Biochemical Indicators for the Bioavailability of Organic Carbon in Ground Water

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Abstract

The bioavailability of total organic carbon (TOC) was examined in ground water from two hydrologically distinct aquifers using biochemical indicators widely employed in chemical oceanography. Concentrations of total hydrolyzable neutral sugars (THNS), total hydrolyzable amino acids (THAA), and carbon-normalized percentages of TOC present as THNS and THAA (referred to as "yields") were assessed as indicators of bioavailability. A shallow coastal plain aquifer in Kings Bay, Georgia, was characterized by relatively high concentrations (425 to 1492 µM; 5.1 to 17.9 mg/L) of TOC but relatively low THNS and THAA yields (~0.2%–1.0%). These low yields are consistent with the highly biodegraded nature of TOC mobilized from relatively ancient (Pleistocene) sediments overlying the aquifer. In contrast, a shallow fractured rock aquifer in West Trenton, New Jersey, exhibited lower TOC concentrations (47 to 325 µM; 0.6 to 3.9 mg/L) but higher THNS and THAA yields (~1% to 4%). These higher yields were consistent with the younger, and thus more bioavailable, TOC being mobilized from modern soils overlying the aquifer. Consistent with these apparent differences in TOC bioavailability, no significant correlation between TOC and dissolved inorganic carbon (DIC), a product of organic carbon mineralization, was observed at Kings Bay, whereas a strong correlation was observed at West Trenton. In contrast to TOC, THNS and THAA concentrations were observed to correlate with DIC at the Kings Bay site. These observations suggest that biochemical indicators such as THNS and THAA may provide information concerning the bioavailability of organic carbon present in ground water that is not available from TOC measurements alone.

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Introduction

Dissolved and particulate organic carbon is present at measurable concentrations (>0.3 mg/L; 25 µM) in ground water produced from a variety of aquifer systems (Thurman 1985). This organic carbon is significant because it can be a substrate for heterotrophic microbial metabolism and drives many of the reduction/oxidation (redox) processes that occur in ground water systems (Böhlke et al. 2002; Puckett et al. 2002). These redox processes, in turn, have important affects on the chemical quality of ground water, including the mobilization/immobilization of naturally occurring contaminants as well as the biodegradation of anthropogenic contaminants (U.S. EPA 2002; McMahon and Chapelle 2008). In particular, bioavailable carbon drives the reductive dechlorination of chlorinated ethenes, a process that contributes to the natural attenuation of these contaminants (U.S. EPA 1998; Rectanus et al. 2007).

Despite this importance, the chemical nature of the dissolved and particulate organic carbon present in ground water systems is poorly understood (Thurman 1985; Aiken 1989). The most commonly measured organic carbon parameters, dissolved organic carbon (DOC) and total organic carbon (TOC), typically involve oxidation of organic carbon to carbon dioxide and thus provide little or no information about the chemical composition of the organic carbon pool. This, in turn, makes it difficult to relate concentrations of TOC or DOC directly to bioavailability.

Natural DOC contains thousands of compounds characterized by complex chemical structures and exhibiting a wide range of bioavailability to microorganisms (Thurman 1985; Aiken 1989; Volk et al. 1997; review by Benner [2003]). Characterizing the chemical structures present in DOC presents significant analytical challenges (Hertkorn et al. 2006), and a variety of approaches, including spectrofluorometric characterization (McKnight et al. 2001) and specific ultraviolet absorbance (Weishaar et al. 2003), have been applied to understanding sources and composition of DOC. Finally, the bioavailability of DOC also depends upon the kinds and amounts of ambient nutrients, trace metals, and vitamins (McDowell et al. 2006) as well as the metabolic capability of microorganisms present in a given system (Trulleyova and Rulik 2004). Given this inherent complexity, the approach for investigating DOC bioavailability in the fields of chemical oceanography and limnology has been to identify biochemical indicators that can be correlated with an operational definition of "bioavailability." This operational definition is often based on observed rates of DOC biodegradation (Servais et al. 1989; McDowell et al. 2006). The principal goal of these approaches has been to develop a biochemical framework for comparing the bioavailability of DOC found in different hydrologic environments (Benner 2003).

The relation between the relative abundance of total hydrolyzable neutral sugars (THNS) and the total hydrolyzable amino acids (THAA) present in sedimentary organic matter (SOM) was initially investigated as an indicator of the degree of diagenetic alteration (Ittekkot 1988; Hedges et al. 1994, 2000; Cowie and Hedges 1994). It was found that yields of THNS and THAA ("yield" is defined as the carbon-normalized percentage of TOC present as THNS or THAA) decreased as the extent of diagenetic alteration increased. Furthermore, since the extent of diagenetic alteration of SOM was observed to be inversely proportional to its bioavailability,

$$\text{Bioavailability of SOM} \propto \frac{1}{\text{Extent of SOM diagenetic alteration}} \quad (1)$$

these results suggested that THNS and THAA yields could also serve as indicators for the bioavailability of organic matter.

Several studies in the oceanograpic and limnologic literature explored this approach experimentally, assessing the bioavailability of DOC in surface water by measuring concentration changes in DOC, THNS, and THAA over time in microcosm experiments with natural assemblages of microflora (Weiss and Simon 1999; Volk et al. 1997; Gremm and Kaplan 1998; Amon et al. 2001). A compilation of these results (Benner 2003) indicates that THNS and THAA accounted for between 29% and 100% of the total bioavailable carbon present in DOC and were preferentially mineralized compared to bulk DOC.

Previous studies have also considered concentrations of THNS as indicators of sources of DOC and its degree of diagenetic alteration in ground water systems (Routh et al. 2001 and references therein). Specifically, Routh et al. (2001) used the sum of certain neutral sugars (fucose + rhamnose) to assess sources, and THNS yield to assess the degree of diagenetic alteration. The results of this study showed that the probable precursor of DOC in ground water of the Yegua Aquifer of Texas was predominantly angiosperm leaves and suggested that shallower oxic ground water was more diagenetically altered than deeper anoxic ground water. The results of Routh et al. (2001) suggest the possibility that concentrations and yields of THNS may be useful indicators of TOC bioavailability in ground water systems as well as in ocean water (Equation 1). The purpose of this article is to investigate the potential of certain classes of biomolecules, specifically neutral sugars and amino acids, to serve as biochemical indicators of TOC bioavailability in two hydrologically distinct ground water systems located in the eastern United States.

Methods and Materials

Hydrologic Settings of the Kings Bay and West Trenton Sites

The Kings Bay site served as a municipal landfill for Naval Submarine Base Kings Bay and Camden County, Georgia, between 1974 and 1981. At some time during its history, chlorinated ethenes were released into the landfill, and the site has been actively remediated for chlorinated ethene contamination using pump-and-treat methods (discontinued in 1998) and Fenton's Reagent injection (initiated in 1999 and described by Chapelle et al. [2005]) and vegetable oil injection into the former source area, which occurred in 2002. The location of the site and the location of monitoring wells used to collect water chemistry data are shown in Figure 1. The well closest to zone of vegetable oil injection is KBA-34 (Figure 1).

The sediments underlying the Kings Bay site are of marginal marine origin and record a prograding shoreline during Pliocene and Pleistocene time (Leeth 1998). Sediments and soils exposed at land surface consist of fine- to medium-grained quartz sands of windblown dune (aeolian) origin that range from 2 to 3 m in thickness. The sands and soils exposed at land surface are relatively permeable, and rainfall easily percolates in the subsurface to recharge the underlying aquifers. Below these surface sands is a layer of organic-rich sands and clays that record sedimentation in a back bay environment (Leeth
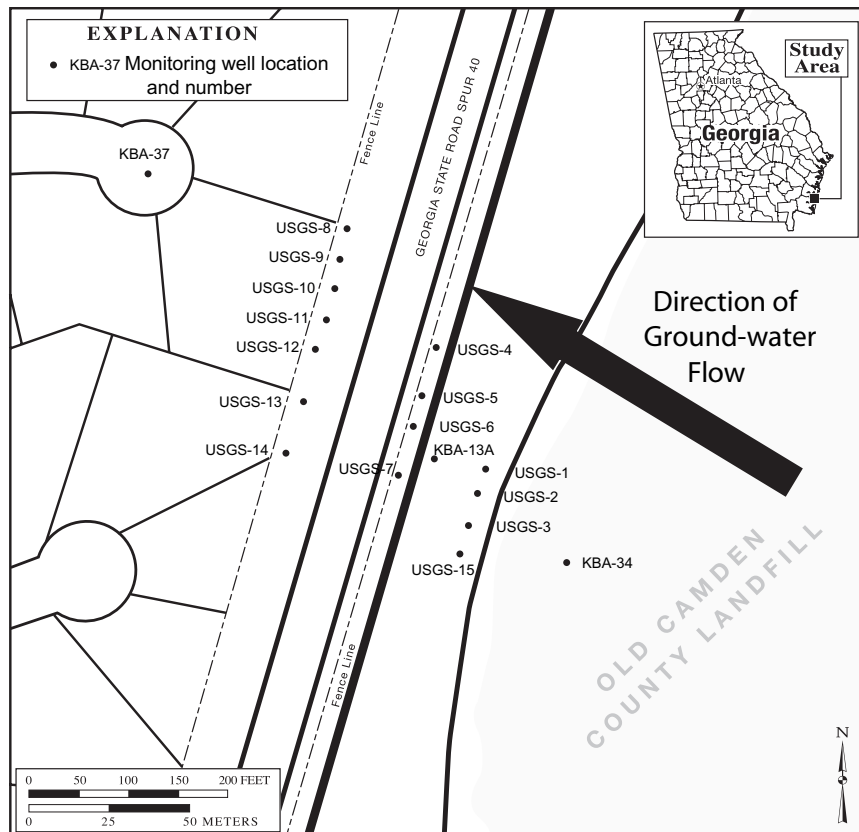
Figure 1. Map showing the location of the Kings Bay site and the location of sampling wells.

1998). This organic-rich layer is typically 3 m thick, is areally extensive in the vicinity of the site, and contains as much as 460 mg/kg of extractable potentially biodegradable organic carbon (Rectanus et al. 2007). Underlying this layer of organic sediments is another layer of fine- to medium-grained aeolian sand that ranges from 6 to 8 m in thickness, is characterized by hydraulic conductivities on the order of 3 to 7 m/d, and functions as a semiconfined aquifer (Leeth 1998). This semiconfined aquifer is tapped by the observation wells shown in Figure 1.

The West Trenton site served as a Naval Aviation Warfare Center (NAWC) from the mid-1950s to the late 1990s. During site operations, chlorinated ethenes were released to the underlying ground water system, and the site has been under active pump-and-treat remediation since the mid-1990s (Lacombe 2000). In addition, a pilot-scale emulsified vegetable oil injection and bioaugmentation remediation system was initiated in 2005 (De Flaun et al. 2006). A map of the West Trenton site and locations of monitoring wells used to collect ground water chemistry data is shown in Figure 2. Well 16BR was used to inject emulsified vegetable oil into the fractured rock aquifer in 2005. Well 68BR was specially instrumented with packers designed to isolate individual water-bearing fracture zones (Williams et al. 2007). Zone A (3 to 13 m) is the shallowest interval tapped by well 68BR followed by zone B (15 to 18 m), zone E (33 to 48 m), and zone F (50 to 57 m).

The West Trenton site is underlain by metamorphosed and fractured mudstones and sandstones of Triassic age

that dip steeply to the northwest (Lacombe 2000; Williams et al. 2007). Overlying the fractured bedrock is a soil zone that varies between 1 and 5 m in thickness. The soils exposed at land surface are relatively permeable and, during precipitation/snowmelt events, water saturates the soil and percolates downward to the underlying bedrock. Once the fractured bedrock is encountered, ground water flows along bedding plane partings and fractures and moves deeper into the subsurface.

Ground Water Sampling and Analysis of DIC and Redox Indicators

Each monitoring well was pumped with a peristaltic pump at a rate of about 500 mL/min, and the temperature, pH, and conductivity continuously monitored until stable readings were obtained. It typically took between 5 and 10 min of pumping for these parameters to stabilize. After stabilization, water turbidity was measured using a spectrophotometer (Hach Inc., Loveland, Colorado). Only water with a turbidity of less than 5 nephelolometric turbidity units (NTU) was collected for analysis. Concentrations of dissolved oxygen (Chemetrics Inc., Calverton, Virginia), ferrous iron (Hach Inc.), and sulfide (Hach Inc.) were measured in the field using colorimetric methods. The lower detection limits were 0.05 mg/L (dissolved oxygen), 0.01 mg/L (ferrous iron), and 0.01 mg/L (sulfide). Concentrations of methane and dissolved inorganic carbon (DIC) were measured by drawing 2 mL of water into a syringe and injecting it through a 0.2-μm filter into a sealed 20-mL
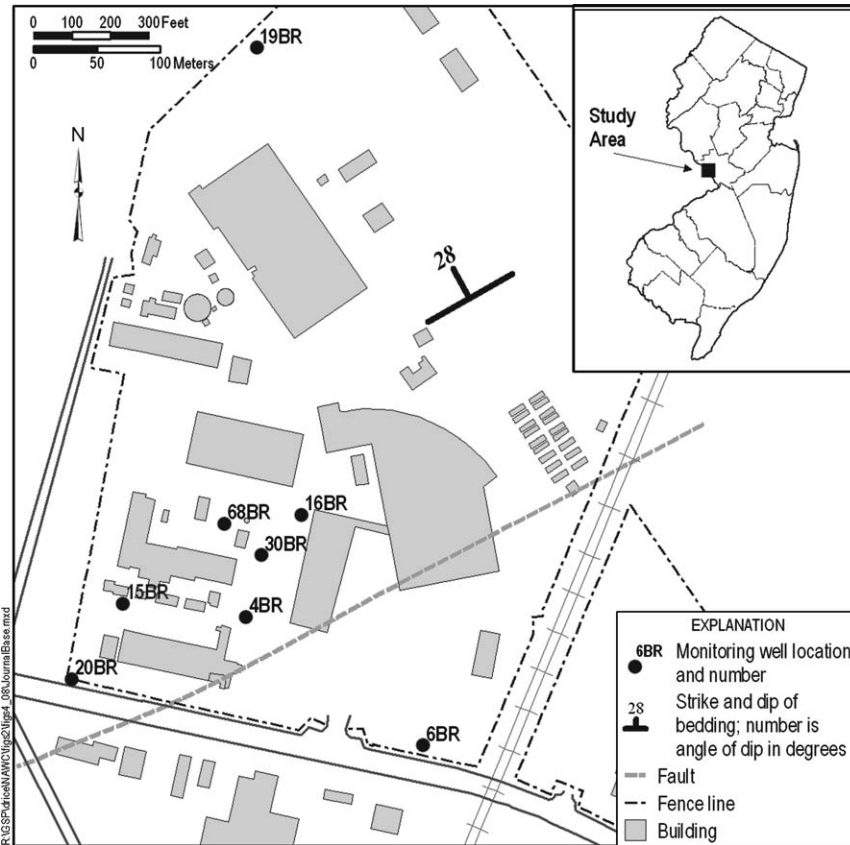
Figure 2. Map showing the location of the West Trenton site and the location of sampling wells.

septated serum vial. In the lab, the water was acidified below pH 3.0, and concentrations of methane and carbon dioxide in the vial headspace were quantified by gas chromatography with thermal conductivity detection. Compound separation was achieved by isocratic (80 °C), packed column (300 cm of 13X molecular sieve) using a Helett-Packard 5890 series II plus GC (Agilent Technologies, Santa Clara, California). Aqueous concentrations were determined using Henry's Law partition coefficients and reported as milligrams per liter of water. The lower detection limits were 0.01 mg/L (methane) and 0.5 mM (DIC).

At the West Trenton site, the alkalinity of freshly pumped water was measured by titrating samples to pH 4.5 on-site, and ground water DIC concentrations estimated from alkalinity using the method of Deffeyes (1965). Direct comparison of DIC concentrations measured at the West Trenton site by acidification and CO_2 quantification as described previously was similar to DIC concentrations estimated from alkalinity measurements using the method of Deffeyes (1965). The lower detection limit for DIC concentrations using the alkalinity method is approximately 0.05 mM. Concentrations of dissolved sulfate were measured in the laboratory using ion chromatography with conductivity detection (Dionex). The lower concentration limit of the sulfate analyses was 0.2 mg/L.

Predominant terminal electron-accepting processes (TEAPs) in ground water were identified using methodology described by Chapelle et al. (1995). Briefly, this method uses the consumption of potential electron acceptors (i.e., dissolved oxygen and sulfate), the production of characteristic end products (i.e., ferrous iron, sulfide, and methane), and concentrations of dissolved molecular hydrogen (H_2) to deduce ambient TEAPs.

Analytical Methods for Organic Carbon Analyses

DOC is operationally defined as organic carbon remaining after passing through filters with a pore size in the 0.2 to 0.7 μm size range (Benner 2003). Because ground water produced from properly constructed and developed wells usually contains little particulate organic carbon (<5 NTU), measurements of TOC typically differ little from measurements of DOC. At the Kings Bay and West Trenton sites, samples filtered through a 0.2-μm filter and analyzed for DOC had statistically indistinguishable concentration ranges from unfiltered TOC samples. This, in turn, is consistent with the expectation that a high percentage of the water borne organic carbon in this ground water is operationally defined DOC. Because filtering increases the possibility of contamination, a significant concern when measuring nanomolar concentrations of neutral sugars and amino acids, unfiltered TOC samples were collected for the biochemical analyses made in this study.

In the field, ground water was used to thoroughly rinse 100-mL acid-rinsed high-density polyethylene bottles (Nalgene), and the bottles were then filled to the top,

capped, immediately chilled to 4 °C, and transported to the laboratory for analysis. In the laboratory, TOC was determined by high-temperature combustion using a Shimadzu TOC-V analyzer (Benner and Strom 1993). The Shimadzu TOC-V analyzer was equipped with a chemiluminescent nitrogen detector (Shimadzu TN-1), allowing for the simultaneous analysis of total nitrogen (TN). Lower detection limits for this methodology were described by Benner and Strom (1993).

THNS were quantified by high-performance anion-exchange chromatography and pulsed amperiometric detection (Skoog and Benner 1997). Samples were hydrolyzed in 1.2 mol/L H_2SO_4 and neutralized with a self-absorbed ion retardation resin (Kaiser and Benner 2000). A Dionex Carbopac PA1 (4 × 250 mm) column coupled to a Carbopac PA1 (5 μm, 4 × 50 mm) guard column was used with 24 mmol/L NaOH as the mobile phase.

THAA were analyzed using an Agilent high-performance liquid chromatography system with fluorescence detection. A Licrosphere RP18 (5 μm, 4 × 250 mm) column was used for separation of amino acids. Samples were hydrolyzed by vapor-phase hydrolysis with 6 mol/L HCl at 150 °C for 32.5 min (Kaiser and Benner 2005). After neutralization, amino acids were separated as o-phthaldialdehyde (OPA) derivatives following the method of Lindroth and Mopper (1979) with minor modifications (Kaiser and Benner 2005). The lower detection limit for this method is 1 nmol/mL.

D and L-amino acids were analyzed using methods described by Kaiser and Benner (2005). Briefly, water samples were hydrolyzed in a CEM Mars 5000 microwave equipped with a protein hydrolysis accessory kit. After neutralization, free amino acid enantiomers were derivatized with a mixture of N-isobutyryl-L-cysteine and OPA or N-isobutyryl-D-cysteine and OPA and separated on a reversed-phase column. Samples were run with both reagents to allow for correction of coeluting peaks. Measured values of enantiomeric amino acids were corrected for acid-catalyzed racemization using the mean of the racemization observed in proteins and free amino acids (Kaiser and Benner 2005). Acid hydrolysis converted D and L-isomers of asparagine and glutamine to D and L-isomers of aspartic acid and glutamic acid, and reported concentrations of these amino acids included both D and L-isomers.

The statistical significance ($p < 0.05$) of possible differences between data sets in this study was assessed using the nonparametric Wilcoxon-Mann-Whitney U rank sum test for comparing two populations (Helsel and Hirsch 1992).

Results

Organic Carbon Mineralization and Ambient Redox Processes

Dissolved oxygen present in percolating water that recharge shallow aquifers may be consumed by heterotrophic microorganism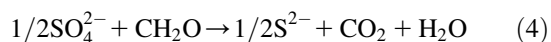s oxidizing dissolved and particulate organic carbon (represented by the formula CH_2O, which assumes carbon of valence zero) present in soils or aquifer materials according to the approximate stoichiometry:

$$CH_2O + O_2 \rightarrow CO_2 + H_2O \qquad (2)$$
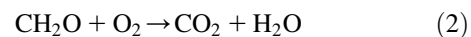
Once dissolved oxygen has been consumed, the mineralization of particulate and DOC proceeds via Fe(III) reduction as follows:

$$4Fe(OH)_3 + CH_2O + 8H^+ + H_2O$$
$$\rightarrow CO_2 + 4Fe^{2+} + 12H_2O \qquad (3)$$
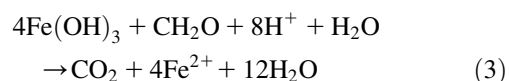
and/or sulfate reduction as follows:

$$1/2SO_4^{2-} + CH_2O \rightarrow 1/2S^{2-} + CO_2 + H_2O \qquad (4)$$

Equations 2 through 4 are written in terms of carbon dioxide production. However, any carbon dioxide produced speciates into dissolved carbonate, bicarbonate, and carbon dioxide depending on the pH of the water (Deffeyes 1965). The sum of these species is referred to herein as DIC. In addition to the production of DIC by reactions 2, 3, and 4, DIC production may be enhanced by the dissolution of carbonate minerals reacting with dissolved carbon dioxide:

$$CO_2 + CaCO_3 + H_2O \rightarrow Ca^{2+} + 2HCO_3^- \qquad (5)$$
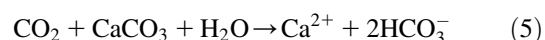
Thus, the production of DIC in ground water systems can reflect ongoing microbial metabolism, which in turn is driven by the presence of bioavailable dissolved or particulate organic carbon.

At the Kings Bay site, ground water chemistry data collected from a network of monitoring wells (Figure 1) at the Kings Bay site indicate that the predominant redox processes occurring in the semiconfined aquifer along the study flowpath are Fe(III) and sulfate reduction (Table 1).

At the West Trenton site (Figure 2), the predominant redox processes in the bedrock aquifer are also observed to be Fe(III) reduction and sulfate reduction (Table 2).

The similar predominant redox processes observed at these sites make a comparison of the bioavailability of TOC present in ground water more feasible than if redox conditions were widely dissimilar.

Composition of Organic Carbon

Concentrations of TOC, DIC, TN, THNS, and THAA measured in ground water from the Kings Bay and West Trenton sites are shown in Table 3.

Overall concentrations of TOC are somewhat higher at the Kings Bay site (425 to 1492 μM) than at the West Trenton site (47 to 325 μM). Similarly, concentrations of TN, THNS, and THAA are typically higher at the Kings Bay site than at the West Trenton site. In contrast, carbon-normalized yields for THNS and THAA are generally lower at the Kings Bay site than they are at the West Trenton site (Table 3). Concentrations of individual neutral sugars, including fucose (Fuc), rhamnose (Rha),

Table 1
Redox Chemistry of Ground Water Produced from the Kings Bay Site, May 2006

Sample	DO (mg/L)	NO_3^- (mg/L)	Fe^{2+} (mg/L)	HS (mg/L)	pH (units)	H_2 (nM)	DIC (mM)	CH_4 (mg/L)	SO_4^{2-} (mg/L)	Redox Process[1]
KBA-34[2]	<0.05	1.09	21.7	0.25	5.07	0.32	8.5	10.2	81	Fe(III)
USGS-2	<0.05	1.43	26.5	0.25	3.96	0.09	8.5	<0.1	263	Fe(III)
USGS-3	<0.05	1.13	22.8	0.25	4.25	0.11	6.9	<0.1	186	Fe(III)
KBA-13A	<0.05	1.10	25.7	6.25	4.13	0.09	9.7	5.4	220	Fe(III)
USGS-5	0.05	<0.1	13.0	1.00	5.31	0.16	9.7	1.3	61	Fe(III)
USGS-6	0.05	0.26	16.8	1.25	5.54	0.25	9.0	<0.1	85	Fe(III)
USGS-10	<0.05	<0.1	0.21	1.50	5.58	2.6	9.1	<0.1	6.1	SO_4
USGS-11	<0.05	<0.1	0.03	1.75	5.62	0.37	8.6	0.9	7.6	Fe(III)
USGS-12	<0.05	<0.1	0.03	1.50	5.47	1.6	7.9	2.3	2.3	SO_4
KBA-37	<0.05	<0.1	0.21	1.75	5.53	0.12	9.2	1.7	13.2	Fe(III)

Notes: Fe(III) = Fe(III) reduction; SO_4 = sulfate reduction.
[1]Predominant terminal electron-accepting process.
[2]Well affected by vegetable oil injection.

arabinose (Ara), galactose (Gal), glucose (Glc), mannose (Man), and xylose (Xyl), present in TOC from the Kings Bay and West Trenton sites are shown in Figure 3A. These data indicate that while TOC concentrations differ between these two sites, concentrations of neutral sugars are fairly similar. This, in turn, reflects the greater THNS yield observed at the West Trenton relative to the Kings Bay site (Table 3). At both sites, glucose is the most abundant neutral sugar.

The mean and standard deviation of individual amino acid concentrations measured in ground water produced from the wells shown in Tables 1 and 2, including aspartic acid (Asp), glutamic acid (Glu), serine (Ser), threonine (Thr), glycine (Gly), arginine (Arg), alanine (Ala), tyrosine (Tyr), valine (Val), and lysine (Lys) present in TOC from the Kings Bay and West Trenton sites, are shown in Figure 3B. Glycine is the most abundant amino acid followed by alanine, and the overall compositions of THAA are similar at both sites (Figure 4A). The concentrations of individual amino acids are typically somewhat higher at Kings Bay than West Trenton, particularly for glycine and alanine (Figure 4B).

Well KBA-34, which is located near a zone of vegetable oil injection at the Kings Bay site, produced ground water with the highest concentration of TOC (Figure 4A). Directly downgradient from well KBA-34 (wells USGS-2 and 3), in the zone affected by chemical oxidation, TOC concentrations were much lower. Concentrations of TOC then increased along the flowpath from wells USGS-2 and 3 to KBA-37. The THNS yield was low near the zone of vegetable oil injection but peaked immediately downgradient (Figure 4B). Subsequent to this peak, the THNS yield decreased downgradient along the flowpath. In contrast, the THAA yield was highest near the zone of vegetable oil injection, decreased immediately downgradient, and remained relatively constant along the flowpath (Figure 4 C). While there was no apparent trend in THAA yield along the flowpath, the mol % composition of D-enantiomers of THAA (Figure 4D) appeared to follow in the same general pattern as the observed changes of TOC (Figure 4A).

Table 2
Redox Chemistry of Ground Water Produced from the West Trenton (NAWC) Site, May 2006

Sample	DO mg/L)	NO_3^- (mg/L)	Fe^{2+} (mg/L)	HS (mg/L)	pH (units)	H_2 (nM)	DIC (mM)	CH_4 (mg/L)	SO_4^{2-} (mg/L)	Redox Process[1]
4 BR	<0.05	0.29	1.8	0.031	7.29	0.22	5.3	0.02	98.7	Fe(III)
15 BR	<0.05	0.2	0.88	na	7.39	0.56	6.8	0.12	58.9	Fe(III)
16 BR[2]	<0.05	0.1	7.94	na	7.72	1.3	3.6	16.7	<0.7	SO_4
19 BR	1.3	0.12	na	0.067	na	0.88	2.4	<0.001	13.8	Fe(III)
30 BR	<0.05	0.2	1.4	0.027	na	1.2	5.2	0.10	115	SO_4
68 BR-A	<0.05	0.2	16.5	na	7.24	0.20	6.5	0.42	23.4	Fe(III)
68 BR-F	<0.05	0.2	2.11	na	8.04	2.7	2.9	0.007	8.2	SO_4
20 BR	<0.05	0.18	1.84	na	7.08	0.69	2.7	0.032	39.3	Fe(III)

Notes: Fe(III) = Fe(III) reduction; SO_4 = sulfate reduction; na = not analyzed.
[1]Predominant terminal electron-accepting process.
[2]Well impacted by vegetable oil injection.

Table 3

Concentrations of TOC, TN, THNS Sum, and THAA Sum in Ground Water from the Kings Bay and West Trenton (NAWC) Sites

Sample	TOC (μM)	TN (μM)	TOC/TN	THNS Sum (nM)	THNS Yield,[1] % TOC	THAA Sum (nM)	THAA Yield,[2] % TOC
Kings Bay (May 2, 2006)							
KBA 34[3]	1248	215.3	5.8	555	0.26	2428	0.71
USGS-2	515	637.9	0.8	606	0.68	129	0.07
USGS-3	425	289.4	1.5	287	0.39	338	0.28
KBA 13A	546	556.4	1.0	504	0.54	754	0.48
USGS-5	633	1211.1	0.5	443	0.41	519	0.26
USGS-6	831	1079.4	0.8	477	0.33	565	0.22
USGS-10	1030	1450.8	0.7	714	0.40	1090	0.37
USGS-11	1228	1920.6	0.6	850	0.40	1131	0.32
USGS-12	840	1131.6	0.7	430	0.29	781	0.33
KBA 37	1106	1625.6	0.7	391	0.20	723	0.22
Kings Bay (July 27, 2006)							
KBA-34[3]	1492	220.8	6.8	1394	0.54	1668	0.35
USGS-2	523	611.2	0.9	669	0.74	962	0.58
USGS-3	431	285.4	1.5	526	0.69	1841	1.26
USGS-5	707	1226.9	0.6	940	0.77	1464	0.65
USGS-6	892	1170.0	0.8	525	0.34	1330	0.48
USGS-10	1029	1659.8	0.6	1937	1.08	1345	0.42
USGS-12	833	1144.6	0.7	1494	1.03	1109	0.42
KBA-37	1040	1676.8	0.6	990	0.55	992	0.30
NAWC (June 5, 2006)							
4 BR	217	22.4	9.7	653	1.73	485	0.80
15 BR	117	6.9	16.9	350	1.72	161	0.47
16 BR[3]	325	19.6	16.6	>5000	>9	7322	9.40
19 BR	47	58.3	0.8	305	3.82	855	7.27
30 BR	160	8.6	18.6	608	2.16	185	0.39
68 BR-A	197	2.8	70.4	557	1.65	198	0.36
68 BR-F	51	2.7	18.8	162	1.86	295	2.23
20 BR	55	8.9	6.2	470	4.88	91	0.60
NAWC (August 28, 2006)							
4 BR	264	18.0	14.7	347	0.76	879	1.31
16 BR[3]	297	18.0	16.5	4062	8.08	3612	4.80
30 BR	173	23.6	7.3	111	0.37	167	0.30
68 BR zone A	191	2.4	80.6	79.2	0.24	245	0.45
68 BR zone B	154	8.3	18.5	177	0.66	303	0.84
68 BR zone E	125	7.4	16.9	274	1.26	369	1.23
68 BR zone F	130	5.9	22.1	255	1.14	309	0.99

[1]Normalized to the number of carbon atoms in each sugar.
[2]Normalized to the number of carbon atoms in each amino acid.
[3]Well influenced by injection of vegetable oil.

In addition to changes in the yield and composition of TOC along the flowpath, changes were also observed over time at the Kings Bay site. The May sampling event followed a period of low rainfall that had extended for several months (3.27 inches; 8.3 cm of precipitation recorded in the 2 months prior to the May sampling event). In contrast, the July sampling event followed a period of heavier rainfall (10.30 inches; 26.2 cm of precipitation in the 2 months prior to the July sampling event) that increased water levels in the shallow aquifer. A comparison of the May and July sampling events shows that TOC concentrations plot close to the 1:1 line (Figure 5A), indicating similar concentrations. There is no statistical difference ($p = 1.0$) between the two data sets. In contrast, concentrations of THNS (Figure 5B) and THAA (Figure 5C) plot predominantly above the 1:1 line, indicating that concentrations were statistically higher in the July relative to the May sampling event ($p = 0.008$ and 0.028, respectively). This shows that while concentrations of TOC were statistically indistinguishable between the two sampling events, the biochemical composition of the TOC was significantly different. Furthermore, concentrations of DIC, a final mineralization product of organic carbon under the ambient redox conditions present at the site (Equations 2 through 4), also increased significantly ($p < 0.001$) from May to July (Figure 5D).
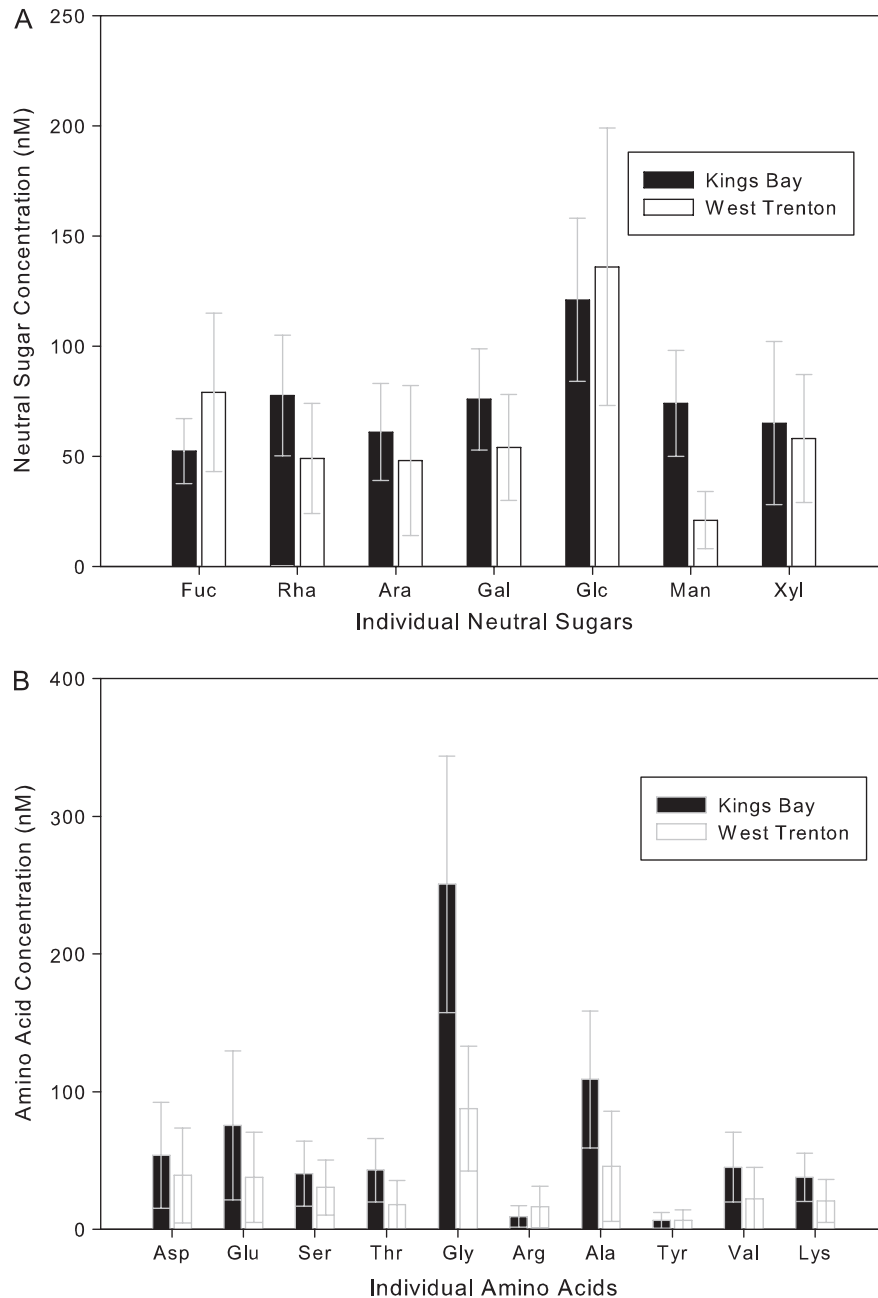
Figure 3. Concentrations of (A) individual neutral sugars and (B) amino acids in TOC measured in ground water from the Kings Bay and West Trenton sites. Data are expressed as the mean and standard deviation of analyte concentrations.

Discussion

The inherent complexity of assessing the composition and bioavailability of TOC present in ground water systems is widely appreciated (Thurman 1985; Aiken 1989; Routh et al. 2001). Furthermore, the various limitations of using TOC as a sole indicator of the reducing potential of ground water systems are well recognized (U.S. EPA 2002). The central question, therefore, is whether indicators of the biochemical composition of TOC, such as concentrations and yields of THAA and THNS, provide insight into the bioavailability of organic carbon that is not provided by TOC measurements alone.

THAA and THNS yields are observed to be higher in surface ocean water, where active photosynthesis produces TOC, and decrease as water sinks into deep abyssal environments isolated from the surface (Benner 2002). These patterns have been interpreted as indicating the selective utilization and removal of THAA and THNS by heterotrophic microorganisms. This leads to an overall decrease in the bioavailability of TOC over time and an increase in concentrations of DIC as TOC is mineralized (Weiss and Simon 1999; Volk et al. 1997; Gremm and Kaplan 1998; Amon et al. 2001; Benner 2002, 2003). This overall pattern, although occurring at a different spatial scale, might be expected in ground water systems as well.

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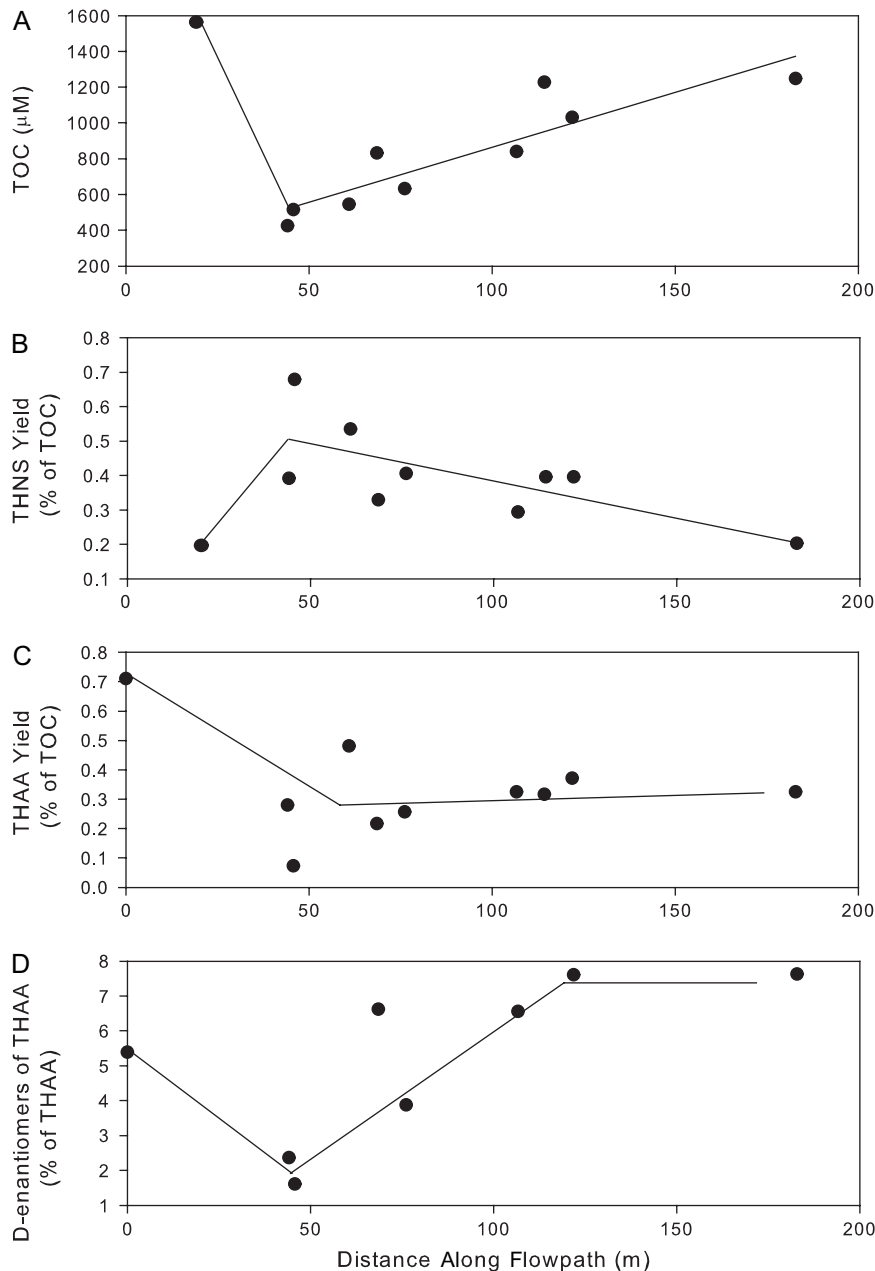F.H. Chapelle et al. GROUND WATER 47, no. 1: 108–121   115

Figure 4. Concentrations of (A) TOC, (B) THNS, (C) THAA, and (D) the percent D-enantiomers of THAA along the flowpath, Kings Bay, Georgia. The lines illustrate apparent trends in the data and do not imply statistical significance.

Shallow aquifers associated with humid climates are typically recharged by rainfall or snowmelt that percolates through soils that contribute photosynthetically derived DOC. This organic carbon can be transported to underlying aquifers where photosynthesis is absent (Thurman 1985; Aiken 1989). One way to interpret the biochemical composition and bioavailability of TOC in ground water, therefore, is to consider the geologic age of organic carbon sources.

Given the relative age (Triassic) and the low-grade metamorphic history, the organic carbon remaining in rocks underlying the West Trenton site has been extensively coalified (Lacombe 2000). Its bioavailability is uncertain but is presumed to be poor. The principal source

of TOC to the fractured rock aquifer appears to be modern soils that support actively photosynthesizing terrestrial plants. This is supported by the observation that the shallowest sampling points at West Trenton, well 4BR and well 68BR zone A, which draw water from weathered bedrock underlying the surface soils, also exhibit the highest concentrations of THNS and THAA of the nonvegetable oil–impacted wells.

While modern soils are also a potential intermittent source of TOC at Kings Bay, the closest continuous source to the aquifer is the relatively ancient (Pleistocene) bed of organic-rich sediment. It might be expected, therefore, that TOC at the West Trenton site would exhibit a higher proportion of bioavailable carbon than at
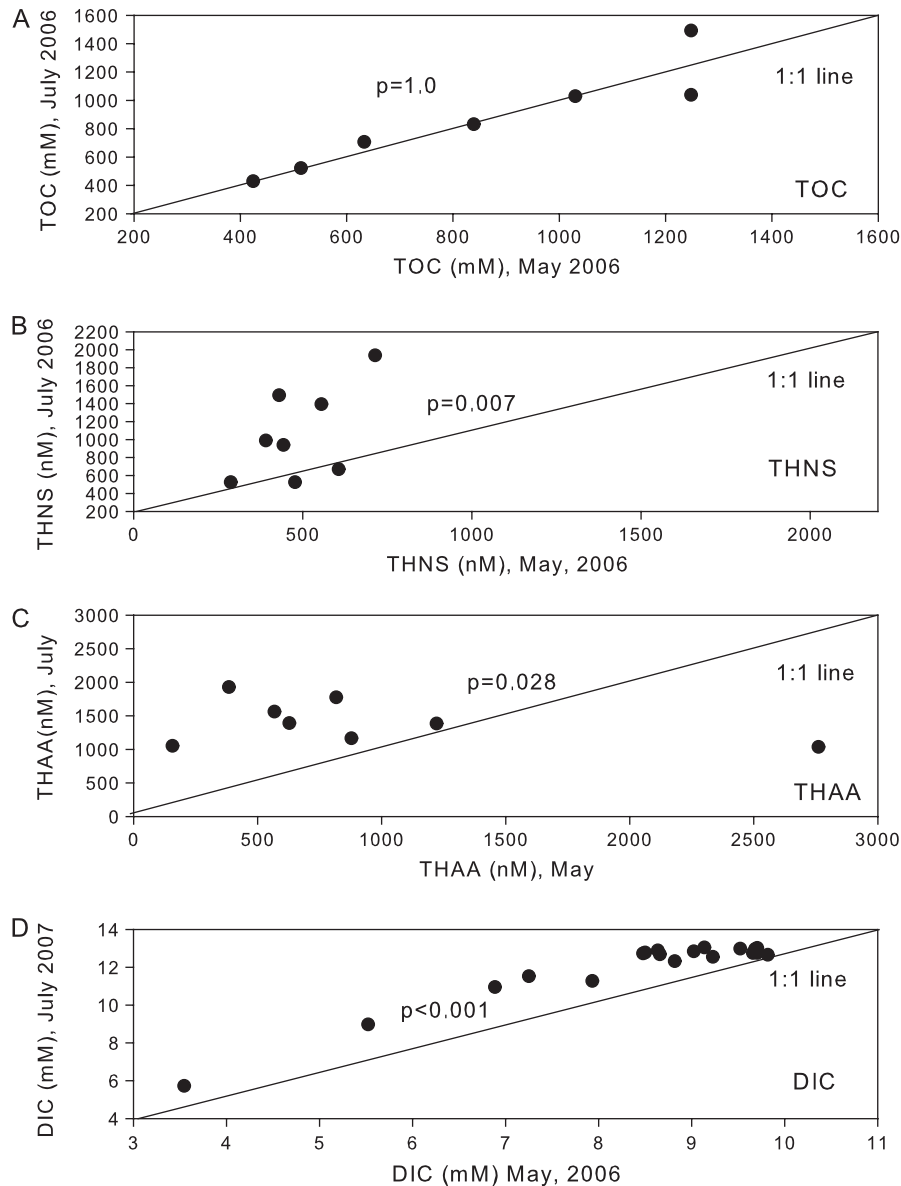
Figure 5. Concentration changes of (A) TOC, (B) THNS, (C) THAA, and (D) DIC from May to July 2006 at the Kings Bay site.

Kings Bay. This expectation is consistent with the observed greater THAA and THNS yields observed at the West Trenton site relative to Kings Bay (Table 3). Furthermore, the amino acid glycine is commonly observed to become enriched in more biodegraded organic matter (Dauwe et al. 1999). The higher glycine concentrations, which are characteristic of Kings Bay TOC (Figure 3B; $p = 0.023$), are another indication that Kings Bay TOC is more biodegraded, and thus less bioavailable, than West Trenton TOC.

The higher bioavailability of TOC at the West Trenton site relative to Kings Bay is also indicated by observed concentrations of DIC. At the West Trenton site, a significant positive correlation ($p = 0.001$) is observed between TOC concentrations and DIC concentrations (Figure 6A). In contrast, no significant positive correlation ($p = 0.5$) was observed at the Kings Bay site (Figure 6B). This

implies that when TOC contains a high proportion of bioavailable carbon and concentrations of DIC, the product of TOC mineralization (Equations 2 through 5) shows an increased positive correlation with TOC (Figure 6A). If the bioavailable component of TOC is depleted, the correlation between TOC and DIC would be expected to attenuate (Figure 6B). Even though TOC and DIC do not correlate at Kings Bay (Figure 6B), significant positive correlations are observed between concentrations of THNS ($p = 0.019$), THAA ($p = 0.005$), and DIC (Figure 7). Again, this observation suggests that THNS and THAA measurements provide information on bioavailability that is not apparent from TOC concentrations alone.

A biochemical characterization of TOC can reveal other patterns of hydrologic interest as well. At the Kings Bay site, concentrations of TOC are highest in the upgradient part of the aquifer affected by the
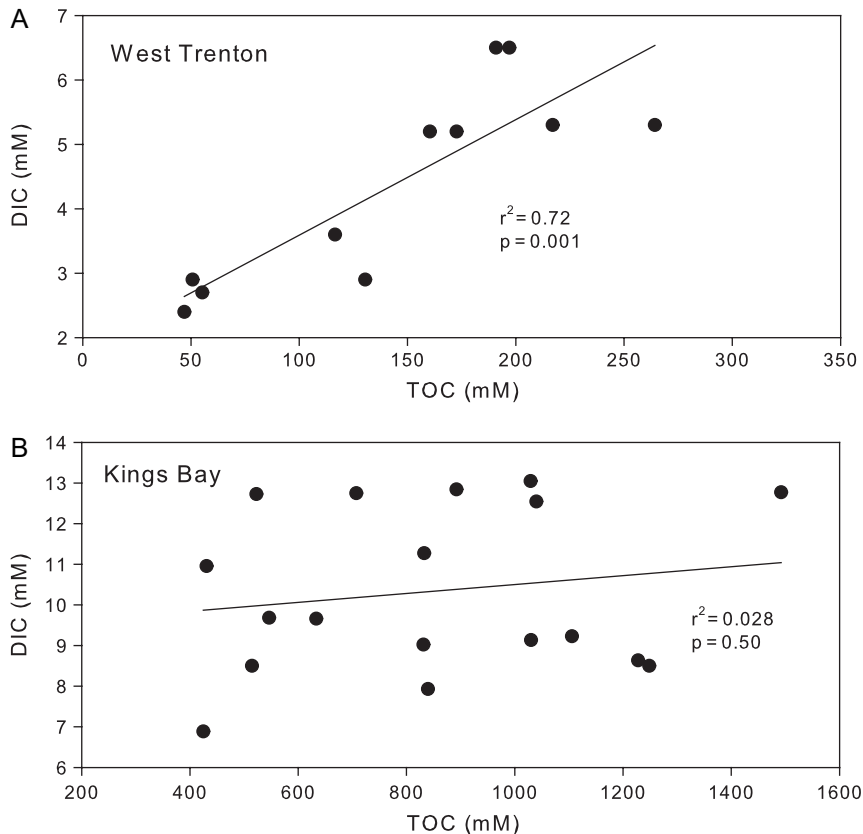
Figure 6. Correlation of TOC and DIC at (A) the West Trenton site and (B) the Kings Bay site.

injection of vegetable oil but decrease immediately downgradient in the zone impacted by Fenton's Reagent injection (Figure 4A). Fenton's Reagent actively oxidizes organic carbon and would be expected to decrease concentrations of TOC where it was applied. As ground water flows downgradient from the TOC minimum, concentrations of TOC steadily increase (Figure 5A). Concentrations of THNS, however, show the opposite pattern, low near the zone of vegetable oil injection and decreasing along the flowpath from the TOC maximum (Figure 4). The low concentrations of THNS upgradient can be attributed to the relative lack of carbohydrates present in vegetable oil. The decrease in THNS along the flowpath—as TOC concentrations increase—is consistent with progressive microbial metabolism of TOC along the flowpath and thus the preferential removal of neutral sugars. Alternatively, or in addition, this trend may reflect the progressive accumulation of low-THNS TOC from the Pleistocene organic-rich bed along the flowpath. In either case, these patterns imply that the bioavailability of TOC decreases along the flowpath (Figure 4B), a possibility that could not be recognized from concentrations of TOC alone (Figure 4A).

Concentration changes of THAA (Figure 4C) and the percentage of D to L-enantiomers of THAA (Figure 4D) also provide insight into the origin of TOC in this system. Concentrations of THAA are high near the vegetable oil injection zone but are relatively constant along

the rest of the flowpath (Figure 4C). The relative composition of the D-enantiomers in the THAA, however, is high near the vegetable oil–injected zone, decreases in the Fenton's-affected zone, and then increases along the flowpath in a manner similar to TOC concentrations (Figure 4 A). D-enantiomer amino acids are present in the cell walls and other macromolecules of bacteria but are absent in organic matter originating from eukaryotes (terrestrial plants) and the archaea (Amon et al. 2001; Kawasaki and Benner 2006). The observed changes of D-enantiomer composition, therefore, suggest an increasing contribution of bacterial cell material to TOC as ground water moves downgradient. This, in turn, suggests that bacterial utilization of TOC systematically consumes THNS (Figure 4B), maintains steady-state concentrations of THAA (Figure 4 C), while increasing the relative contribution of bacterial cell material to TOC in this system (Figure 4D). Similar patterns have been previously reported in the oceanographic literature as shallow surface water grades into deeper abyssal water (Benner 2003).

The potential utility of THNS and THAA as indicators of bioavailability is also suggested by temporal concentration changes observed between May and July 2006 (Figure 5). Prior to the May sampling, the Kings Bay area experienced a period of low rainfall, with 8.3 cm of precipitation recorded in the preceding 2 months. During the summer, however, the amount of rainfall increased and the site recorded 26.12 cm in the 2 months preceding the July sampling event. If TOC concentrations were the only
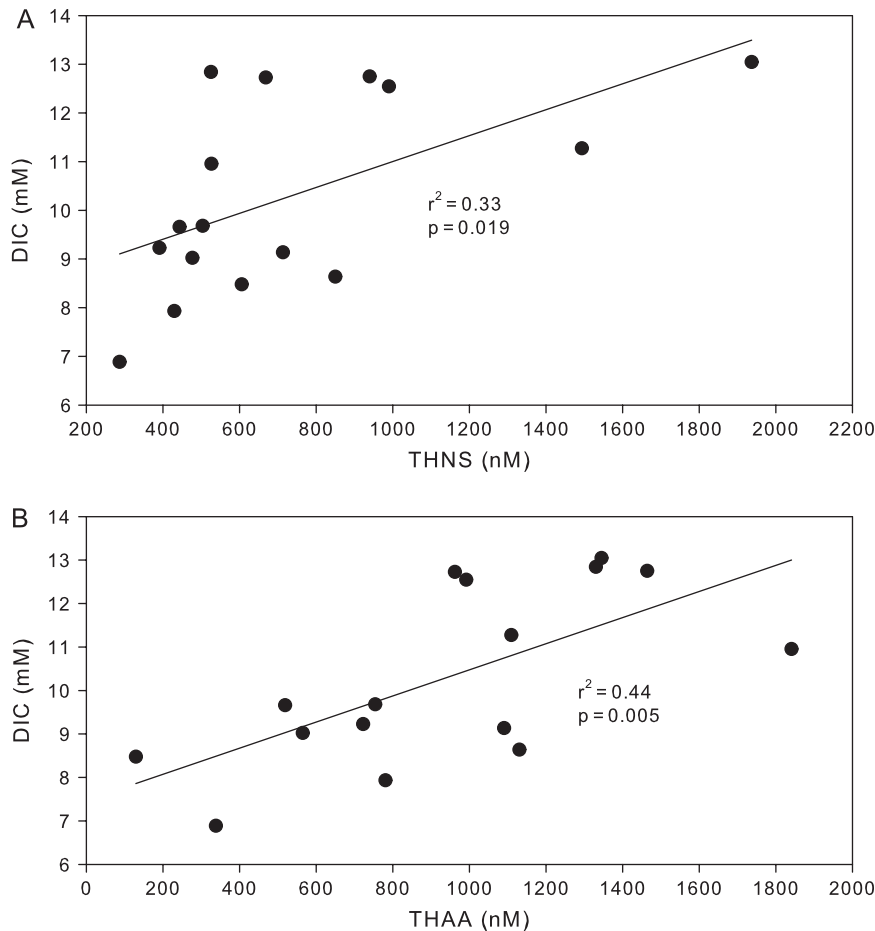
Figure 7. Correlation between DIC and (A) THNS and (B) THAA concentrations at the Kings Bay site.

metric available, it might be concluded that there was no difference in bioavailable organic carbon between these two sampling events (Figure 5A). However, concentrations of THNS (Figure 5B) and THAA (Figure 5C) were higher in July than in May. The fact that concentration of DIC increased as well (Figure 5D) and is consistent with the interpretation that bioavailable TOC was delivered to the aquifer between May and July by percolating recharge. This raises the possibility that recharge events carry relatively bioavailable carbon from the modern soil layer to the underlying aquifer. In between recharge events, however, the predominant source of TOC to the aquifer appears to be from the contiguous and more biodegraded Pleistocene organic-rich bed. These observations indicate that the TOC bioavailability in this system varies temporally (Figure 5) as well as spatially (Figure 4).

Conclusions

The results of this study are consistent with the hypothesis that considering the biochemical composition of TOC can provide insight into the bioavailability of organic carbon present in ground water. This biochemical characterization includes quantifying concentrations of neutral sugars and amino acids. However, because neutral sugars and amino acids compose such

a small percentage of the TOC present, they are more properly thought of as indicator compounds that correlate with a qualitative notion of "bioavailability." It is possible that as our ability to characterize the biochemical composition TOC present in ground water improves, a more quantitative definition of bioavailability may become feasible.

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