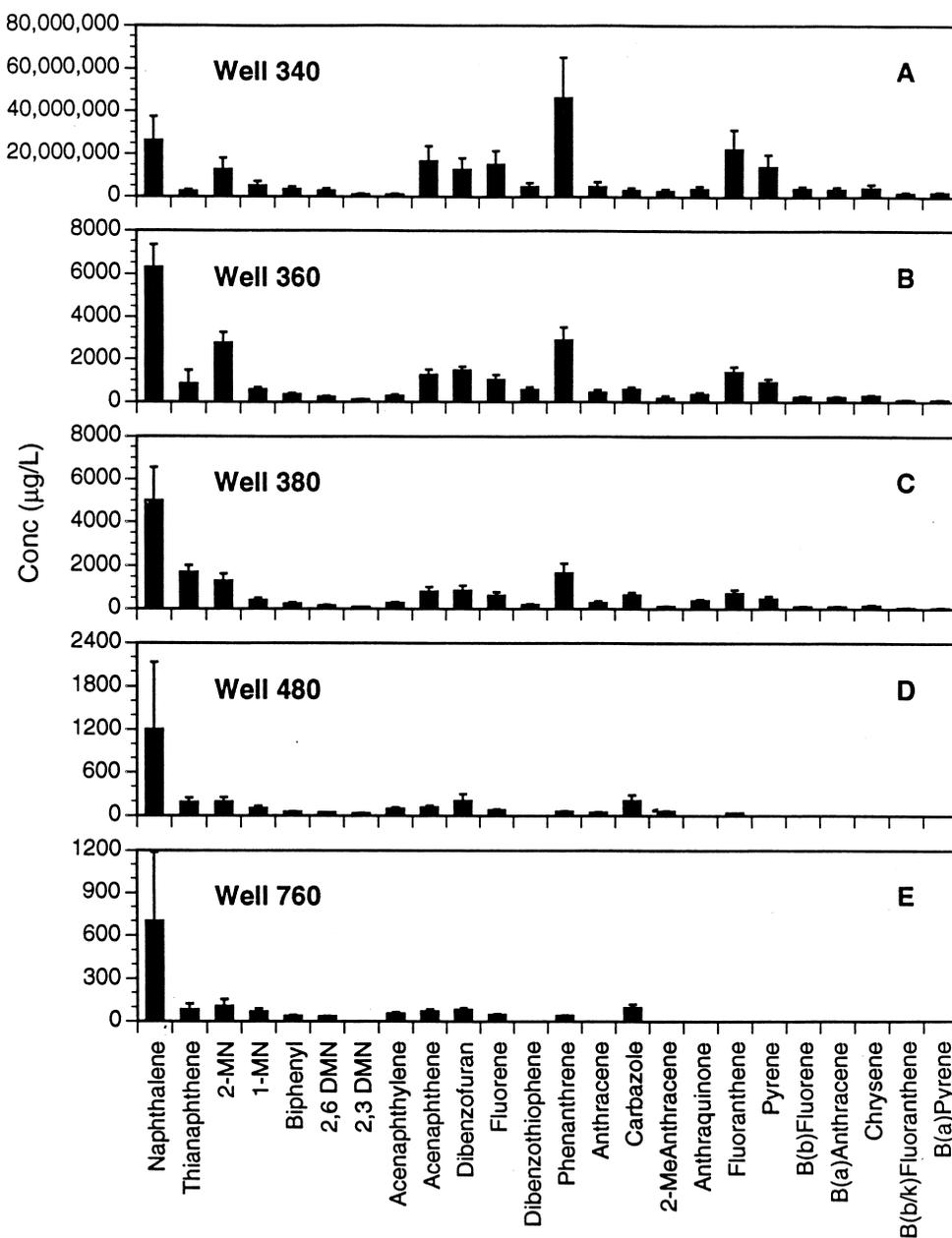
Fig. 3. Concentrations of PAHs in groundwaters collected from ACW Wells on September 9, 1994. The panels (A through E) are in order of increasing distance from the source of contamination. Error bars represent the standard deviation of triplicate samples. Note the changes in scale on the concentrations axes. The PAH compounds are listed in the order that they elute from the GC column.

fered the most between the wells on the September 9, 1994 sampling date. Whereas biphenyl had $\delta^{13}C$ values of $-20.65$ to $-21.16‰$ at the '300' wells, this compound was depleted in $^{13}C$ downstream at

Well 760, measuring $-27.48‰$. This value for biphenyl at Well 760 was statistically different from all three '300' wells ($p < 0.01$, Student's $t$-test). Similarly, 1-methylnaphthalene measured $-23.48$ to

Table 1
The $\delta^{13}C$ values of PAHs extracted from groundwaters collected at the American Creosote Works (ACW) in June and September 1994

| Compound | $\delta^{13}C$ (‰) | | | | | | | | | | | | All Well average[a] | | Total average[b] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Well 340 | | Well 360 | | Well 380 | | Well 400 | | Well 480 | | Well 760 | | | | |
| | 6/94 | 9/94 | 6/94 | 9/94 | 6/94 | 9/94 | 6/94 | 9/94 | 6/94 | 9/94 | 6/94 | 9/94 | 6/94 | 9/94 | |
| Naphthalene | −24.72 | −25.07 | −24.73 | −24.41 | −24.29 | −24.35 | −24.45 | | | −23.56 | | −24.45 | −24.55 | −24.37 | −24.45 |
| Thianaphthene | −24.01 | −24.21 | −24.77 | −24.00 | −23.01 | −26.93 | −21.50 | | | −23.47 | | −23.57 | −23.32 | −24.44 | −23.94 |
| 2-Methylnaphthalene | −24.00 | −24.24 | −24.60 | −24.11 | −23.97 | −23.46 | −21.76 | | | −23.31 | | −23.49 | −23.58 | −23.72 | −23.66 |
| 1-methylnaphthalene | −23.48 | −24.04 | −23.61 | −23.48 | −24.01 | −24.80 | −31.27 | | | −26.59 | | −28.63 | −25.59 | −25.51 | −25.55 |
| Biphenyl | −22.72 | −20.65 | −21.19 | −21.12 | −23.77 | −21.16 | −23.71 | | | – | | −27.48 | −22.85 | −22.60 | −22.73 |
| 2,6-Dimethylnaphthalene | −23.91 | −24.26 | −23.22 | −23.95 | −24.24 | −21.59 | – | | | – | | – | −23.79 | −23.27 | −23.53 |
| 2,3-dimethylnaphthalene | −22.34 | −24.96 | −24.60 | −25.58 | −21.50 | – | – | | | – | | – | −22.81 | −25.27 | −23.80 |
| Acenaphthylene | −20.09 | – | – | – | – | – | – | | | – | | −22.90 | −20.09 | −22.90 | −21.50 |
| Acenaphthene | −23.72 | −24.10 | −23.47 | −23.73 | −23.53 | −23.48 | −22.52 | | | −23.15 | | −22.97 | −23.31 | −23.49 | −23.41 |
| Dibenzofuran | −23.65 | −24.09 | −23.42 | −23.31 | −23.51 | −22.88 | −22.66 | | | −27.04 | | −23.36 | −23.31 | −24.14 | −23.77 |
| Fluorene | −23.88 | −24.05 | −24.35 | −25.30 | −23.98 | −23.99 | −22.53 | | | −23.45 | | −23.96 | −23.69 | −24.15 | −23.94 |
| Dibenzothiophene | −26.79 | −30.61 | – | −32.94 | −30.76 | −28.91 | – | | | – | | – | −28.78 | −30.82 | −30.00 |
| Phenanthrene | −24.41 | −23.95 | −23.88 | −24.27 | −24.20 | −23.89 | −23.44 | | | −22.14 | | −23.25 | −23.98 | −23.50 | −23.71 |
| Anthracene | −24.24 | −23.32 | −22.82 | −20.66 | −23.83 | −23.42 | – | | | – | | – | −23.63 | −22.47 | −23.05 |
| Carbazole | −28.16 | – | −25.61 | −24.26 | −25.77 | −23.43 | – | | | −23.20 | | −24.26 | −26.51 | −23.79 | −24.96 |
| 2-Methylanthracene | −24.82 | – | – | – | – | – | – | | | – | | – | −24.82 | – | −24.82 |
| Anthraquinone | – | – | – | – | – | −26.89 | – | | | – | | – | – | −26.89 | −26.89 |
| Fluoranthene | −24.11 | −23.78 | −23.58 | −24.37 | −23.69 | −23.50 | −25.04 | | | – | | – | −24.11 | −23.88 | −24.01 |
| Pyrene | −23.65 | −23.57 | −23.43 | −23.91 | −23.96 | −23.20 | – | | | – | | – | −23.68 | −23.56 | −23.62 |
| Benz(a)anthracene | −23.41 | – | – | −22.69 | – | – | – | | | – | | – | −23.41 | −22.69 | −23.05 |
| Chrysene | −26.72 | – | – | −24.70 | – | – | – | | | – | | – | −26.72 | −24.70 | −25.71 |

[a]All Well average = average of all the wells on that date.
[b]Total average = average of All Well average 6/94 and All Well average 9/94.

−24.80‰ at the '300' wells on September 9, 1994 but was a more negative −28.63‰ at Well 760 on the same date. However, the value for 1-methylnaphthalene at Well 760 was only significantly different from the value at Well 360 ($p < 0.05$, Student's $t$-test). Besides biphenyl, only three other compounds differed significantly between the '300' wells and either Well 480 or 760 ($p < 0.05$, Student's $t$-test). These were 2-methylnaphthalene, acenaphthene, and phenanthrene. Only naphthalene was significantly different between Well 480 and Well 760 ($p < 0.05$, Student's $t$-test).

There was one well at the ACW site that had distinct $\delta^{13}C$ values of PAHs. On the June 8, 1994 sampling date, $\delta^{13}C$ values for eight of the 10 compounds at Well 400 were statistically different ($p < 0.05$, Student's $t$-test) from at least two of the three '300' wells measured on the same date. The only two compounds that were not different between the '300' wells and Well 400 were naphthalene and biphenyl. As mentioned above, the '300' wells did not differ isotopically from Wells 480 or 760 for the majority of compounds at the September 1994 sampling, so it is interesting that Well 400, which is situated close to Well 480, albeit 6.1 m deeper, was statistically different from the '300' wells at the June sampling. It is possible that there was a second source of PAHs to Well 400 that did not contribute to the contamination at the other wells. In order to determine if Well 400 was unique from all wells, Well 400 was also compared to Wells 480 and 760, even though they were not sampled on the same date. In this comparison, only thianapthene and 2-methylnaphthalene differed between Well 400 and both Well 480 and Well 760. Furthermore, these two compounds were not found to differ between the '300' wells and Wells 480 and 760 when they all were sampled on September 9, 1994. All of these information together make it difficult to conclude that a secondary source of PAHs was contributing to the contamination in Well 400.

Because the majority of PAHs for which $\delta^{13}C$ values were obtained were not statistically different between the wells at the ACW site, the values for each individual compound were averaged across the wells to produce an 'All Well' average for each compound on both the June and September 1994 sampling dates (Table 1). In order to determine if the $\delta^{13}C$ values of the individual PAHs changed over the 3-month period of time between samplings, the 'All Well' averages for each compound were compared statistically between the two dates. Of all the compounds, only carbazole differed between the two dates ($p < 0.05$, Student's $t$-test).

### 3.3. Concentrations and $\delta^{13}C$ values of PAHs at the Gainesville site

Concentrations of 24 PAHs were measured in groundwaters collected from shallow (3.7 m below ground surface) and deep (6.1 m below ground surface) ports at each of 12 monitoring wells in February and May 1995. Concentrations of these PAHs were summed, and the resulting 'Total PAH' values are plotted in Fig. 4 for both shallow and deep wells on the two sampling dates.

Generally, concentrations of PAHs were higher in the deep ports relative to the shallow ports at any one well. For example, in February 1995, Total PAH concentration in the deep port of Well 5 was 8.9 mg/l, whereas it was only 0.2 mg/l in the shallow port. Similarly, in May 1995, the Total PAH concentration in the deep port of Well 5 was 6.4 mg/l, whereas it was 0.2 mg/l in the shallow port. Being one of the more water-soluble constituents of creosote, naphthalene was the most abundant PAH in 17 of the 24 water sampling ports on both dates. Comparing concentrations between the wells along Transect 1 (Wells 1–3, 10–12) for the February sampling, PAH concentrations tended to decrease with increasing distance from the north lagoon area. Total PAH concentrations in the shallow wells decreased from a maximum value of 10.3 mg/l at Well 1 to a minimum of 0.6 mg/l at Well 12. Similarly, Total PAH concentrations in the deep wells decreased from a maximum of 12.7 mg/l at Well 3 to a minimum of 1.6 mg/l at Well 12. Along Transect 2 (Wells 4–9), concentrations in the wells showed no regular pattern. Concentrations in the shallow ports varied between 0.2 mg/l Total PAHs (Well 5) and 16.6 mg/l Total PAHs (Well 9), and in the deep ports they ranged from 1.2 mg/l Total PAHs (Well 4) to 10.0 mg/l Total PAHs (Well 6). The lack of a regular pattern along Transect 2 was not unexpected, as these wells lie approximately equidistant from the source area.
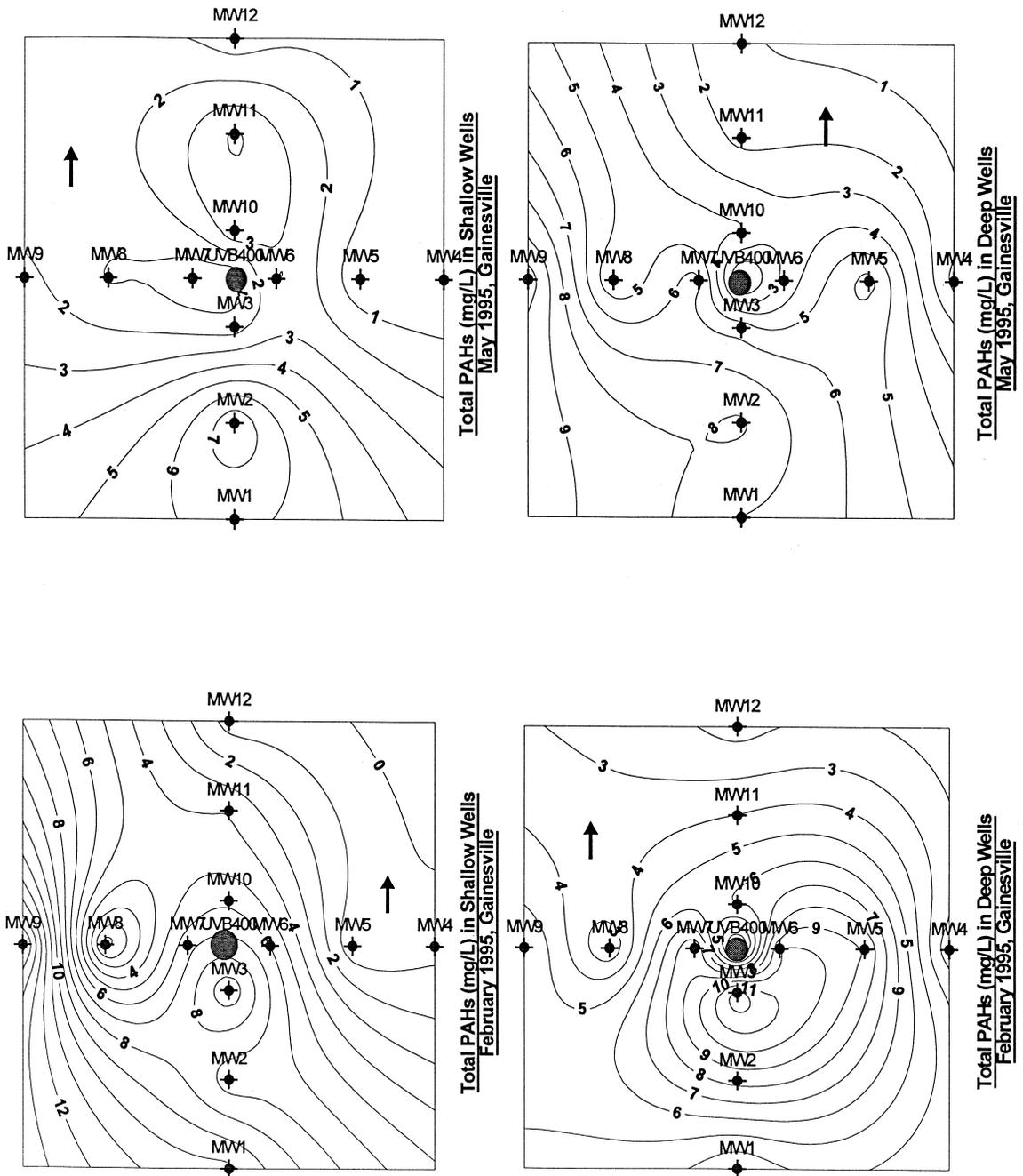
Fig. 4. Contour plots of Total PAH concentrations in groundwaters collected from shallow (3.7 m) and deep (6.1 m) ports at the Gainesville site on February 6, 1995 and May 31, 1995.

Trends in concentrations along the two transects were generally the same in May 1995 as in February 1995. In May 1995, PAH concentrations were found to have decreased from the February 1995 levels in 18 sampling ports, whereas they had increased in the remaining six wells. For example, Total PAH con-

Table 2
The $\delta^{13}C$ values of PAHs extracted from groundwaters collected at the Cabot Carbon/Koppers site in Gainesville, FL on May 31, 1995

| Well | $\delta^{13}C$ (‰) | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Naph | Thia | 2-MN | 1-MN | Biph | 2,6-DMN | 2,3-DMN | Acy | Acen | DiBF | Fluor | Phen | Anth | Carb | Fla | Pyr |
| 1-Shallow | −23.72 | −25.77 | −23.19 | −23.47 | −23.68 | −23.36 | −23.02 | −22.10 | −24.43 | −23.34 | −24.41 | − | − | −22.08 | − | − |
| 1-Deep | −23.27 | −25.02 | −22.73 | −23.04 | −21.22 | −22.82 | −21.19 | −22.50 | −21.74 | −22.18 | −22.64 | − | − | − | − | − |
| 2-Shallow | −23.63 | −25.80 | −23.76 | −24.22 | −21.94 | −21.06 | −20.97 | −20.99 | −22.43 | −24.09 | −22.89 | − | − | − | − | − |
| 2-Deep | −23.95 | −26.36 | −24.41 | −24.36 | −22.39 | −21.42 | −20.64 | −20.35 | −22.38 | −22.31 | −22.59 | −22.21 | −23.76 | −22.81 | − | − |
| 3-Shallow | −23.72 | −23.52 | −24.03 | −25.15 | −21.60 | −23.56 | −21.04 | − | −22.95 | −23.38 | −22.70 | −22.80 | −23.53 | −22.52 | − | − |
| 3-Deep | −24.10 | − | −23.19 | −24.12 | −22.24 | −20.05 | −18.87 | − | −22.48 | −22.82 | −22.37 | −22.09 | −23.42 | −22.33 | − | − |
| 4-Shallow | − | − | − | − | − | − | − | − | −24.53 | −23.06 | −23.84 | −23.15 | − | − | − | − |
| 4-Deep | −23.36 | −26.37 | − | − | − | −21.74 | − | − | −23.55 | −23.26 | −23.54 | −23.48 | −24.46 | −22.65 | − | − |
| 5-Shallow | − | − | − | − | − | − | − | − | −25.35 | −23.86 | −23.63 | −23.14 | − | − | − | − |
| 5-Deep | −23.88 | −26.97 | −23.45 | −24.87 | −21.93 | −22.36 | −21.83 | − | −22.77 | −22.87 | −22.74 | −22.82 | −25.32 | −22.30 | − | − |
| 6-Shallow | −23.94 | −26.83 | −23.32 | −24.61 | −21.88 | −25.09 | − | − | −23.44 | −23.67 | −23.37 | −23.32 | − | − | − | − |
| 6-Deep | −24.19 | −26.52 | −23.36 | −25.83 | −22.99 | − | − | − | −23.17 | −22.47 | −23.18 | −23.10 | −26.11 | − | − | − |
| 7-Shallow | − | −23.67 | − | − | −20.07 | − | − | − | −24.58 | −22.99 | −23.40 | −23.15 | −25.94 | − | − | − |
| 7-Deep | −23.79 | −26.98 | −23.29 | −24.84 | −22.42 | − | − | − | −22.76 | −22.45 | −22.85 | −22.13 | − | − | − | − |
| 10-Shallow | −23.82 | −25.82 | −23.06 | −23.83 | − | −21.58 | − | − | −22.82 | −22.42 | −23.01 | −23.72 | −25.79 | − | − | − |
| 10-Deep | −24.34 | −26.52 | −23.16 | −23.84 | − | −19.18 | − | − | −23.33 | −22.71 | −22.91 | −22.67 | − | − | − | − |
| 11-Shallow | −24.17 | −27.05 | −23.24 | −24.49 | −22.85 | − | − | − | −22.48 | −22.39 | −23.20 | −22.63 | − | − | − | − |
| 11-Deep | −23.55 | −25.73 | −22.54 | − | −21.08 | − | − | − | −23.08 | −22.26 | −22.76 | −22.91 | −26.27 | −22.90 | − | − |
| 12-Shallow | − | − | − | − | − | −20.91 | − | − | − | −23.08 | −23.55 | −22.96 | −25.60 | −24.83 | − | − |
| 12-Deep | −23.08 | −25.47 | −20.78 | − | −21.33 | −21.92 | − | −21.49 | −22.06 | −21.80 | −21.89 | −22.05 | −26.55 | −21.31 | − | − |
| AWA 5/95 | −23.78 | −25.90 | −23.17 | −24.36 | −21.97 | −21.93 | −21.08 | −21.78 | −23.17 | −22.87 | −23.07 | −22.84 | −25.16 | −22.64 | − | − |
| AWA 2/95 | −24.27 | −25.00 | −23.73 | −24.99 | −22.51 | −23.47 | −19.93 | −21.64 | −22.88 | −23.24 | −22.79 | −22.51 | −24.43 | −21.87 | −23.26 | −23.25 |
| Total average | −24.03 | −25.45 | −23.45 | −24.68 | −22.24 | −22.70 | −20.51 | − | −23.03 | −23.06 | −22.93 | −22.68 | −24.80 | −22.26 | −23.26 | −23.25 |

Abbreviations are as follows: Naph = naphthalene; Thia = thianaphthene; MN = methylnaphthalene; DMN = dimethylnaphthalene; Acy = acenaphthylene; Acen = acenaphthene; DiBF = dibenzofuran; Phen = phenanthrene; Anth = anthracene; Carb = carbazole; Fla = fluoranthene; Pyr = pyrene; AWA 5/95 = All Well average for May 1995; AWA 2/95 = All Well average for February 1995; Total average = average of AWA 5/95 and AWA 2/95.

centration was 10.1 mg/l in the deep port of Well 6 in February, but it was only 3.3 mg/l in May. In contrast, Total PAH concentration in the deep port of Well 9 measured 6.1 mg/l in February, and it increased to 10.6 mg/l by May. The reason for the variability in concentrations between sampling dates is unknown, but it perhaps can be attributed partly to the start-up of the groundwater circulation system on February 16, 1995, which increased groundwater flow in the area of the monitoring wells.

At the Gainesville site, $\delta^{13}C$ values of the various PAHs ranged from $-18.87‰$ to $-27.05‰$, with 70% of the values falling between $-22.06‰$ and $-24.53‰$ (Table 2). Similar to the ACW site, $\delta^{13}C$ values did not vary greatly between the wells, even when concentrations ranged from fairly contaminated (Wells 1, 2, and 3) to only trace amounts (Well 12). In a statistical comparison of individual compounds between different wells (e.g., naphthalene at Well 1 shallow vs. naphthalene at Well 10 deep), the $\delta^{13}C$ values of all the PAHs were found to be significantly different in 35% or less of the comparisons ($p < 0.05$, Student's $t$-test). For example, anthracene only differed between wells in 5% of the

cases, phenanthrene differed in 24%, and 1-methylnaphthalene differed in 35%.

Because the majority of $\delta^{13}C$ values for the PAHs at the Gainesville site were not significantly different between the different wells, an 'All Well' average for each compound was calculated by averaging the values for each well (shallow and deep). An 'All Well' average was calculated for both the February 1995 and May 1995 samplings (Table 2). Comparing the $\delta^{13}C$ values of the compounds between the two dates, only naphthalene, thianaphthene, and 2-methylnaphthalene differed statistically ($p < 0.05$, Student's $t$-test).

## 3.4. Comparison of $\delta^{13}C$ values of PAHs at the ACW and Gainesville sites

The $\delta^{13}C$ values of PAHs at the Gainesville site, averaged over all wells and sampling times (Table 2, last row), are plotted along with the corresponding values for the ACW site in Fig. 5. Twelve of the 16 compounds agreed within 1.0‰ for the two sites. There were five compounds which were statistically different between the sites ($p < 0.05$, Student's $t$-
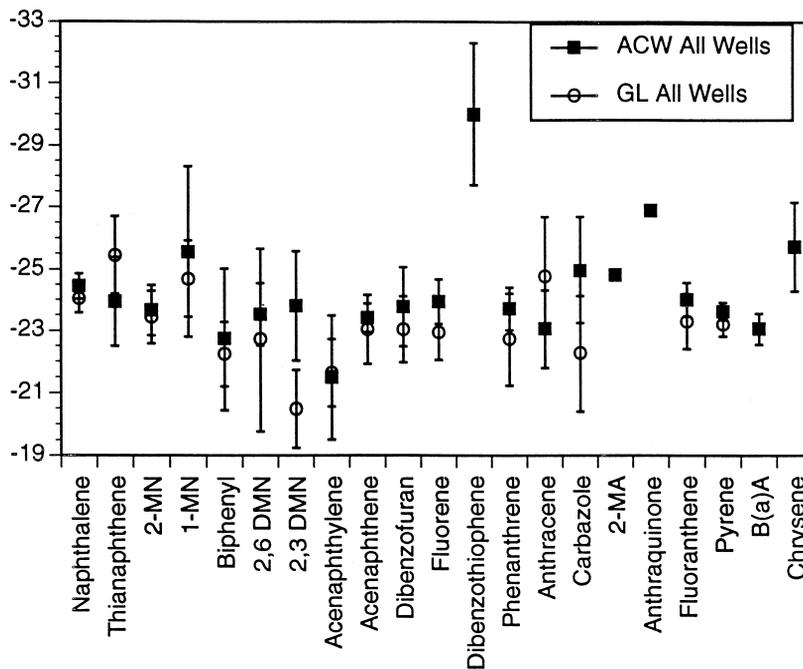


Fig. 5. Comparison of $\delta^{13}C$ values of PAHs at the ACW site with those at the Gainesville site. Values for each site are averages of all wells sampled over both sampling dates. Error bars represent $\pm 1$ standard deviation.

test). These compounds are naphthalene (differed by 0.42‰), thianaphthene (differed by 1.51‰), 2,3-dimethylnaphthalene (differed by 3.29‰), fluorene (differed by 1.01‰), and carbazole (differed by 2.70‰). Anthracene differed by 1.75‰ between the two sites, but this difference was not statistically significant.

## 4. Discussion

The combined GC/ITMS/IRMS system has proven both useful and reliable in measuring the stable carbon isotopic composition of individual PAHs in groundwater samples. In order for $\delta^{13}C$ values to be effective in examining the fate of these compounds in bioremediation scenarios, two basic criteria need to be satisfied. First, the $\delta^{13}C$ values of the individual PAH compounds need to be maintained over distance, i.e., migration, from the contaminated area, and secondly, they need to be maintained through time. Once these criteria are satisfied, stable isotopic measurements of contaminant compounds could be used to fingerprint contaminant sources and to trace their fate in the environment. Furthermore, comparisons between $\delta^{13}C$ values of contaminants, indigenous organic matter and respired carbon dioxide may provide evidence that microorganisms are participating in the remediation of a polluted site and may lead to greater understanding of biodegradation processes.

Our data provide evidence that the $\delta^{13}C$ values of individual PAH compounds are maintained over distance from the source area as the contaminants migrate both horizontally and vertically through a groundwater aquifer. At the ACW site, concentrations of PAHs decreased by over four orders-of-magnitude both in a horizontal transect from Well 340 to Well 760 and in a vertical transect from Well 340 to Well 380. Concentration changes were most likely due to a combination of biodegradation and dilution. Unlike concentrations, stable carbon isotopic values for the majority of PAHs, especially the higher molecular weight compounds, did not change dramatically or systematically across the wells (Table 1); therefore, it appears that neither dilution nor degradation significantly alters the stable isotopic composition of PAHs. The same results were ob-

served along Transect 1 at the Gainesville site (Table 2).

Most bioremediation programs monitor concentrations of the contaminant compounds through time. For stable isotopic analyses to be useful in such programs, contaminant compounds need to maintain their isotopic composition through time as well as during migration. Samples of groundwater obtained in June and again in September 1994 from the ACW wells had similar suites of $\delta^{13}C$ values for PAHs. Likewise, groundwaters collected 3 months apart at the Gainesville site had similar isotopic compositions for contaminants. These results indicate that $\delta^{13}C$ values are maintained through time, at least over short periods, and that the measuring techniques presented in this paper are reproducible and reliable.

The constancy of PAH $\delta^{13}C$ values with both migration through a groundwater aquifer and time indicates that stable carbon isotopes could be useful indicators of bioremediation at contaminated environments. Typically, microorganisms are only able to degrade contaminants which are in the dissolved phase. High molecular weight compounds such as PAHs tend to have very low aqueous solubilities; therefore, most PAHs become adsorbed to soil particles and are unavailable for biodegradation. Because there is an equilibrium between the dissolved and adsorbed PAHs, as the dissolved PAHs are biodegraded or otherwise removed from the area, adsorbed PAHs dissolve to replenish the supply (e.g., Morris et al., 1995). Thus, in bioremediation scenarios, changes in aqueous PAH concentrations are incomplete indicators of in situ bioremediation because concentrations of PAHs in groundwaters tend not to decrease or are highly variable, even though biodegradation is occurring. In these cases, $\delta^{13}C$ values of bacterial biomass and respired carbon dioxide could be measured at both a contaminated environment and an uncontaminated control site. The values could then be compared to those of the contaminants and/or background indigenous organic matter, providing evidence that biodegradation is occurring. By coupling stable isotope measurements with carbon mass balances, it would be possible to estimate the amount of the contaminant that is being degraded over time.

Although the majority of $\delta^{13}C$ values for individual PAHs were consistent both with migration and

time at the two creosote-contaminated sites, it should be pointed out that there were a few compounds that were variable. At the ACW site, thianaphthene, dibenzothiophene, anthracene, and biphenyl showed the most variability with migration, and carbazole was the most variable with time. At the Gainesville site, $\delta^{13}C$ values of the compounds were well-conserved with migration, but 'All Well' averages of naphthalene, thianaphthene, and 2-methylnaphthalene were statistically different with time.

Anthracene has been documented to become isotopically enriched in $^{13}C$ when it undergoes photolytic decomposition (O'Malley et al., 1994). Perhaps, this compound also fractionates isotopically when it is subjected to other degradation mechanisms in the environment. Of the other compounds that were the most variable, thianaphthene, dibenzothiophene, and carbazole are heterocyclic compounds. Perhaps, the sulfur or nitrogen atom imparts a relative instability to these compounds which causes them to be isotopically fractionated during transport or degradation. Lastly, because naphthalene, 2-methylnaphthalene, and biphenyl are fairly easy to degrade relative to higher molecular weight PAHs (Mueller et al., 1996), they also may be subjected to isotopic fractionations that do not affect the higher molecular weight compounds.

It is not likely, however, that high molecular weight compounds such as PAHs are isotopically fractionated during chemical or physical transformations. For example, O'Malley et al. (1994) found that the $\delta^{13}C$ of naphthalene, which is the lightest compound analysed in this study, did not change during microbial degradation, even after a 95% reduction in naphthalene concentration occurred. In similar studies, $\delta^{13}C$ values of acenaphthene, fluorene, phenanthrene, and fluoranthene have also been found to be unaffected by microbial degradation (Kelley et al., 1995; Trust et al., 1995). It is also unlikely that isotopic fractionation occurs during other transformations, such as the partitioning of compounds between dissolved and adsorbed phases.

A more likely explanation for the variability of the few aforementioned PAHs is that the $\delta^{13}C$ values of these compounds are variable across different creosote sources. The creosote facilities at both the ACW and Gainesville sites were in operation for over 50 years. Over this time period, it is likely that many different creosote sources were added to the lagoons and holding ponds at these sites. It is also likely that these various sources had different suites of $\delta^{13}C$ values associated with them which in turn produced the differences in $\delta^{13}C$ values observed between the wells for the more variable compounds.

It is important to stress that even though a few of the compounds were variable at any one site, the bulk of the compounds were not. Similarly, in a comparison of the $\delta^{13}C$ values of the PAHs between the sites (i.e., ACW vs. Gainesville), the majority of compounds (11 out of 16) were also found not to be statistically different. Furthermore, of the five compounds that were statistically different, naphthalene, thianaphthene, and carbazole have already been mentioned above as being variable at either the ACW or the Gainesville site with migration or time. These results indicate that the majority of PAHs in creosote may have characteristic $\delta^{13}C$ values. In other words, there may be a distinctive 'isotopic fingerprint' of $\delta^{13}C$ values which identifies PAHs at a contaminated site as being from creosote. In addition, the compounds that are more variable could serve to differentiate between two or more possible creosote sources. In future studies, the $\delta^{13}C$ values of more PAHs, both of creosote and non-creosote origin, should be measured to see, first of all, if there is a characteristic pattern of $\delta^{13}C$ values for creosote and if this pattern is distinct from other PAH sources (we did not have enough compounds in common with O'Malley et al. (1994) to compare our creosote PAHs with their PAHs of various origin), and secondly, if there are a few individual compounds that vary across different creosotes and thus can be used as tracers of specific creosotes.

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