ELSEVIER

Chemical Geology 218 (2005) 3–13

CHEMICAL GEOLOGY
INCLUDING
ISOTOPE GEOSCIENCE

www.elsevier.com/locate/chemgeo

Compound Specific Isotopic Analysis (CSIA) of landfill leachate DOC components

Hossein Mohammadzadeh[a,b,*], Ian Clark[a], Mark Marschner[a], Gilles St-Jean[a]

[a]*Ottawa-Carleton Geosciences Centre, University of Ottawa, 140 Louis Pasteur, Ottawa, ON, Canada K1N 6N5*
[b]*Department of Geology, Faculty of Science, Ferdowsi University of Mashhad, Iran*

Received 8 March 2004; received in revised form 15 October 2004; accepted 16 January 2005

Abstract

In an attempt to better characterize the biogeochemical evolution of dissolved organic carbon (DOC) in landfill leachates, and to interpret the origin of DOC in groundwater, we have developed a new analytical technique for the compound specific isotope (^{13}C) analysis (CSIA) of DOC. This is a new operational system that measures chromatographically separated DOC compounds with a total inorganic/organic carbon analyzer (TCA) interfaced with an isotope ratio mass spectrometer (IRMS), and it represents a significant contribution to analytical technology.

Leachate samples were collected from the Trail Road Landfill (TRL) site located about 25 km to the west of the city of Ottawa, Canada. Measurements of Eh, pH, electrical conductivity, dissolved oxygen, total dissolved solids, and temperature were completed at the field site. High performance liquid chromatography (HPLC) was used to separate DOC components into fractions for separate analysis on TCA. The TCA is operated in-line with a Thermo-Finnigan Delta^{Plus} continuous-flow isotope ratio mass spectrometer (CF-IRMS) that oxidises organic carbon to CO_2 for measurement of both concentration, by infrared absorption, and $\delta^{13}C$. DOC fraction collection was based on the detection of discrete peaks of individual compounds, allowing identification of key peaks, such as acetate, with recoveries of up to 100%. The difference in $\delta^{13}C$ values for leachate acetate ($-10.7‰$ to $-16.9‰$ VPDB) and the bulk DOC ($-24.7‰$ VPDB) can be used to distinguish landfill leachate derived DOC and identify biogeochemical reactions. The enrichment of $\delta^{13}C$ in the acetate suggests that this biologically derived compound has become a substrate for secondary biogeochemical reaction, likely methanogenesis.

Keywords: Landfill leachate; Dissolved organic carbon (DOC); Chromatographically separated DOC components; Compound Specific Isotopic Analysis (CSIA); Carbon-13; Acetate; Trail Road Landfill (TRL) site

1. Introduction

The presence of dissolved organic components in landfill leachates is of some concern where such solutions may contaminate groundwaters. Their tox-

* Corresponding author. Department of Earth Sciences, University of Ottawa, 140 Louis Pasteur, Ottawa, ON, Canada K1N 6N5. Tel.: +1 613 562 5800x6282; fax: +1 613 562 5192.
E-mail addresses: hmoha769@science.uottawa.ca (H. Mohammadzadeh), idclark@uottawa.ca (I. Clark).

0009-2541/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.chemgeo.2005.01.022

icity is not only dependent on the nature of dissolved organic matter (DOM), but also on concentration and availability (Alkalay et al., 1998). Dissolved organic carbon (DOC) is a complex component of groundwaters and leachate that includes high molecular weight humic and fulvic acids as well as lighter weight fatty acids such as acetate. DOC largely originates from biodegradation of solid organic wastes in the landfill. Even in a landfill that is decades old, the leachate may contain DOC at the level of thousands of milligrams per litre (Christensen et al., 1998). Although natural DOC plays an important role in freshwater systems for the mobility of toxic heavy metals and other pollutants, DOC may itself be a groundwater contaminant (Christensen et al., 1998; Drever, 1997).

Tracing the sources and biotransformations of DOC in groundwaters is aided by isotope analysis of DOC fractions (e.g., Wassenaar et al., 1990). Traditional separations of DOC are methodological in nature, focusing on acid–base separations and hydrophobicity. Here we present a new method that characterizes DOC on the basis of concentrations and ^{13}C of discrete DOC compounds, mainly light fatty acids, separated by liquid chromatography. The method is applied to a methanogenic leachate from the Trail Road municipal Landfill (TRL).

2. Site description and sampling

The TRL is owned and operated by the City of Ottawa and is located in the former municipality of Nepean, about 25 km west of the city centre (Fig. 1). Site operation commenced at the Nepean landfill site, just beside the TRL site in the early 1960s, and

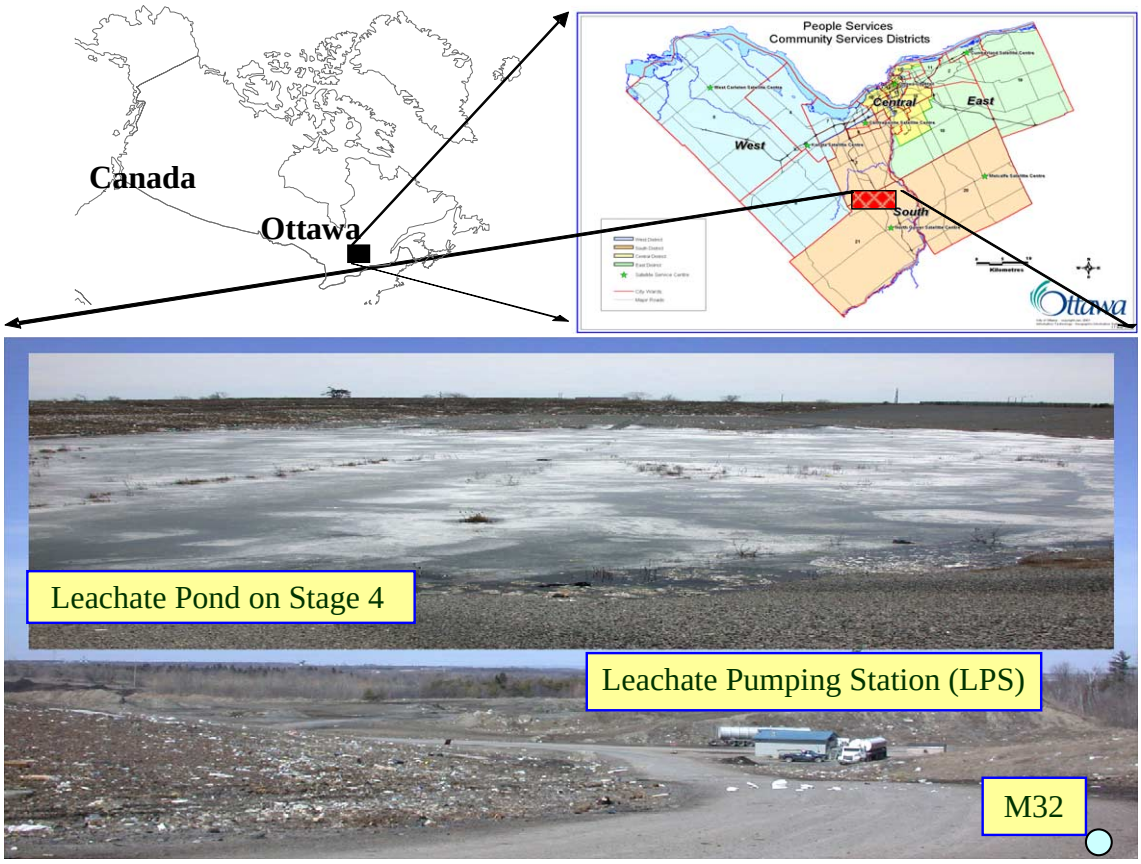


Fig. 1. Trail Road Landfill (TRL) site, the leachate pumping station and the leachate pond of the TRL Site.

continued at the Nepean landfill for approximately 20 years. The landfill was extended in 1980 into the TRL site. Right now the TRL accepts annually about 2.47×10^5 tons of residential, industrial, commercial and institutional refuse, plus construction and demolition waste from the City of Ottawa. Landfill leachate is the main source of groundwater pollution in this area (City of Ottawa, 2001). The previous physical and chemical hydrogeological studies show there is some leachate influence on groundwater beneath older stages of the TRL site which has no bottom liner or leachate collection system (City of Ottawa, 2002).

Sampling was done at three locations within the TRL site; at the leachate pumping station (LPS), in M32 monitoring well and at the leachate pond (Fig. 1). Samples were collected in amber glass bottles and stored at 4 °C to limit any continued microbial activity. Samples taken back to the lab were filtered using 0.45 μm cellulose nitrate membrane filters (25 mm ø circles, Cat. No. 7184002, Whatman International Ltd, Maidston, Germany) and were analyzed immediately in the laboratory. Samples were diluted with deinonized water (DIW) to get a sufficient volume for the TCA. Then, the leachate's DIC and DOC concentration and the $\delta^{13}C$ values of DIC and DOC were measured by TCA and IRMS, respectively. Field measurements of Eh, pH, electrical conductivity, dissolved oxygen, total dissolved solids, and temperature were performed in the field. Table 1 shows the measured field parameters, DIC/DOC concentrations and stable isotopic composition ($\delta^{13}C$, $\delta^{18}O$, $\delta^{2}H$) of the leachate.

3. Methodology

3.1. Compound specific isotope analysis of DOC

A high performance liquid chromatograph (HPLC), a total inorganic/organic carbon analyzer (TCA), and subsequent continuous-flow isotope ratio mass spectrometry (CF-IRMS) were used to separate DOC components and to measure the concentrations and ^{13}C-isotopic compositions of each component in standards and leachate samples. Since the main aim of this work was to recover discrete organic fractions, the use of carrier solvents containing organic or inorganic carbon was precluded. After several experiments with different mobile phase systems (e.g., H_2SO_4, H_3PO_4, NaH_2PO_4) under different HPLC parameter conditions, the best separation was achieved on a reverse C-18 column using a dilute H_3PO_4 mobile phase. Samples were eluted using 5 mM H_3PO_4 (pH 3) with a gradient flow rate of 3 to 8 ml min^{-1}, a UV detector wavelength setting of 230 nm, and a column temperature of 60 °C (see Table 2). A 5 mM H_3PO_4 mobile

Table 1
Field parameters, DIC, DOC concentrations and stable isotope values of Trail Road Landfill (TRL) leachate

Sample location	Sample no.	Date of sampling	Field measurements					
			T (°C)	pH	Eh (mv)	EC (μm/cm)	TDS (mg/l)	DO (mg/l)
Leachate pumping station (LPS)	L1	6-Nov-03	15.4	6.69	169	5120		3.03
		11-Apr-03	17.6	6.77	306	6090	3080	
Stage 4 leachate pond	L2	11-Apr-03	10.3	6.07	376	4786	2630	

			Lab. measurements					
			DIC (mgl^{-1})	DOC (mgl^{-1})	$\delta^{13}C_{DOC}$ (‰)	$\delta^{13}C_{DIC}$ (‰)	$\delta^{18}O$ (‰)	$\delta^{2}H$ (‰)
Leachate pumping station (LPS)	L1	6-Nov-03	509	245	−24.6	9.9	−12.4	−73.2
		11-Apr-03	832	202	−24.4		−15.4	−92.4
		10-Aug-02	647	300	−25.0	15.4	−8.4	−42.3
Stage 4 leachate pond	L2	11-Apr-03	42	10	−24.8		−15.6	−116.5
Monitoring well on landfill	M32	6-Nov-03	200	4871	−28.8	7.0	−9.8	−68.4
		12-Jul-04	339	5125	−17.5	10.7	−10.0	−70.4

The sample locations are shown in Fig. 1.
A large percentage of Stage 4's pond was precipitation, even snow and ice was seen in pond.

Table 2
HPLC, TCA and Delta^{Plus} IRMS operating conditions

HPLC
Column: C18 column 25*0.94 cm (Preparative column)
Pre-column: Cartridge precolumn
Mobile phase: 5 mM H_3PO_4, pH 3.00
UV wavelength: 230 nm
Sample loop: 1.00 ml
Injection volume: 1 ml
Temperature: 60 °C
Solvent flow rate: gradient and isocratic time events
• Gradient program for standards
0:00–19:00 min 3 ml min^{-1}
19:00–20:00 min transition 3 to 8 ml min^{-1}
20:00–26:00 min 8 ml min^{-1}
26:00–27:00 min transition 8 to 3 ml min^{-1}
27:00–40:00 min 3 ml min^{-1}
• Isocratic program for leachate samples and acetic acid:
0.00–32:00 min 2 ml min^{-1}
Fractions collector procedures:
1. Set HPLC optimized conditions
2. Set fraction windows in FC
3. Prepare samples and standards
4. Prepare suitable time events, method and sequence
5. Run powerstream
6. Inject samples
7. Run FC
8. Load injected samples in HPLC column
9. Run UV detector of HPLC
TCA
Model: 1010 TCA
TIC reagent: 300 µl 5% H_3PO_4
• Reaction time: 2:00 min
• Detection time: 1:00 min
• Reaction temperature: 90 °C
TOC reagent: 13001 of 100 gl^{-1} $Na_2S_2O_8$
• Reaction time: 3:00 min
• Detection time: 1:30 min
• Reaction temperature: 97 °C
Rinse: 25 ml DIW
Rinse per sample: 2
Rinse temperature: 85 °C
Standby temperature: 85 °C
Sample loop (calibrated): 1.00 ml
Carrier gas: N_2 and He
Calibration standards:
• 0 ppmC DIW (H_2O)
• 5 ppmC citric acid ($C_6H_8O_7CH_2O$)
• 10 ppmC KHP
• 20 ppmC sucrose ($C_{12}H_{22}O_{11}$)
Delta^{Plus} IRMS
Model:
ConFlow interface: III
Carrier gas: He
Flow rate: 110 ml min^{-1}

phase was flushed through the column for 10 min at 8 ml min^{-1} to remove any organic materials retained on the column between samples. After each multi-sample injection session, the column was cleaned with acetonitrile and DIW. The fraction collector was used to collect dissolved organic compound fractions based on time and on peak detection. Finally, the concentration and isotopic composition of DOC components were measured from the collected fractions on the TCA interfaced with CF-IRMS.

Quantitative DOC and DIC measurements were made with an OI Instruments Model 1010 Total Carbon Analyzer where organic carbon was converted to CO_2 by a wet oxidation method (St-Jean, 2001). Phosphoric acid (5% H_3PO_4) and sodium persulfate ($Na_2S_2O_8$) were used to convert DIC and organic materials to CO_2, respectively. DIC was removed from the sample by acidification with H_3PO_4, and then DOC was reacted separately with $Na_2S_2O_8$; the procedure could not be reversed due to the volatility of DIC which could have caused an isotopic shift in the residual aliquot. Likewise, volatile organic species (VOS) could not be analyzed quantitatively by the TCA method.

The purged CO_2 following sample oxidation was measured by a non-dispersive infrared detector (NDIR) and was subsequently sent to a gas scrubber followed by the ConFlow III interface for $\delta^{13}C$ IRMS analysis of DIC and DOC (Table 2; Fig. 2) (St-Jean, 2001, 2003). All concentrations in this paper are expressed in mg·l^{-1} of carbon. Isotope results are expressed in standard δ-‰ notation against the international VPDB standard (Vienna PeeDee Belemnite).

The combination of HPLC for separation, TCA for quantifying the DOC components, and IRMS for measuring the $\delta^{13}C$ values is ideal for the sensitive determination of dissolved organic materials in leachate and leachate-polluted groundwater.

3.2. Verification with standard solutions

Initial standardization using some simple short-chain organic acid standards was preformed prior to analysis of samples (Marschner et al., 2005). This initial experimentation was done in order to optimize the HPLC parameters and to test the integrity of fraction recovery both quantitatively and isotopically

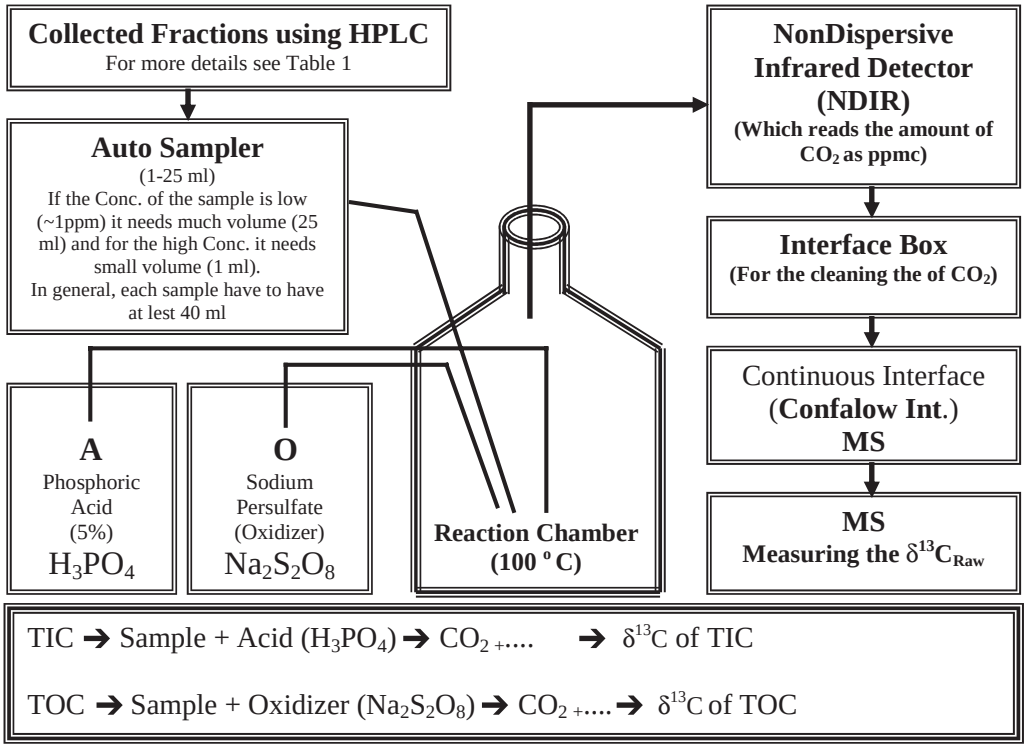
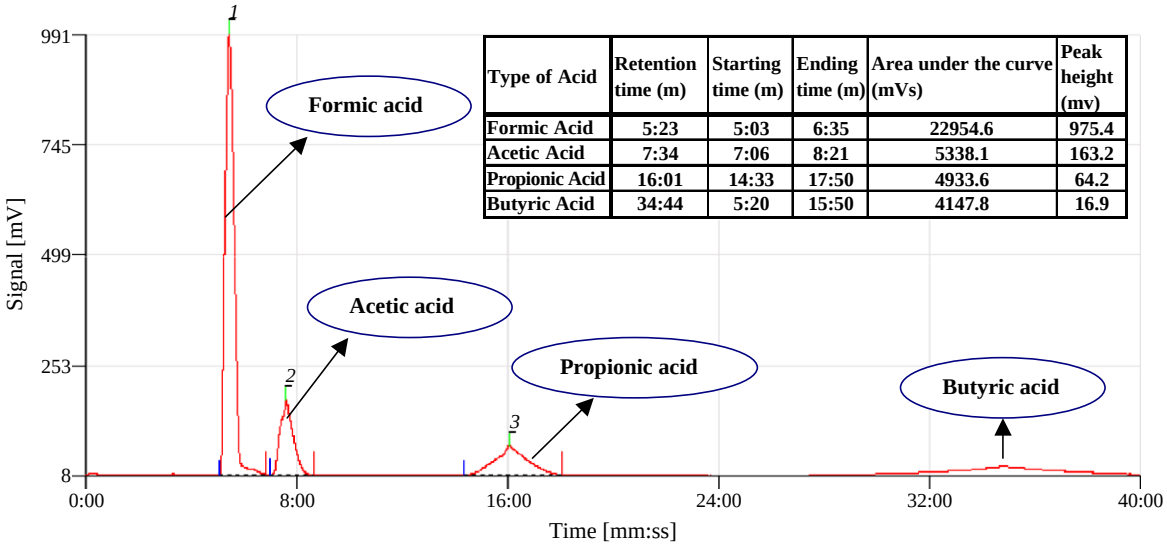


Fig. 2. Schematic illustration of TCA and CF-IRMS interface.



Type of Acid	Retention time (m)	Starting time (m)	Ending time (m)	Area under the curve (mVs)	Peak height (mv)
Formic Acid	5:23	5:03	6:35	22954.6	975.4
Acetic Acid	7:34	7:06	8:21	5338.1	163.2
Propionic Acid	16:01	14:33	17:50	4933.6	64.2
Butyric Acid	34:44	5:20	15:50	4147.8	16.9

Fig. 3. A typical HPLC chromatogram and its attribute table of a standard solution containing formic acid, acetic acid, propionic acid and butyric acid in a concentration of 1000 mgl^{-1} each. The experimental parameters are shown in Table 2.

after separation by HPLC. The reproducibility of the retention times was determined by several successive injections of a standard "cocktail" solution. After optimization of the chromatographic parameters, the column resolved the four separate and sharp peaks of the standard cocktail. Fig. 3 shows a typical HPLC chromatogram and the attribute table of these standards.

Standard DOC components, classified by their column retention times, were collected by a programmable fraction collector. Windows in the Fraction Collector (FC) were determined by: (1) the HPLC's time event, (2) the attribute and peak table of the out coming chromatogram, (3) the flow rate of the mobile phase, and (4) the travel time of the mobile phase between the UV detector and the FC's tubes. Finally, after adding some DIW to obtain the required volume, the collected DOC fractions were run on the TCA/CF-IRMS system for concentration and $\delta^{13}C$ measurement.

The results of two different runs are summarized in Table 3. Sample blanks, the carrier itself, were prepared before starting to collect fractions and concentration of the each DOC components were corrected by subtracting the blank concentration. The reproducibility of the analytical results for $\delta^{13}C$ in the

Table 3
DOC concentrations and $\delta^{13}C$ values of standards and leachate fractions based on peak retention times and 5 min time intervals in the chromatogram

Sample ID		HPLC				TCA and IRMS	
		Fraction collector	Flow rate (ml min^{-1})	Number of injections	Total collected volume (ml)	Recovered carbon (mgl^{-1})	$\delta^{13}C_{VPDB}$ (‰)
Standards							
Acetic acid 1000 mgl^{-1}		10:30–13:30	2	2	12	1019	−36.6
Cocktail first run	Formic acid fraction	5:00–07:00	3	1	6	1014	−19.0
	Acetic acid fraction	07:00–09:00	3	1	6	822	−36.5
	Propionic acid fraction	14:30–18:30	3	1	12	932	−27.9
	Butyric acid fraction	29:00–40:00	3	1	33	894	−28.0
Cocktail second run	Formic acid fraction	5:00–07:00	3	1	6	938	−18.8
	Acetic acid fraction	07:00–09:00	3	1	6	855	−37.0
	Propionic acid fraction	14:30–18:30	3	1	12	884	−28.9
	Butyric acid fraction	29:00–40:00	3	1	33	897	−26.4
Coctail (containing FA, AA, PA and BA in a concentration of 1000 mg l^{-1})					# 1	3859	−27.9
					# 2	3709	−28.2
					# 3	3960	−28.3
Leachate samples							
Acetic acid in LPS #1		10:30–13:30	2	3	18	24	−16.9
Acetic acid in LPS #2		10:30–13:30	2	3	18	23	−17.0
Acetic acid in LPS #3		10:30–13:30	2	3	18	25	−16.8
Acetic acid in LPS+AA 1000 mg l^{-1}		10:30–13:30	2	1	6	542	−36.4
Acetic acid in M32[a] #1		10:30–14:00	2	1	7	1666	−10.7
Acetic acid in M32 #2		10:30–14:00	2	1	7	1755	−10.8
Propionic acid in M32 #1		20:30–29:30	2	1	18	961	−19.3
Propionic acid in M32 #2		20:30–29:30	2	1	18	1313	−20.6
Acetic acid+Propionic acid in M32			2	1	25	2518	−14.8
5-min time interval fractions in LPS chromatogram							
Fraction #1		00:00–05:00	na	na	na	na	na
Fraction #2		05:00–10:00	2	1	10	86	−21.1
Fraction #3		10:00–15:00	2	1	10	61	−25.1
Fraction #4		15:00–20:00	2	1	10	45	−25.4
Fraction #5		20:00–25:00	2	1	10	39	−24.7

[a] Sample taken from M32 monitoring well represent leachate.

acetic acid (−36.6‰ VPDB), its fraction (−36.5/−37.0‰ VPDB) and for the concentration of acetic acid are satisfactory (1019 $mg \cdot l^{-1} \cong 1000\ mg \cdot l^{-1}$). However, as noted in Table 3, in order to ensure that we will not have any contributions from the adjacent peak of the other acids, the reproducibility of acetic acid fraction for the assigned windows in fraction collector is not exactly 1000 $mg \cdot l^{-1}$. Since the $\delta^{13}C$ values are the target, the tail of the chromatogram was not significant to the results and therefore not collected. Dilution of samples is another reason for this discrepancy. As shown in Table 3 the recovered concentration of the standard DOC components and their $\delta^{13}C$ in both runs are close in value. This indicates the high precision of the measurements.

3.3. Identification and analysis of acetate in leachate

Acetic acid (CH_3COOH), a simple short-chain fatty acid, is a low molecular weight molecule that can be produced during the oxidation of complex organic molecules in anoxic environments such as landfill leachate by fermentive bacteria. Typically, municipal landfill leachate has a pH range of 4.5–9.0 (Christensen et al., 2001). In this pH range, which for the landfill leachate exceeds the p*K*a of acetic acid, at 4.7,

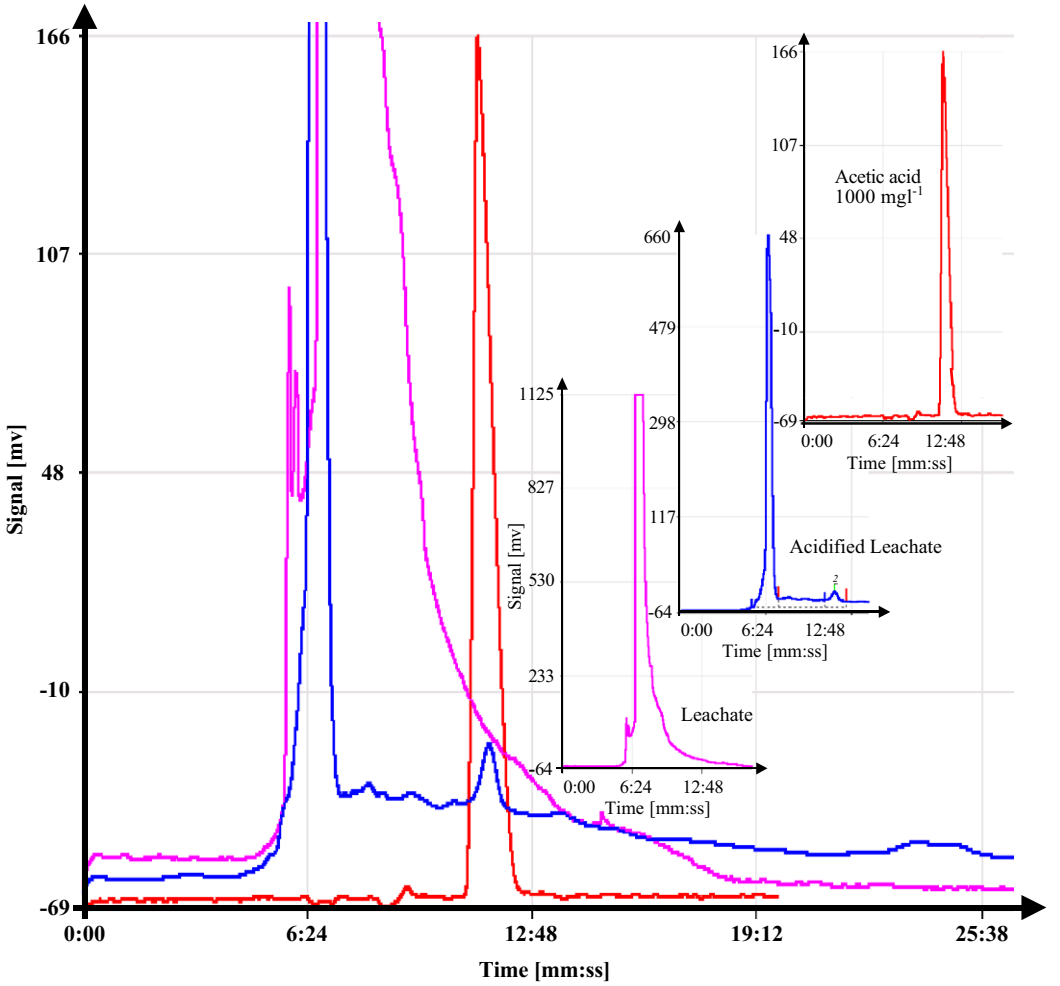


Fig. 4. The HPLC chromatograms of leachate, acidified leachate and 1000 mgl^{-1} acetic acid. The acetate peak appears after acidifying the leachate using concentrated (85%) phosphoric acid. Four drops of H_3PO_4 added to 20 ml leachate sample reduce the pH of the leachate to 2.2.

most acetic acid will be present as a deprotonated ion, CH_3COO^-. This form of acetic acid has a different retention time than the acetic acid in the HPLC system. CH_3COO^- was converted to CH_3COOH by acidification concentrated (85%) phosphoric acid (H_3PO_4). The CH_3COOH peak was easily detected by the UV detector and can be identified on the HPLC chromatogram (Fig. 4). For the leachate and the standard acetic acid, all the HPLC parameters except for time events, were assigned the same values as applied for the standard DOC components (see Table 2). Acetic acid in the leachate sample was identified by retention time (Rt) on the basis of the Rt determined for commercial acetic acid (Fig. 4).

Quantification of leachate acetate concentration was done by running acidified leachate samples on the HPLC under the same conditions as were applied for a series of standards. The concentrations were determined by measuring the area under the curve (AUC) and comparing the results with the calibration curves. Verifications were performed by spiking leachate samples with the acetic acid standard solution (50% leachate and 50% 100 $mg \cdot l^{-1}$ acetic acid). The concentration of acetic acid in this spiked solution based on our calibration curve, was 70.9 $mg \cdot l^{-1}$ within 15% the calculated value of 61.3 $mg \cdot l^{-1}$ from the average of leachate (23.5 ± 1.63 $mg \cdot l^{-1}$) and the standard solution (100 $mg \cdot l^{-1}$).

3.4. DOC and $\delta^{13}C$ of the leachate acetic acid based on peak retention times

As noted earlier, the HPLC parameters for the leachate, with the exception of the time events, were assigned the same values as were applied for the standard DOC components. In order to attain separate and sharp peaks for the leachate, the time event was changed to isochratic condition with 3 ml min^{-1} flow rate Table 2. Fig. 4 shows the acidified leachate HPLC chromatogram which contains two peaks. The first peak is the solute front, present due to acidifying the leachate by H_3PO_4. The second peak, with the peak retention time of 11.5 min, represents elution of the acetic acid. For acetic acid, the HPLC eluent was collected three times between 10.5 and 13.5 min of the run time. The collected fraction was diluted by a factor of 2 to bring up to volume for the TCA, then, run on the TCA/CF-IRMS system for the concentration and $\delta^{13}C$ measurements. The results are summarized in Table 3.

4. Discussion

The aim of this work is the integration of such compound-specific isotope and concentration data with other geochemical and isotope parameters of the solution for insights to the origin and biogeochemical reactions taking place in the DOC of groundwater. Relevant data are summarized in Table 1. The DOC concentration of the TRL pumping station leachate varies between 300 $mg \cdot l^{-1}$, in August 2002, and 202 $mg \cdot l^{-1}$ in April 2003, reflecting the amount of dilution from precipitation during different seasons. This variation in seasonal contributions is demonstrated by the strong shift in stable isotopes of the leachate water ($\delta^{18}O$ and $\delta^{2}H$, Table 1), which vary from −8.4‰ for $\delta^{18}O$ in August to −15.6‰ in April. The average DOC concentration and $\delta^{13}C$ values of the leachate are 249 $mg \cdot l^{-1}$ and −24‰ VPDB (Table 1).

By contrast, the samples from M32, a monitoring well completed at the base of the unlined Stage 1 landfill, has DOC concentrations varying between 4871 and 5125 $mg \cdot l^{-1}$ for two sampling dates, and little to no change in the stable isotopes of water. This site is clearly unaffected by seasonal dilution and can be considered an undiluted leachate sampling site. The $\delta^{13}C$ of this DOC is also remarkable, in that it varies from −28.8‰ (November 2003) to −17.5‰ (July 2004) between the two seasons. This remarkable shift suggests a considerable turnover of DOC likely due to variable rates of biological activity.

In order to assess the retention time distribution of the DOC components in leachate, the fractions were collected by: (1) 5-min intervals, and (2) peak retention times using the programmable fraction collector. Data from the analysis of leachate from LPS and from M32 are summarized in Table 3. For the LPS data, the 5- to 10-min fractions, which resulted in the highest peak in the HPLC chromatogram, had the highest DOC concentration of 86 $mg \cdot l^{-1}$, and a $\delta^{13}C$ value of −21.1‰ VPDB. The DOC concentrations of the subsequent 5-min fractions were lower than that of 5- to 10-min fractions. Each equal time interval had a specific

amount of DOC and $\delta^{13}C$, which may be used for tracing leachate derived DOC. Peak separation was then attempted as a more effective means to identify and isotopically characterize the specific compounds.

The acetate peak in leachate was identified by injecting acidified leachate at pH 2.3 in the HPLC under optimized conditions. Note that at this pH, humic acids become largely insoluble, and so this component of the DOC was precipitated from solution prior to injection into the HPLC. The concentration of acetate peak, determined by the area under the curve measurement, was 23 ± 1 mg·l^{-1} for the LPS leachate, and by running the collected acetate fraction on the TCA/CF-IRMS system was 24 ± 1 mg·l^{-1} (Table 3). The $\delta^{13}C$ value of acetate in the leachate is variable between collection sites and sample dates, ranging between −10.7‰ and −17.0‰ VPDB, which is significantly enriched above the precursor DOC of the leachate (−24.7‰ VPDB). This difference of $\delta^{13}C$ values implies that microbial processes are involved in the degradation of acetate fraction leading to the enrichment of $\delta^{13}C$ in leachate's acetate. As acetate is a product of anaerobic degradation of vegetation (typically represented as carbohydrate, CH_2O), and subsequently used in methanogenic reactions, one can begin to trace the cycling of carbon in such systems.

The $\delta^{13}C$ values for M32 acetate and bulk DOC show a pattern of enrichment between the two sample dates that is reflected by other parameters as well. The $\delta^{13}C$ of DIC experiences an enrichment of close to 4‰ over this period. Similarly, the $\delta^{13}C$ of methane (−50.8‰) is highly enriched in the later sample as compared with biogenic methane from other landfills, which is generally in the range of <−60‰ (Clark and Fritz, 1997). Enrichment trends in acetate, bulk DOC and other carbon components are evidence of substrate consumption, which results in an Rayleigh type enrichment in ^{13}C. While preliminary, these data reflect a series of biogeochemical reactions that vary in rate through time.

In order to assess the efficiency of HPLC fraction collection and preservation of isotope signals during separations, experimental data were compared with theoretical values in a carbon mass balance. First, the mass balance was performed based on the following equation using $\delta^{13}C$ and the recovered carbon concentration of each four standard DOC component fractions (formic acid (FA), acetic acid (AA), propionic acid (PA), butyric acid (BA)) and their mixture:

$$\begin{aligned}\delta^{13}C_{\text{Cocktail}} \times C_{\text{Cocktail}} = &\left(\delta^{13}C_{\text{FA}} \times C_{\text{FA}}\right) \\ &+ \left(\delta^{13}C_{\text{AA}} \times C_{\text{AA}}\right) \\ &+ \left(\delta^{13}C_{\text{PA}} \times C_{\text{PA}}\right) \\ &+ \left(\delta^{13}C_{\text{BA}} \times C_{\text{BA}}\right)\end{aligned}$$

where $\delta^{13}C$ is the per mil (‰) value of each component and C is the carbon concentration.

For calculating the mass balance of the leachate acetate, the same equation was applied for the M32 leachate sample. Relevant data are summarized in Table 4. Calculated errors are approximately 7% and 6% for the standards and leachate data, respectively (Table 4). Comparison of the recovered mass of carbon and $\delta^{13}C$ with initial data also shows the high

Table 4
Mass balance data for the standards and leachate fractions

Parameter		Standards					Leachate in M32			
		Formic A	Acetic A	Propionic A	Butyric A	Cocktail	Acetic peak #1	Unknown peak #2	Propionic peak #3	Whole chromatogram (peak #1, 2, 3)
Initial carbon	mass (mg)	1.00	1.00	1.00	1.00	4.00				
	$\delta^{13}C_{VPDB}$	−18.7	−37.6	−28.7	−27.5	−27.9				
Recovered carbon	mass (mg)	0.970	0.832	0.902	0.890	3.859	1.665	0.060	0.96	2.59
	$\delta^{13}C_{VPDB}$	−18.9	−36.8	−28.4	−27.2	−27.9	−10.7	−18.8	−19.3	−15.4
$\delta^{13}C^{*}M_C$		−18.17	−31.27	−25.92	−24.46	−107.73	−17.80	−1.12	−18.51	−39.91
Sum		−99.82					−37.43			
Error (%)		7.34					6.22			

efficiency of HPLC fraction collection. Consequently, the precision of chromatographically separated DOC compounds and the measured concentration and $\delta^{13}C$ values of these DOC fractions using a TCA interfaced with IRMS is high and this technique can be used to: (1) distinguish landfill-derived DOC, (2) identify metabolic pathways, and (3) identify biogeochemical reactions in leachate contaminated aquifers.

5. Conclusion

HPLC, TCA and IRMS are important analytical instruments for characterizing dissolved organic matter in groundwater. However, we show that they can be interfaced sequentially in series for the quantitative and ^{13}C-isotopic analyses of discrete organic compounds in naturally occurring waters. This new methodology generates reproducible results, which prove that chromatographically separated organic fractions can be used for compound-specific isotopic analysis (CSIA). Such analyses complement routine isotopic characterization of DOC components using methodological separations such as on acid and base solubilities and hydrophobicity. Further, it provides access to low concentrations using reasonable sample sizes.

Optimal setting for the HPLC parameters and the reproducibility of the peak retention times were determined by several successive injections of a standard solution containing formic acid, acetic acid, propionic acid, and butyric acid (1000 $mg \cdot l^{-1}$ each). Standard DOC components, acetate peak, and 5-min time intervals of the TRL leachate chromatogram, classified by time and peak retention times were collected with a programmable fraction collector and later run on the TIC-TOC/CF-IRMS system for concentration and measurement of $\delta^{13}C$. Each peak in the leachate chromatogram is related to a specific DOC compound (e.g., acetic acid). While time interval fractions can be used, peak separation and collection is the preferable method for quantification of DOC components and their subsequent isotope analysis.

The significant difference of $\delta^{13}C$ values of the leachate's acetate (−10.7‰ to −16.9‰ VPDB) versus $\delta^{13}C$ value of the leachate (−24.7‰ VPDB) provides insights into cycling of carbon and biogeochemical reactions of DOC in this preliminary case study of a landfill leachate. Further applications will include other groundwaters and waste waters contaminated by elevated levels of DOC, as well as the analysis of natural DOC in methanogenic and other groundwaters.

Acknowledgments

Access to the Trail Road landfill was provided by Brent Loney, Eric Shilts (Dillon Consulting Limited), Trish Johnson, Peter Filipowich and Blaine Coons (City of Ottawa). Engineers are thanked for their generous assistance during the July 2004 sampling, and Paul Smolkin, Berend Velderman, Marc Lafleche, Jaime Oxtobee, Charles Albert (Golder Associates Ltd., 2001) during sampling and data gathering in 2003. We gratefully acknowledge Wendi Abdi, Patricia Wickham, Paul Middlestead and Monika Walemany for their support in the isotope and geochemistry laboratories. We also wish to thank Shane W. Greene and Annie Bourbonnais for their help on the field. **[LW]**

References

Alkalay, D., Guerrero, L., Lema, J.M., Mendez, R., Chamy, R., 1998. Review: anaerobic treatment of municipal sanitary landfill leachates: the problem of refractory and toxic components. World J. Microbiol. Biotechnol. 14 (3), 309–320.

Christensen, J.B., Jensen, D.L., Gron, C., Filip, Z., Christensen, T.H., 1998. Characterization of the dissolved organic carbon in landfill leachate-polluted groundwater. Water Res. 32 (1), 125–135.

Christensen, T.H., Kjeldsen, P., Bjerg, P.L., Jensen, D.L., Christensen, J.B., Baun, A., et al., 2001. Review biogeochemistry of landfill leachtae plumes. Appl. Geochem. 16, 659–718.

City of Ottawa, 2001. Trail and Nepean landfill sites for the 2000 monitoring and operating program.

City of Ottawa, 2002. Trail Road waste facility landfill operating and expantion project, EA/EPA Document, App. B.

Clark, I.D., Fritz, P., 1997. Environmental Isotopes in Hydrogeology. Lewis Publishers, Boca Raton, FL. 328 pp.

Drever, J.J., 1997. The Geochemistry of Natural Waters: Surface and Groundwater Environments, Third Edition. Prentice Hall. 436 pp.

Golder Associates, Ltd., 2001. Historical leachate data of Trail Road and Nepean landfills Report.

Marschner, M., Middlestead, P., Clark, P., 2005. Using a simple HPLC separation and fraction collection methodology to achieve compound-specific isotopic analysis (CSIA) for dissolved organic compounds. Rapid Commun. Mass Spectrom. 19, 261–268.

St-Jean, G., 2001. ^{13}C analysis of DIC and DOC in CF-IRMS using a TOC analyser. The 4th International Symposium on Applied Isotope Geochemistry (AIG-4). California, June 21–25, 2001 (http://wwwrcamnl.wr.usgs.gov/kinwater/default.htm).

St-Jean, G., 2003. Automated quantitative and isotope (^{13}C) analysis of dissolved inorganic carbon and dissolved organic carbon in continuous-flow using a total organic carbon analyser. Rapid Commun. Mass Spectrom. 17, 419–428.

Wassenaar, L., Aravena, R., Fritz, P., Barker, J., 1990. Isotopic composition (^{13}C, ^{14}C, 2H) and geochemistry of aquatic humic substances from groundwater. Org. Geochem. 15, 383–396.