

Combined ^{14}C and $\delta^{13}\text{C}$ analysis of petroleum biodegradation in a shallow contaminated aquifer

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Abstract The contamination of soil and groundwater by the accidental release of petroleum hydrocarbons is a common problem that affects drinking water supplies, and in situ biodegradation is a promising option for removing contaminants, with relatively low costs and minimal health risks. Total petroleum hydrocarbons (TPH), $\delta^{13}\text{C}$ and ^{14}C isotope ratios in dissolved inorganic carbon (DIC), and other geochemical indicators have been analyzed to demonstrate the effectiveness of in situ biodegradation at a contaminated site. The $\delta^{13}\text{C}$ and ^{14}C isotope ratios ranged from -5.4 to -18.4 ‰ and from 35.1 to 67 pmc, respectively. Some groundwater samples were contaminated with petroleum hydrocarbons with low ^{14}C and $\delta^{13}\text{C}$ contents, indicating the existence of microbial non-methanogenic biodegradation, whereas low ^{14}C and high $\delta^{13}\text{C}$ contents were associated with the highest TPH concentrations, confirming that microbial methanogenic biodegradation occurred in these areas. Differences in the $\delta^{13}\text{C}$ and ^{14}C isotope ratios between monitoring wells in uncontaminated and contaminated areas suggest that the percentage of the DIC produced by methanogenic biodegradation decreased from 28.5–32.0 to 5.94–6.67 % along the flow direction, and became almost zero in downgradient areas and at the edge of the contamination plume.

Keywords Groundwater · Petroleum hydrocarbon contamination · Radiocarbon isotope

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Introduction

Petroleum hydrocarbon spills are some of the most widespread causes of soil and groundwater contamination (Conrad et al. 1997). Intrinsic biodegradation, which involves using naturally occurring microorganisms to convert organic contaminants into harmless forms, is gaining widespread acceptance as a viable method for remediating sites contaminated with hydrocarbon compounds (Conrad and Depaolo 2004). However, a major barrier to the widespread acceptance of in situ bioremediation as a clean-up technology is whether the contaminants are actually degraded in the field (US National Research Council 1993). Evidence for the microbial degradation process is commonly acquired by monitoring the decrease in the contaminant concentrations (Douglas et al. 1992; Dong et al. 2014), monitoring electron acceptors, measuring increases in the degradation products (Borden et al. 1995; Anderson and Lovley 1997; Lesage et al. 1997; Kao and Wang 2001), and measuring biomass concentrations (Madsen et al. 1991; Fleming et al. 1993). However, effects that are similar to those expected from contaminant biodegradation (e.g., the biodegradation of organic matter or the dissolution of carbonate minerals) can occur for other reasons, often giving ambiguous results.

The $\delta^{13}\text{C}$ of dissolved inorganic carbon (DIC) in groundwater has commonly been used to provide further verification of petroleum biodegradation, because hydrocarbon compounds are generally relatively depleted in $\delta^{13}\text{C}$ relative to most other sources of C (Schoell 1984). The microbial metabolism of compounds derived from petroleum hydrocarbons therefore tends to produce CO_2 with relatively low $\delta^{13}\text{C}$ values where significant degradation of hydrocarbons is occurring, and this effect has been seen in some field studies (Aggarwal and Hinchee 1991; National

Research Council 1993; Bolliger et al. 1999; Hunkeler et al. 1999; Fang et al. 2000; Topinkova et al. 2007).

However, certain processes and CO₂ sources can lead to ambiguous results when only δ¹³C analysis is used to investigate petroleum biodegradation. For example, in a system that has become anaerobic because of contaminant inputs, methane production yields δ¹³C-depleted CH₄ and δ¹³C-enriched CO₂ (Lollar et al. 2001), so the δ¹³C values for CO₂ and/or DIC produced from petroleum hydrocarbon degradation in areas of significant methanogenic activity can be higher rather than lower (Baedecker et al. 1993; Revesz et al. 1995; Landmeyer et al. 1996). There can also be significant overlaps between the δ¹³C values of petroleum hydrocarbon contaminants and of indigenous plants. In such areas, radiocarbon (¹⁴C) measurements of the groundwater DIC is an alternative technique that can be used to better identify the origin of microbial CO₂ and give an estimate of the amount of microbial CO₂ production from aerobic petroleum mineralization (Aelion et al. 1997).

The ¹⁴C content can be used to distinguish between carbon sources of different ages (Bauer et al. 1990, 1992, 1995) because it has a half-life of approximately 5,680 years (Libby 1955). Petroleum hydrocarbons of geological origins are, therefore, radiocarbon free (0 pmc) and provide a definitive end-member when analyzed against photosynthesis-based carbon-containing chemicals, which contain modern CO₂ from the atmosphere. Given these two extreme end-members, one can readily assess the relative contributions of petroleum and plant biomass degradation to the total respired CO₂ pool (Suchomel et al. 1990; Bhupathiraju et al. 2002; Conrad and Depaolo 2004; Bugna et al. 2005), and in situ ¹⁴C and δ¹³C measurements have been used in several studies under methanogenic environmental conditions (Conrad et al. 1999; Coffin et al. 2008). However, only rarely have there been reports of the processes that take place, including methanogenic and non-methanogenic degradation, mineral dissolution, oxidation of organic matter, and other CO₂ sources, being quantified.

The shallow aquifer at the study site was contaminated by petroleum hydrocarbons because of oil from oil wells penetrating an aquiclude, and total petroleum hydrocarbons (TPH), geochemical indicators, δ¹³C_{DIC}, and δ³⁴S_{SO4} have been discussed in previous work, in which the occurrence of biodegradation at the site was proved (Su et al. 2013). However, it could not be determined whether the δ¹³C-enriched DIC measured was derived from methanogenic degradation. Here, a study have been presented in which DIC concentrations and δ¹³C and ¹⁴C data were collected, with the objectives of (1) demonstrating the effectiveness of combined δ¹³C and ¹⁴C measurements in providing direct evidence of in situ petroleum hydrocarbon biodegradation, especially where there are significant levels of methanogenesis, and (2) quantifying the contributions of

the methanogenic and non-methanogenic biodegradation processes to the DIC production.

Method

Study area

The contaminated site is located at Songyuan, Jilin province in the northeast China (Fig. 1), near the Songhua River. The quaternary-unconsolidated-rock pore-water aquifer is the main water supplying stratum in the area. Figure 2 shows the lithology and thickness of the shallow formations, which are relative stable, the aquifer lithology being, from top to bottom, fine sand, silt, and gravel, with a total thickness of about 20 m. Above the aquifer, there is an impermeable clay layer about 2 m thick, and a lower silt–clay layer forms the bottom of the aquifer. The main source for recharging the aquifer is lateral runoff from hillock groundwater, and the aquifer discharges through lateral runoff and artificial extraction. Groundwater level measurements indicate that the depth of the groundwater table is about 3.5 m and the groundwater flows from the southeast to the northwest, with a hydraulic gradient of 0.47 %.

The shallow aquifer of the test site was contaminated by petroleum hydrocarbon due to aquiclude penetration of oil from oil wells. Several oil–water ponds were formed by the accidental spill at the site (Figs. 2, 3), the petroleum hydrocarbons in nonaqueous phase was found in the boreholes near the wells Z23, E1, E2, E3, Z1-1, Z6, Z7, Z8, and the vertical extension of the it was between 0.5 and 12 m, and the petroleum hydrocarbons concentration varied between about 200 and 3,400 mg/kg from the contaminated soil.

Data from the monitored wells have shown that the groundwater is contaminated with petroleum hydrocarbons. The TPH plume reaches a steady-state distribution



Fig. 1 Location of the study area at the northeast China

and the direction the plume spreads in is controlled by the groundwater flow (i.e., from the southeast to the northwest). The groundwater pollution is most severe near the abandoned oil well and gradually decreases along the direction of groundwater flow.

Sampling and analysis

Sampling

There are 19 monitoring wells at the contaminated site. Groundwater and soil samples were collected in November

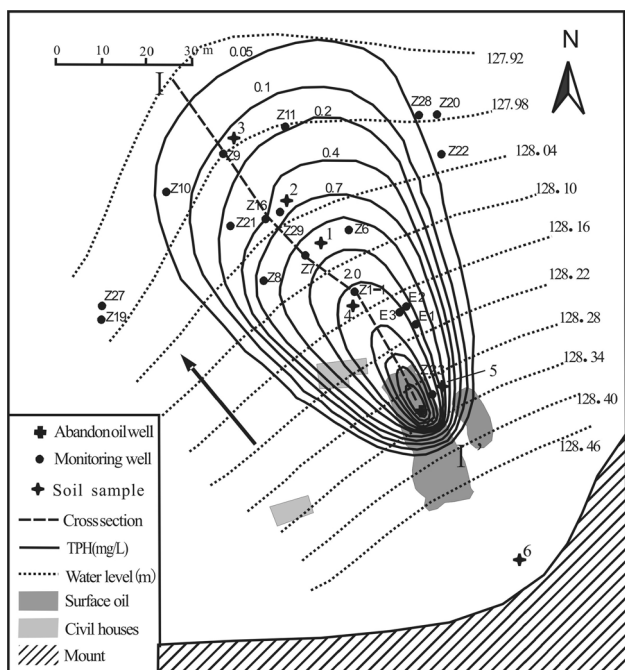


Fig. 2 Map of the groundwater flow regime and the plume of TPH contaminated groundwater

2011 and the main parameters that were measured in the samples were TPH, organic and inorganic chemical compositions, pH, oxidation–reduction potential (E_h), and dissolved oxygen (DO). Seven samples for the analysis of ^{14}C and $\delta^{13}C$ in DIC were collected from the wells located along the central line and along the in of the contamination plume.

Soil sample collection

The soil samples were collected from different depths using an auger. After drilling to the target depth, the sample at the tip of the drill was collected quickly. The samples were stored in 60 mL amber glass bottles, with no headspace.

Groundwater sample collection

Monitoring wells were purged for at least 3–5 well volumes before sampling. Groundwater samples were collected for laboratory analyses once the pH, temperature, electrical conductivity, and E_h values had stabilized.

Groundwater samples for TPH analysis were collected in 1 L amber glass bottles and immediately acidified with HCl (to pH <2) and sealed without a headspace. Ground water samples for the analyses of CH_4 were taken into 40 mL glass vials fitted with Teflon-lined screw caps, without headspace. Temperature, pH, electrical conductivity, and E_h were measured on site using a W-23XD multiparameter meter (Japan). The groundwater samples for cation and dissolved metal analysis were acidified with 0.1 % HNO_3 and stored in polyethylene bottles. The samples for $\delta^{13}C$ analysis were collected in polyethylene bottles with airtight caps and preserved with $HgCl_2$ to prevent biological activity. All sample lids were sealed with parafilm.

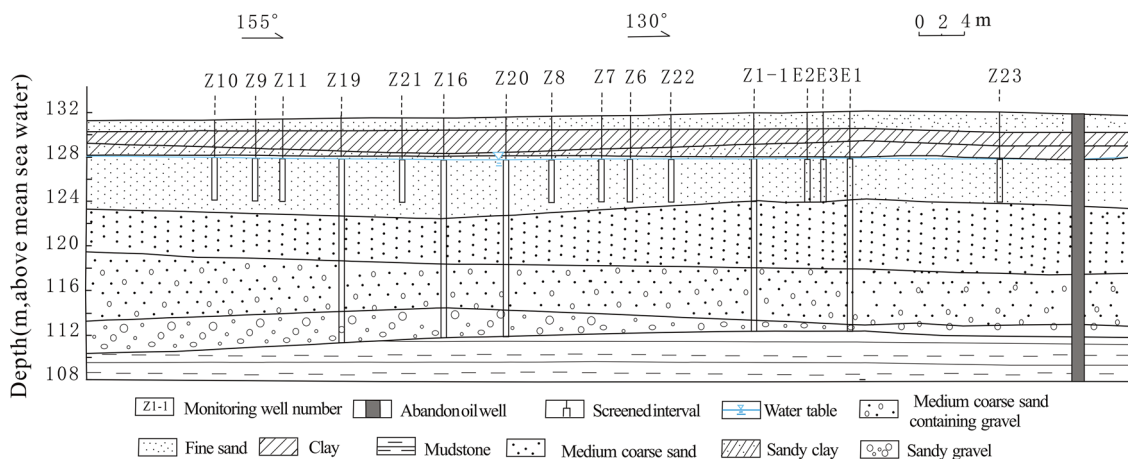


Fig. 3 Hydrogeological cross section of the aquifer, from I to I' in Fig. 1 (from Su et al. 2013)

Chemical analysis

TPH in groundwater samples was determined by infrared spectral colorimetry (JDS-108U+, China). Concentration of dissolved CH₄ was determined using a headspace equilibration method using a gas chromatograph equipped with a flame ionization detector. The concentrations of K⁺, Na⁺, Ca²⁺, Mg²⁺, were determined using an atomic absorption spectrophotometer (Perkin Elmer AAnalyst 700, USA). The ions NH₄⁺, NO₃⁻, and NO₂⁻ were quantified using an ultraviolet spectrophotometer (CARY50, USA). The sample alkalinity was measured by acid–base titration, and HCO₃⁻ was determined by phenolphthalein titration.

Stable isotope analysis

Stable isotopes were measured using a method published by Krishnamurthy et al. (1997) with some modifications. A 10-mL water sample was injected using a syringe, into a glass bottle containing 1 mL of 85 % phosphoric acid and a magnetic stirrer bar. The acid–water reaction began immediately upon injection and the mixture was allowed to stand for several hours. CO₂ was extracted at 50 °C for 10 min, with stirring, into a vacuum line that passed through a N₂ cooled ethanol trap, to remove H₂O, and was finally frozen into a manometer cold finger in a liquid N₂ trap. The frozen CO₂ was cryogenically transferred into a tube for isotope measurement. The C isotope ratios in the DIC were determined on a Finnigan MAT 252 mass spectrometer.

Soil samples for organic carbon analysis were dried at room temperature, and, depending on the amount of organic carbon anticipated to be present in each sample, 100–300 mg aliquots of each soil were loaded into Vycor tubes. CuO and Cu were added to the tubes, and they were then evacuated and sealed. The samples were then heated to 850 °C for 2 h, to oxidize the C. The CO₂ produced was separated and sealed in glass tubes. The total weight percent organic C in the samples was then calculated from the CO₂ yield divided by the total weight of the sample.

Soil inorganic carbon for isotopic analyses was cleaned in deionized water, air-dried, and crushed. Approximately 10 mg of powder was reacted with phosphoric acid overnight at 25 °C. The resulting CO₂ was purified and collected in glass tubes for isotopic analyses.

The δ¹³C isotope values are reported using the δ notation in ‰ relative to PDB,

$$\delta \text{‰} = \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \times 1,000 \quad (1)$$

where *R* is ¹³C/¹²C. The overall precision of the δ¹³C measurements was better than ±0.1 ‰.

Radiocarbon analysis

The ¹⁴C content of the DIC sample aliquots taken from the stable isotope samples was analyzed by Beta Analytic Inc. CO₂ was converted to graphite following the procedures documented by Loyd et al. (1991), and the ¹⁴C content of the graphite was analyzed using an accelerator mass spectrometer. The results from these analyses are reported as fractions of modern (pre-1950) C values (values >100 pmc come from samples containing radiocarbon produced during the aboveground testing of nuclear weapons). The precision of the analyses was ≤0.01 × modern C values.

X-ray diffraction

XRD analysis was performed using a D8 Advance instrument (Bruker, Billerica, MA, USA). The routine operating conditions were 40 kV/40 mA, step scanning at 0.05/30 s in the 2θ range 3–70°.

The content of the indicators are shown in Tables 1 and 2.

Results and discussion

Background carbon isotopic signatures

The shallow confined aquifer at the contaminated site has a relatively closed structure that is difficult for air and precipitation to infiltrate. The groundwater *p*CO₂ is about 10⁻¹ atm, which is much larger than the atmosphere *p*CO₂ (10^{-3.5} atm), so the impact of atmospheric CO₂ on the groundwater DIC can be ignored.

The clay layer above the aquifer has a high organic C content, of up to 2.224 %, indicating that the aquifer media is rich in organic C. The δ¹³C value of organic C in the soil is between -24.4 and -25.8 ‰, with a mean of -24.9 ‰ (Table 2), which is in the range typical for δ¹³C value of soil or aquifer organic carbon (Clark and Fritz 1997).

According to the X-ray diffraction mineral analysis, the calcite contents of all of the aquifer media are up to approximately 5 %. But on the other hand, The carbon isotope data for the soil inorganic carbon samples are consistent (averaging 9.18 ‰), it is much more likely that they are secondary minerals precipitated from soil processes (root and microbial respiration of CO₂). Furthermore, calculations of carbonate equilibrium using the available data for all the monitoring wells (including wells located in upgradient of the site) indicate that the system is at saturation with respect to calcite, so the contribution of carbonate dissolution to the groundwater DIC can also be ignored.

Table 1 Data for groundwater samples

Well	Na ⁺ (mg/L)	Ca ²⁺ (mg/L)	Mg ²⁺ (mg/L)	Cl ⁻ (mg/L)	SO ₄ ²⁻ (mg/L)	HCO ₃ ⁻ (mg/L)	NO ₃ ⁻ (mg/L)	DO (mg/L)	E _n (mv)	pH	TPH (mg/L)	Temp (°C)	δ ¹³ C (‰)	¹⁴ C (pmc)
Z1-1	459.68	58.46	21.27	154.01	7.78	1277.23	1.24	0.96	-213.70	7.65	0.78	10.60	-14.40	37.45
Z8	310.94	150.39	47.36	210.39	45.93	1111.31	1.10	2.60	-2.80	7.32	0.74	11.30	-25.00	35.10
Z11	127.84	100.69	30.66	51.65	117.55	573.66	0.44	1.27	-109.30	7.31	0.13	11.00	-13.90	64.28
Z16	216.55	127.98	37.54	142.55	25.30	892.65	0.59	1.37	-134.50	7.34	0.43	10.40	-14.80	55.50
Z19	279.66	151.85	51.98	146.33	83.29	1090.73	1.12	1.59	25.40	7.29	0.11	10.80	-15.50	52.08
Z20	330.64	159.16	42.25	446.80	142.07	646.98	0.74	0.86	-12.30	7.28	0.08	11.30	-12.10	67.06
E1	377.84	56.52	16.94	161.84	0.82	998.12	0.74	0.99	-163.40	7.64	1.47	11.40	-5.40	44.30

Table 2 Data for soil samples

Site	δ ¹³ C—organic carbon (‰)	δ ¹³ C—inorganic carbon (‰)	δ ¹³ C—petroleum hydrocarbons (‰)
1	-24.96	-10.38	-31.10
2	-24.41	-9.81	
3	-24.72	-10.59	
4	-24.98	-8.57	
5	-25.77	-8.37	
6	-24.56	-7.32	

The TPH content of soil sample collected in the up-gradient zone (soil sample 6) of site is almost 0 mg/kg, which suggests that the upgradient zone was unimpacted by the fuel and represents ‘background’ conditions. The δ¹³C value of soil organic carbon for sample 6 is -24.6 ‰, during the process of degasification the δ¹³C value has about 4 ‰ fractionation (Cerling et al. 1991), so the δ¹³C value of soil gas CO₂ is about -20.9 ‰, according to the fractionation factors of carbonates in the underground environment with the temperature about 10 °C, the stable carbon isotope fractionation between soil gas CO₂ and HCO₃⁻ dissolved in groundwater is 9.6 ‰, while between soil gas CO₂ and calcite is 12.7 ‰ (Clark and Fritz 1997), so the calculated δ¹³C value of the HCO₃⁻ in groundwater was calculated to be about -11.4 ‰, which is in good agreement with the δ¹³C value found in the slightly polluted groundwater downgradient of the contaminant plume (Z11-1 and Z20, showing low concentration of TPH and HCO₃⁻, while high content of SO₄²⁻ and Eh); and the calculated δ¹³C value of the precipitated calcite should be -7.9 ‰, which is also consistent with the measured δ¹³C value of soil inorganic carbon δ¹³C value (-7.3 ‰).

Evidence for methanogenic and non-methanogenic biodegradation

As discussed above, using groundwater sample Z20 to represent the uncontaminated condition, the δ¹³C and ¹⁴C

values in the uncontaminated groundwater samples were about -12.1 ‰ and 67.06 pmc, respectively, and the wells’ downgradient and at the edge of the contaminated plume had similar δ¹³C and ¹⁴C values. However, the ¹⁴C values in water from the wells in the upgradient portion of the plume (E1, Z1-1, and Z8) were clearly lower than the ¹⁴C values downgradient and at the edge of the plume (Fig. 4). There are two possible sources of low ¹⁴C DIC, the biodegradation of petroleum hydrocarbons and carbonate dissolution, but the relatively low concentration of Ca²⁺ and the positive saturation index for carbonate in these wells showed that carbonate dissolution was not the main source of DIC in the contaminated groundwater. Therefore, the relatively low ¹⁴C values in the contaminated groundwater were caused by the biodegradation of petroleum hydrocarbons. It should be noted that the petroleum hydrocarbons mentioned here not only refer to the dissolved phase, but also the free-phase hydrocarbons in the aquifer in the core of the plume, because the highest measure values of TPH in groundwater are about 2 mg/L, that would not be enough to significantly shift the concentration of the background DIC (about 600–1,200 mg/L).

On the other hand, the δ¹³C values in the contaminated groundwater had different characteristics (Fig. 4). The groundwater from well Z8 had δ¹³C-depleted values and a high DIC content, which suggests that the DIC was derived from the non-methanogenic biodegradation of petroleum hydrocarbons (with δ¹³C = -31.1 ‰); the δ¹³C value of DIC from E1 was almost 7 ‰ higher than that of DIC obtained from the Z20, and there is not any source of high δ¹³C with low ¹⁴C value carbon at this site, so it can be explained that the methanogenic biodegradation of petroleum hydrocarbons was important (with δ¹³C = 10–15 ‰). Well Z1-1, which is located between wells E1 and Z8, had medium δ¹³C values and DIC contents, and appeared to be influenced by both biodegradation processes.

The spatial variations in the δ¹³C and ¹⁴C values further proved the analysis described above, in that the ¹⁴C values

indicated relatively modern C was present (Fig. 5) along the TPH plume, with decreasing TPH and DIC concentrations. This indicates that the biodegradation of the petroleum hydrocarbons contributed more DIC with low ^{14}C values to the groundwater upgradient of the contaminated plume than downgradient of it.

The $\delta^{13}\text{C}$ values increased from well Z8 to downgradient of the plume, indicating that non-methanogenic TPH degradation processes had occurred, generating DIC with low $\delta^{13}\text{C}$ values (about -31.1‰). Because the upgradient TPH content was relatively high, more CO_2 is formed there by biodegradation, and the low $\delta^{13}\text{C}$ value for the CO_2 resembles that of TPH.

The $\delta^{13}\text{C}$ values in the groundwater DIC in the wells near the contamination source (E1 and Z1-1) were higher than the values in the slightly contaminated groundwater. The higher petroleum concentrations around the core of the contaminant plume led to stronger degradation, and the electron acceptor (such as DO , SO_4^{2-} , and NO_3^-) concentrations were decreased to very low levels. Therefore, according to thermodynamic principles, the methanogenic degradation process should be initiated there and produce DIC enriched in $\delta^{13}\text{C}$ ($10\text{--}15\text{‰}$). From the spatial variation of dissolved CH_4 in groundwater (Fig. 5), it can also find that more CH_4 has been produced in groundwater near the contamination source than the downgradient of the plume, which verified the analysis above. The changes in

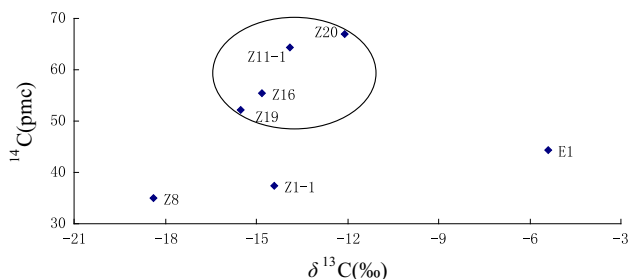
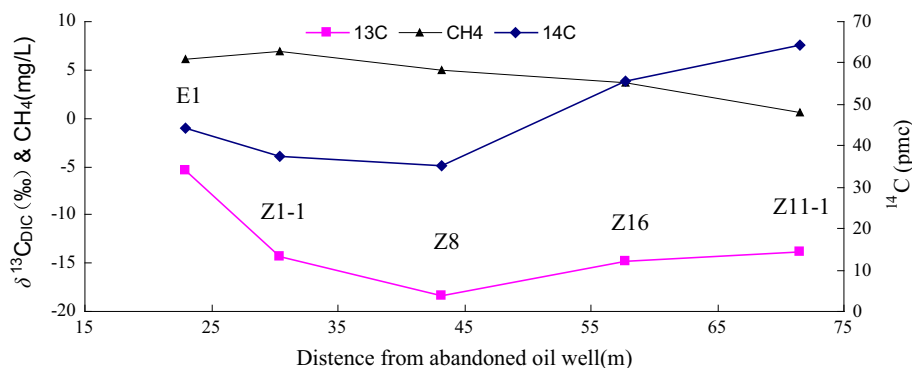


Fig. 4 ^{14}C variations relative to $\delta^{13}\text{C}$ variations in the groundwater dissolved inorganic carbon. The circled wells are all located in the downgradient and edge of contaminated plume, represent the uncontaminated and slightly contaminated groundwater

Fig. 5 Variations of the CH_4 , $\delta^{13}\text{C}_{\text{DIC}}$ and $^{14}\text{C}_{\text{DIC}}$ values along the groundwater flow direction



the $\delta^{13}\text{C}$ values in the DIC therefore reflect both non-methanogenic and methanogenic degradation processes.

Quantification of contaminated groundwater biodegradation

Based on the analysis above, the percentages of DIC produced from non-methanogenic and methanogenic degradation processes were quantified using Eqs. 2–4.

$$\begin{cases} x + y + z = 1 & (2) \\ x \times ^{13}\text{C}_{\text{unc}} + y \times ^{13}\text{C}_{\text{non-m}} + z \times ^{13}\text{C}_{\text{meth}} = ^{13}\text{C}_{\text{sam}} & (3) \\ x \times ^{14}\text{C}_{\text{unc}} + y \times ^{14}\text{C}_{\text{non-m}} + z \times ^{14}\text{C}_{\text{meth}} = ^{14}\text{C}_{\text{sam}} & (4), \end{cases}$$

where x , y , and z are the fractions of DIC derived from uncontaminated groundwater, non-methanogenic petroleum mineralization, and methanogenic petroleum mineralization, respectively, and $^{13}\text{C}_{\text{unc}}$, $^{13}\text{C}_{\text{non-m}}$, $^{13}\text{C}_{\text{meth}}$, $^{14}\text{C}_{\text{unc}}$, $^{14}\text{C}_{\text{non-m}}$, and $^{14}\text{C}_{\text{meth}}$ are the C isotope signatures for uncontaminated groundwater, non-methanogenic petroleum mineralization, and methanogenic petroleum mineralization, respectively. Well Z20 was chosen to represent the uncontaminated groundwater, and $^{13}\text{C}_{\text{sam}}$ and $^{14}\text{C}_{\text{sam}}$ are the isotope values for the contaminated groundwater samples (E1, Z1-1, and Z8), assuming that the $^{13}\text{C}_{\text{non-m}}$ and $^{14}\text{C}_{\text{non-m}}$ values would be -31.1‰ and 0 pmc, respectively, and that the $^{13}\text{C}_{\text{meth}}$ and $^{14}\text{C}_{\text{meth}}$ values would be $10\text{--}15\text{‰}$ and 0 pmc, respectively. The percentage ranges for each contribution can, therefore, be calculated.

The well Z8 results show that approximately 40.9–41.7 % (40.9 % using 10 ‰ as the $^{13}\text{C}_{\text{meth}}$ and 41.7 % using a $^{13}\text{C}_{\text{meth}}$ of 15 ‰) of the DIC was derived from non-methanogenic biodegradation and only 5.94–6.67 % was derived from methanogenic biodegradation. In well E1, 1.88–5.36 % of the DIC was derived from non-methanogenic biodegradation and 28.5–32.0 % was derived from methanogenic biodegradation. Therefore, the groundwater at Z8 appears to be mainly influenced by non-methanogenic biodegradation, but methanogenic

Table 3 The dissolved inorganic carbon contributions (%) from uncontaminated groundwater and non-methanogenic biodegradation

Well	Calculate by ¹⁴ C		Calculate by ¹³ C	
	x	y	x	y
Z16	0.828	0.172	0.858	0.142
Z11-1	0.959	0.041	0.905	0.095
Z19	0.777	0.223	0.821	0.179

biodegradation processes are the major source of DIC in groundwater near E1. In well Z1-1, as discussed before, the non-methanogenic and methanogenic contributions were in between the contributions found in wells E1 and Z8, the actual values being 29.3–30.9 % from non-methanogenic biodegradation and 13.2–14.8 % from methanogenic biodegradation.

The z value for the other wells appeared to be negative, as discussed above, and the δ¹³C and ¹⁴C values and the DIC and TPH contents of the samples from the wells located in the downgradient and at the edge of the TPH plume indicated that the groundwater there was slightly contaminated, so the contribution of methanogenic biodegradation to the DIC can be ignored in those areas. Therefore, the contributions of the different sources to the DIC according to the individual δ¹³C and ¹⁴C values (using Eqs. 2, 3 or 2, 4) can be calculated. The results are shown in Table 3.

It can be seen that the results using Eqs. 2 with 3 and 2 with 4 were consistent with the results shown in Table 3, verifying the reliability of the ¹³C and ¹⁴C model. The percentage contributions show that the DIC in groundwater from these locations is mainly derived from uncontaminated groundwater, which is in agreement with the conclusions drawn earlier.

Conclusion

Analysis using only the δ¹³C values may produce ambiguous results in methanogenesis zones and where the δ¹³C values in the soil organic carbon overlap those of the petroleum hydrocarbons. Combining the use of stable carbon and radiocarbon isotope analysis can improve the quality of the evidence for the relative importance of methanogenic and non-methanogenic biodegradation processes. Moreover, because of there being more control variables, the contributions of various dissolved inorganic carbon sources can be quantified. The analysis shows that the DIC in groundwater near the contaminant plume is mainly controlled by petroleum hydrocarbon biodegradation, with methanogenesis being dominant close to the core of the plume; the dominant biodegradation process

gradually changes to non-methanogenesis downgradient, the methanogenic contribution to DIC decreasing from 28.5–32.0 % at well E1 to 5.94–6.67 % in the downgradient well Z8, and approaches zero further from the source than well Z8, which is then similar to the uncontaminated environment.

Because of the high cost of ¹⁴C testing (approximately USD650 per sample), the study focused the research on samples collected from the central line and the edge of the contaminant plume, and previously derived ¹⁴C isotope signatures for carbon source have been used. In future studies, samples for each end member (including the isotope for CH₄) and all of the monitoring wells should be collected and analyzed, so that the range of influence of the methanogenic biodegradation process can be delineated and its contribution to the DIC quantified more rigorously.

References

Aelion CM, Kirtland BC, Stone PA (1997) Radiocarbon assessment of aerobic petroleum bioremediation in the vadose zone and groundwater at an AS/SVE site. *Environ Sci Technol* 31:3363–3370

Aggarwal PK, Hinchee RE (1991) Monitoring in situ biodegradation of hydrocarbons by using stable carbon isotopes. *Environ Sci Technol* 25:1178–1180

Anderson RT, Lovley DR (1997) Ecology and biogeochemistry of in situ groundwater bioremediation. *Adv Microb Ecol* 15:289–350

Baedecker MJ, Cozzarelli IM, Eganhouse RP, Siegel DI, Bennett PC (1993) Crude oil in a shallow sand and gravel aquifer-III. Biogeochemical reactions and mass balance modeling in anoxic groundwater. *Appl Geochem* 8:569–586

Bauer JE, Spies RB, Vogel JS, Nelson DE, Southon JR (1990) Radiocarbon evidence of fossil-carbon cycling in sediments of a nearshore hydrocarbon seep. *Nature* 348:230–232

Bauer JE, Williams PM, Druffel ERM (1992) ¹⁴C activity of dissolved organic carbon fractions in the north-central Pacific and Sargasso Sea. *Nature* 357:667–670

Bauer JE, Reimers CE, Druffel ERM, Williams PM (1995) Isotopic constraints on carbon exchange between deep ocean sediments and sea water. *Nature* 373:686–689

Bhupathiraju VK, Krauter P, Holman HYN, Conrad ME, Daley PF, Templeton AS, Hunt JR, Hernandez M, Alvarez-Cohen L (2002) Assessment of in situ bioremediation at a refinery waste-contaminated site and an aviation gasoline contaminated site. *Biodegradation* 13:79–90

Bolliger C, Höhener P, Hunkeler D (1999) Intrinsic bioremediation of a petroleum hydrocarbon-contaminated aquifer and assessment of mineralization based on stable carbon isotopes. *Biodegradation* 10:201–217

Borden RC, Gomez CA, Becker MT (1995) Geochemical indicators of intrinsic bioremediation. *Ground Water* 33(2):180–189

Bugna GC, Chantona JP, Stauffer TB, MacIntyre WG, Libelo EL (2005) Partitioning microbial respiration between jet fuel and native organic matter in an organic-rich long time-contaminated aquifer. *Chemosphere* 60:177–187

Cerling TE, Solomon DK, Quade J, Bowman JR (1991) On the isotopic composition of carbon in soil carbon dioxide. *Geochim Cosmochim Acta* 55:3403–3405

- Clark I, Fritz P (1997) Environmental isotopes in hydrogeology. CRC press, New York
- Coffin RB, Pohlman JW, Grabowski KS, Knies DL, Plummer RE, Magee RW, Boyd TJ (2008) Radiocarbon and stable carbon isotope analysis to confirm petroleum natural attenuation in the vadose zone. *Environ Forensics* 9:75–84
- Conrad ME, Depaolo DJ (2004) Carbon isotopic evidence for biodegradation of organic contaminants in the shallow vadose zone of the radioactive waste management complex. *Vadose Zone J* 3:143–153
- Conrad ME, Daley PF, Fischer ML, Buchanan BB, Leighton T, Kashgarian M (1997) Combined ^{14}C and ^{13}C monitoring of in situ biodegradation of petroleum hydrocarbons. *Environ Sci Technol* 31:1463–1469
- Conrad ME, Templeton AS, Daley PF, Cohen LA (1999) Isotopic evidence for biological controls on migration of petroleum hydrocarbons. *Org Geochem* 30:843–859
- Dong WH, Zhang Y, Lin XY, Du SH (2014) Prediction of 1,2,4-trichlorobenzene natural attenuation in groundwater at a landfill in Kaifeng, China. *Environ Earth Sci* 71:941–948
- Douglas GS, McCarthy JK, Dahlen DT, Seavey JA, Steinhauer WG, Prince RC, Elmendorf DL (1992) The use of hydrocarbon analyses for environmental assessment and remediation. *J Soil Contam* 1:197–216
- Fang J, Barcelona MJ, Krishnamurthy RV (2000) Stable carbon isotope biogeochemistry of a shallow sand aquifer contaminated with fuel hydrocarbons. *Appl Geochem* 15:157–169
- Fleming JT, Sanseverino J, Saylor GS (1993) Quantitative relationship between catabolic gene frequency and expression in predicting PAH degradation in soils at town gas manufactured sites. *Environ Sci Technol* 27:1068–1074
- Hunkeler D, Höhener P, Bernasconi S (1999) Engineered in situ bioremediation of a petroleum hydrocarbon-contaminated aquifer: assessment of mineralization based on alkalinity, inorganic carbon and stable isotope balances. *J Contam Hydrol* 37:201–233
- Kao CM, Wang YS (2001) Field investigation of the natural attenuation and intrinsic biodegradation rates at an underground storage tank site. *Environ Geol* 40(4–5):622–631
- Krishnamurthy RV, Atekwana EA, Guha H (1997) A simple, inexpensive carbonate–phosphoric acid reaction method for the analysis of carbon and oxygen isotopes of carbonates. *Anal Chem* 69(20):4256–4258
- Landmeyer JE, Vroblesky DA, Chapelle FH (1996) Stable carbon isotope evidence of biodegradation zonation in a shallow jet-fuel contaminated aquifer. *Environ Sci Technol* 30:1120–1128
- Lesage S, Hao X, Kent SN (1997) Distinguishing natural hydrocarbons from anthropogenic contamination in groundwater. *Groundwater* 35(1):149–160
- Libby WF (1955) Radiocarbon dating, 2nd edn. University of Chicago Press, Chicago
- Lollar BS, Slater GF, Sleep B (2001) Stable carbon isotope evidence for intrinsic bioremediation of tetrachloroethene and trichloroethene at area 6, Dover Air Force Base. *Environ Sci Technol* 35:261–269
- Lloyd DH, Vogel JS, Trumbore S (1991) Lithium contamination in AMS measurements of C-14. *Radiocarbon* 33:297–301
- Madsen EL, Sinclair JL, Ghiorse WC (1991) In situ biodegradation: microbiological patterns in a contaminated aquifer. *Science* 252:830–833
- National Research Council (1993) In situ Bioremediation when does it work?. National Academy Press, Washington, DC
- Revesz K, Coplen TB, Baedecker MJ, Glynn PD (1995) Hult M. Methane production and consumption monitored by stable H and C isotope ratios at a crude oil spill site, Bemidji, Minnesota. *Appl Geochem* 10:505–516
- Schoell M (1984) Stable isotopes in petroleum research. *Adv Petroleum Geochem* 1:215–245
- Su XS, Lv H, Zhang WJ, Zhang YL, Jiao X (2013) Evaluation of petroleum hydrocarbon biodegradation in shallow groundwater by hydrogeochemical indicators and C, S-isotopes. *Environ Earth Sci* 69(6):2091–2101
- Suchomel KH, Kreamer DK, Long A (1990) Production and transport of carbon dioxide in a contaminated vadose zone: a stable and radioactive carbon isotope study. *Environ Sci Technol* 24:1824–1831
- Topinkova B, Nesetril K, Datel J (2007) Geochemical heterogeneity and isotope geochemistry of natural attenuation processes in a gasoline-contaminated aquifer at the Hnevice site, Czech Republic. *Hydrogeol J* 15:961–976