

Crude Oil Metabolites in Groundwater at Two Spill Sites

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Abstract

Two groundwater plumes in north central Minnesota with residual crude oil sources have 20 to 50 mg/L of nonvolatile dissolved organic carbon (NVDOC). These values are over 10 times higher than benzene and two to three times higher than Diesel Range Organics in the same wells. On the basis of previous work, most of the NVDOC consists of partial transformation products from the crude oil. Monitoring data from 1988 to 2015 at one of the sites located near Bemidji, MN show that the plume of metabolites is expanding toward a lakeshore located 335 m from the source zone. Other mass balance studies of the site have demonstrated that the plume expansion is driven by the combined effect of continued presence of the residual crude oil source and depletion of the electron accepting capacity of solid phase iron oxide and hydroxides on the aquifer sediments. These plumes of metabolites are not covered by regulatory monitoring and reporting requirements in Minnesota and other states. Yet, a review of toxicology studies indicates that polar metabolites of crude oil may pose a risk to aquatic and mammalian species. Together the results suggest that at sites where residual sources are present, monitoring of NVDOC may be warranted to evaluate the fates of plumes of hydrocarbon transformation products.

Introduction

Recognition of problems with source removal is driving renewed interest in natural attenuation processes associated with residual petroleum hydrocarbon sources (ITRC 2009). A retrospective study of closed leaking underground storage tank sites in Wisconsin (Evanson et al. 2009) concluded that at least 36 of 113 studied sites still have residual petroleum hydrocarbon mass in soil. At sites where residual crude oil or petroleum hydrocarbon fuel contaminants are present in the environment, partial oxidation products form from biodegradation reactions (Cozzarelli et al. 1994; Beller et al. 1995; Thorn and Aiken 1998; Gieg et al. 1999; Lundegard and Sweeney 2004)

and from photo-oxidation in the case of surface exposures (e.g. Lewan et al. 2014). These transformation products are more soluble than the parent petroleum hydrocarbons due to their greater polarity and corresponding low volatility (Zemo and Foote 2003; Lang et al. 2009; Melbye et al. 2009; Zemo et al. 2013). Transformation products from residual source zones partition into the aqueous phase and are transported from the source forming a groundwater plume (Cozzarelli et al. 1990; Thorn and Aiken 1998; Lundegard and Knott 2001; Lundegard and Sweeney 2004; Lang et al. 2009; Ng et al. 2015). Several studies suggest that some of these transformation products may exhibit human and aquatic toxicity (Neff et al. 2000; Rogers et al. 2002; Mao et al. 2009; Melbye et al. 2009).

Current regulatory monitoring and data reporting requirements at petroleum-contaminated sites typically include concentrations of benzene, toluene, ethylbenzene, and xylene (BTEX), polycyclic aromatic hydrocarbons, and total petroleum hydrocarbons (TPH) defined as gasoline range organics (GRO, C6-C12), diesel range organics (DRO, C10-C28), or oil range organics (C20-C35). For example, Minnesota's Pollution Control Agency uses a 1 mg/L concentration of DRO, in combination with other analyses, as a regulatory action level (Minnesota Pollution Control Agency 2008). These reporting requirements do not provide data on the concentrations or properties of the individual compounds that form as transformation products. Yet at study sites where residual

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product is present, the total concentration of transformation products can exceed BTEX concentrations by one to two orders of magnitude (Lundegard and Knott 2001; Lundegard and Sweeney 2004; Lang et al. 2009; Amos et al. 2012; Ng et al. 2015). In addition to possible concerns about toxicity, these plumes of metabolites consume the electron-accepting capacity of the contaminated aquifer resulting in the slowing of biodegradation of contaminants of concern (Amos et al. 2012; Ng et al. 2015). Studies that evaluate the fate of these metabolite plumes including biodegradation rates and potential for expansion to receptors including surface water bodies are needed.

The goal of this paper is to present observations of metabolite concentrations in two crude oil plumes. Metabolite concentrations are approximated based on the nonvolatile dissolved organic carbon (NVDOC) content of the groundwater. Twenty years of monitoring data at one of the study sites have been compiled to illustrate the growth rate and natural attenuation of the NVDOC plume. The NVDOC concentrations are compared to DRO results at both sites to quantify the concentrations of metabolites that are not reported under current regulatory practice. Previous research concludes that most of the NVDOC consists of partial transformation products (Thorn and Aiken 1998) although a small fraction could be polar compounds from the crude oil itself. Eganhouse et al. (1993) concluded that nonvolatile hydrocarbons from the oil are only a minor component of the NVDOC. Thus, for simplicity in this paper we equate NVDOC to polar metabolites of the crude oil, but future studies of these compounds are needed to better understand their relationship to the crude oil source. A discussion of the implications of the observations covers three issues: the fate of the plume and potential for surface water discharge, comparison of the concentrations to existing toxicity literature, and the shortcomings of current analytical methods requirements for understanding the extent of these metabolite plumes.

Site Description

Both study sites are located in north-central Minnesota in a surficial glacial outwash formation. At both sites only crude oil was spilled with no co-occurring organic contaminants. The first site, located near Bemidji (Figure 1), was contaminated in 1979 when an oil pipeline ruptured spilling about 1.7 million liters of light crude oil. The oil sprayed over an area of about 6500 m² and collected in topographic depressions (Essaid et al. 2011). Clean-up operations recovered approximately 75% of the spilled oil, but much of the remainder infiltrated the aquifer forming three residual oil bodies at the water table. According to Eganhouse et al. (1993) the oil has a molecular composition of 58% to 61% saturated hydrocarbons, 33% to 36% aromatics, 4% to 6% resins and 1% to 2% asphaltenes, with 0.56% sulfur and ~0.28% nitrogen for the whole oil. The surficial aquifer consists of about 20 m

Table 1
Dissolved Organic Carbon (DOC) Fractionation in mg C per Liter for 1987 Bemidji Well Samples

Well	310E	603	522	532B	530B	515A
Distance (m)	-200	-100	26	45.7	91.3	136.9
DOC	2.9	15	41.5	31.5	21	8.1
Hydrophobic acid	1.3	7	26	17.5	11.7	4.4
Hydrophobic neutral	0.1	5	1	0.5	0.9	0.8
Hydrophilic acid	0.2	1.5	8.5	10.5	4.5	1.6

Table 2
Concentrations of NVDOC and DRO Measured at Bemidji in 2015

Well ID	Distance (m)	Elevation (m)	NVDOC (mg/L)	DRO (ug/L)
310B	-200.5	406.8	1.93	<110
310E	-200.1	423.1	1.49	<110
1217C	328.4	421.4	2.84	170
1217B	327.5	414.5	1.42	<110

of calcareous silty sand with gravel and silt lenses overlying a clayey till. Research conducted since 1983 at the site has focused mainly on the north oil pool and associated groundwater plume (Figure 1). The water table is 6 to 8 m below the surface in this area and groundwater flows east-northeast at an estimated average linear or pore velocity of 22 m/year (Essaid et al. 2011). In 1996 the 10 µg/L benzene contour extended about 125 m beyond the edge of the oil (Figure 1) and its position has been fairly stable since then (Cozzarelli et al. 2001). A detailed site description and summary of study results is provided by Essaid et al. (2011).

The second site is located at the South Cass Lake pumping station near the town of Cass Lake, Minnesota (Figure 2). At this site a leaking flange discovered in 2002 spilled crude oil for an unknown period. The estimated volume of crude oil at the water table was 182,000 L in 2005 (Wenck Associates Inc. 2005). Composition of the leaking oil may have varied over time, as evidenced by the range in oil densities of 0.85- to 0.94 g/cm³ for oil samples collected from different monitoring wells. The surficial aquifer is 8 to 14 m thick and consists of fine to medium grained sand and silt with some gravel. The water table is 7 to 9 m below the land surface (Natural Resources Engineering Company 2003) and groundwater flows east-southeast with an estimated average linear or pore velocity of ~26 m/year (Drennan et al. 2010). In 2007 the 10 µg/L benzene contour extended about 150 m beyond the edge of the oil. The hydrocarbon plume, redox zonation, and degradation rates are described by Drennan et al. (2010).

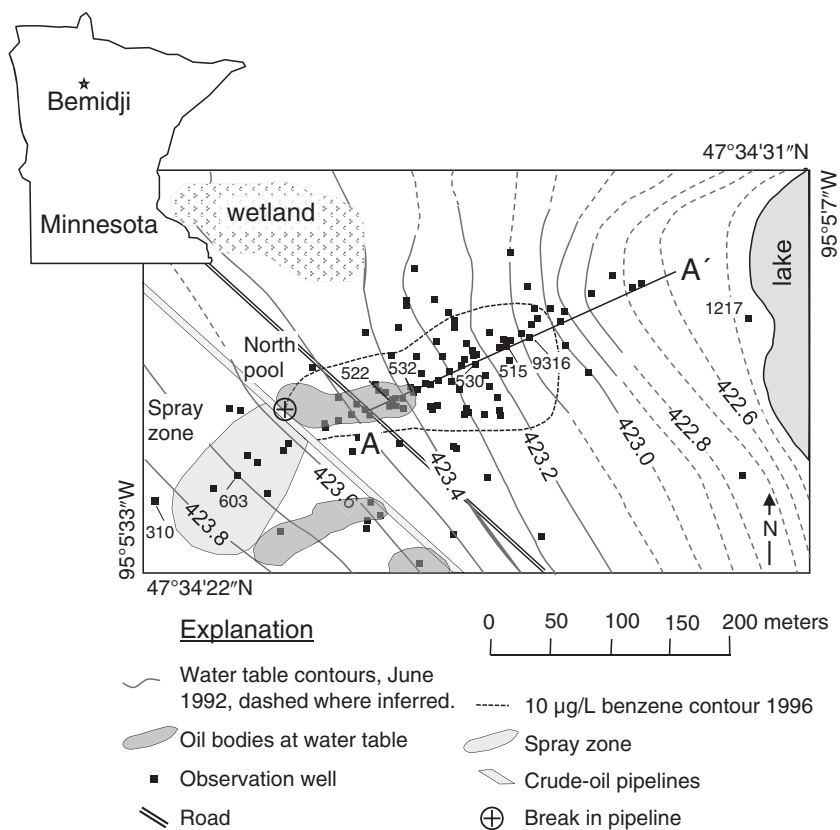


Figure 1. Map of the National Crude Oil Spill Fate and Natural Attenuation Research Site located near Bemidji MN showing oil bodies at the water table, oil spray zone, monitoring wells, 10 µg/L benzene concentration contour, water table contours in meters, line of section for Figures 3 and 4, and well locations for Figure 5 and Tables 1 and 2. Figure modified from Bekins et al. (2001).

The focus of this study is on the groundwater NVDOC plumes that originate from dissolution and biodegradation of the residual oil at the two sites. NVDOC is an operationally defined fraction consisting of the dissolved organic carbon remaining after purging of the volatile dissolved organic carbon (VDOC). Total dissolved organic carbon (TDOC) is given by the sum of VDOC and NVDOC. In 1987 at the Bemidji site, Eganhouse et al. (1993) found 48.2 mg/L TDOC composed of 58% NVDOC and 42% VDOC at a well located immediately downgradient from the residual oil. A small amount of the NVDOC was from the native groundwater but the majority was from partial oxidation of petroleum hydrocarbons (Eganhouse et al. 1993). Low molecular weight organic acids were 1% to 11% of the NVDOC fraction (Eganhouse et al. 1993; Thorn and Aiken 1998). To better understand the composition of the majority of the NVDOC, Thorn and Aiken (1998) isolated three fractions designated hydrophobic neutrals (HPO-N), hydrophobic acids (HPO-A), and hydrophilic acids (HPI-A). These fractions were characterized using elemental analyses, ¹⁴C ages, number average molecular weight determinations, and liquid-phase ¹H and ¹³C NMR. The results show that the HPO-N, HPO-A, and HPI-A fractions form a progression of increasing oxygen content, with decreasing carbon content and aromaticity, respectively. On the basis of the sulfur

contents, average number of carbons per molecule, and ¹³C NMR aromaticities, the HPO-N fraction represents partial oxidation products of C₂₂ or greater components from the aromatic, resin, or asphaltene components of the crude oil. Although partial degradation products from the saturate fraction of the crude oil could not be directly confirmed, these were likely present in the fractions studied. The possibility that unaltered constituents from the polar fractions of the crude oil (carboxylic acids, resins, asphaltenes, etc.) could contribute to the NVDOC was not directly addressed in Thorn and Aiken (1998), and must also remain a consideration. However, in the case of the unaltered resins and asphaltenes, the limited aqueous solubilities of these materials would in principle limit their dissolution and transport in the aquifer.

Methods

Groundwater samples were collected after the well was purged at least three-well volumes and the field parameters pH, dissolved oxygen, temperature and specific conductance reached stabilization. The plume scale NVDOC data for Bemidji are from sampling campaigns conducted in July 1988 (22 wells), 1995 (23 wells), and 2010 (23 wells). Samples for NVDOC were collected from contaminated wells at the Cass Lake site in June 2008 (15 wells), and one background well in

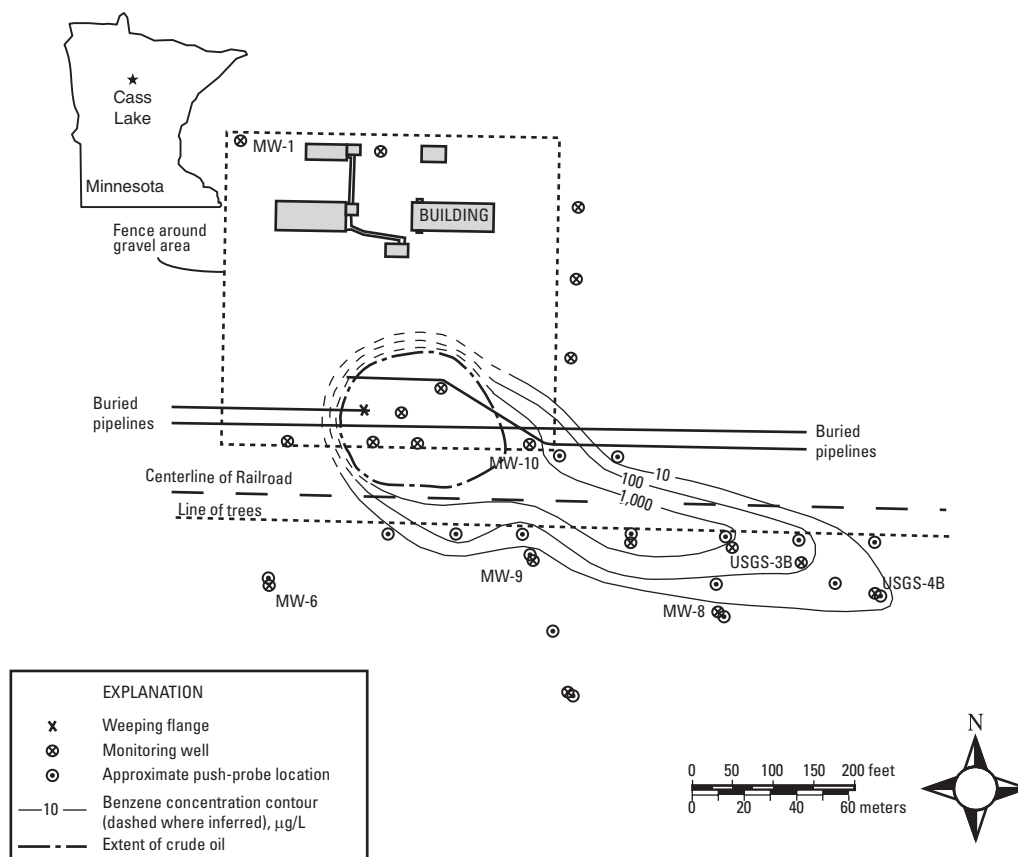


Figure 2. Map of the South Cass Lake Pumping Station located near Cass Lake, MN showing oil body at the water table, monitoring wells, push-probe locations with benzene concentrations and benzene concentration contours in µg/L. Figure modified from Drennan et al. (2010).

August 2009. Samples for NVDOC were filtered through 0.20-µm Supor® or Nuclepore filters into baked glass bottles, preserved with hydrochloric acid to a pH of <2. Concentrations of NVDOC were measured after purging with N₂ to remove inorganic carbon and volatile organics. Samples collected from Bemidji in 1986 to 1995 were analyzed by the persulfate wet-oxidation technique using a carbon analyzer (Baedecker and Cozzarelli 1992). Samples collected from Bemidji in 2009 to 2015 and from Cass Lake in 2008 and June 2009 were analyzed by the high temperature combustion technique using a Shimadzu TOC Vcsn analyzer (Shimadzu Corporation, Kyoto, Japan). As used in this paper, the term NVDOC is equivalent to non-purgeable organic carbon (NPOC) analyzed on a filtered sample (St-Jean 2003).

Samples for DRO from the Bemidji site were collected into 1-L amber bottles with Teflon-lined caps in July 2014 and August 2015. Samples were preserved with 5 mL of 50% HCl at the time of collection and then stored at 4 °C until extraction, within 7 days of sample collection. Samples were analyzed utilizing extraction of the organic constituents with hexane. The extract was dried, concentrated, and injected into a capillary column gas chromatograph. The gas chromatograph was temperature programmed to facilitate separation of

organic compounds. Detection was achieved by a flame ionization detector (FID). Quantitation was based on FID detector response to a diesel component standard (Wisconsin Department of Natural Resources 1995). The detection limit of this method is 0.1 mg/L. Samples for DRO extended range C₁₀ to C₃₂ (ERDRO) values used in the comparisons were collected at the Cass Lake site on 26 June 2008 from MW6, MW8, MW9, and MW10 and on 21 August 2009 from MW-1 (Natural Resources Engineering Company 2010). The samples were analyzed by En Chem, Green Bay, WI, for ERDRO using the Wisconsin-modified DRO method (Wisconsin Department of Natural Resources 1995). The detection limit for this method was 0.11 mg/L.

For this study we present benzene data on water samples that were collected from the Bemidji site in 1988 (20 wells), 1995 (24 wells) and in 2010 (39 wells) and from the contaminated wells at the Cass Lake site in 2008 (15 wells). Benzene concentrations in 1995 for the Bemidji site were measured by purge-and-trap capillary gas chromatography (GC) with mass spectrometric detection as described by Eganhouse et al. (2001). Benzene concentrations for Bemidji in 2010 (Ng et al. 2014) and for Cass Lake in 2008 (Drennan et al. 2010) were analyzed by the USGS National Water Quality Laboratory (NWQL) in Denver, CO (<http://nwql.usgs.gov/nwql>).

shtml) using heated purge and trap, GC, and mass spectrometry.

Methods for isolation of NVDOC fractions and their analyses were described by Thorn and Aiken (1998). Briefly, water samples were collected in 10 gallon stainless steel cans, acidified to pH 2 with HCl and pumped through a two-column array of XAD-8 and XAD-4 resins. HPO-A and HPO-N fractions adsorb to XAD-8 resins and HPI-A adsorb to XAD-4 resins. Number average molecular weights were determined by vapor pressure osmometry. Elemental analyses and analytical DOC fractionations (Table 1) were performed by Huffman Laboratories, Golden, CO. Carbon number per average molecule was computed from number average molecular weights and elemental analyses.

Results

Bemidji Site

Figure 3 shows contour plots of benzene concentrations at the Bemidji site along the plume centerline in 1988, 1995 and 2010. During this 22-year period, concentrations of benzene near the crude oil source decreased from values of 4 through 7 mg/L to 2 through 3 mg/L, whereas the position of the 0.1 mg/L contour was stable. The observed groundwater concentration decreases are comparable to observed decreases of benzene concentrations in the crude oil source (Bekins et al. 2011). Baedecker et al. (2011) analyzed oil samples collected in 2008 and found that in four of five sampled wells benzene concentrations had decreased by 37% to 87% compared to the reference sample for the spilled oil. These results show that the benzene groundwater plume is decreasing in concentration as the benzene content of the oil becomes depleted by dissolution followed by biodegradation. Several studies have shown that benzene degrades anaerobically under iron-reducing conditions at the Bemidji site (Baedecker et al. 1993; Anderson et al. 1998; Cozzarelli et al. 2010). The stable position of the 0.1 mg/L benzene concentration contour indicates that the previously documented processes of anaerobic biodegradation by iron-reduction are effective in decreasing groundwater benzene concentrations over the interval 50 to 150 m from the center of the oil body source (Baedecker et al. 1993; Bekins et al. 2001; Cozzarelli et al. 2001).

Figure 4 illustrates contour plots of NVDOC in 1988, 1995, and 2010. In 2010, concentrations of NVDOC near the center of the oil body are over 10 times higher than benzene concentrations. In contrast to benzene, the NVDOC concentrations near the source have increased from a range of 20 through 30 mg/L in 1988 and 1995 to 40 through 50 mg/L in 2010. In all the plots the NVDOC concentrations decrease with distance. The area between 75 and 125 m corresponds to the present-day iron-reducing zone of the plume (Cozzarelli et al. 2015). Decreases in 2010 NVDOC concentrations over this zone (Figure 4) support earlier studies suggesting the NVDOC compounds

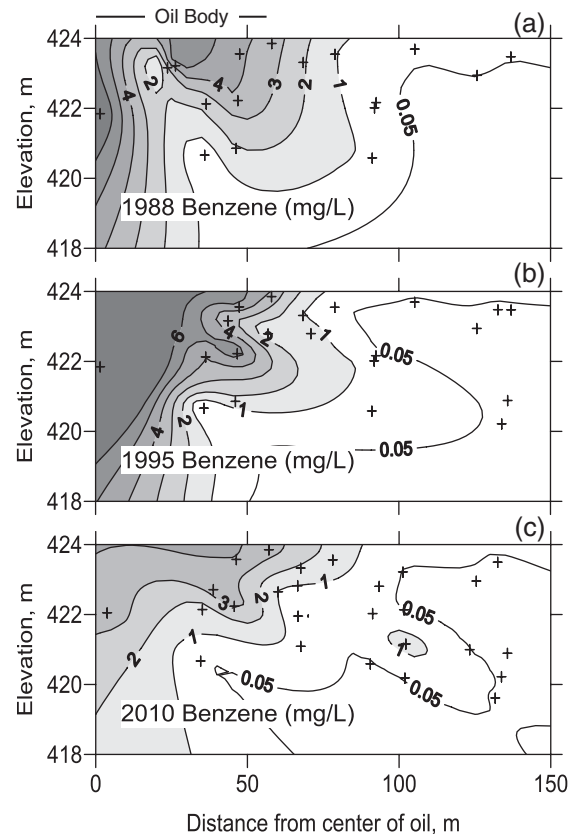


Figure 3. Contour plots of benzene concentrations in mg/L along section line A-A' at the Bemidji site (Figure 1) for (a) 1988 (b) 1995, and (c) 2010. Crosses mark centers of sampled monitoring well screens. Data indicate benzene concentrations near the oil decreased during the 22-year interval 1988 to 2010.

are susceptible to biodegradation coupled to ferric iron reduction (Baedecker et al. 1993; Eganhouse et al. 1993). Recent evidence for degradation of NVDOC under iron-reducing conditions includes calculations showing that losses of NVDOC, VOC and methane are balanced with measured decreases in sediment iron oxy-hydroxides between 1995 and 2007 (Amos et al. 2012; Ng et al. 2014). Several studies also have documented increases in dissolved inorganic carbon (DIC) consistent with biodegradation of NVDOC and VOC in the iron-reducing portion of the aquifer (Baedecker et al. 1993; Bennett et al. 1993; Ng et al. 2014).

The NVDOC plume extends well beyond the benzene plume, and the position of the 5 mg/L contour has expanded from 125 to over 200 m from the center of the oil in the 22-year time frame (Figure 4). To quantify the expansion we computed the area encompassed by the 3 mg/L contour line. This contour reflects the concentration level that exceeds all previous measurements of background NVDOC in the aquifer. The two-dimensional area of the plume cross section expanded from 872 m² in 1988 to 901 m² in 1995 and 1305 m² in 2010. In 2010, the concentration of 2.9 mg/L measured in the well located 235-m downgradient was only slightly larger than the average uncontaminated background value of 1.9 mg/L measured

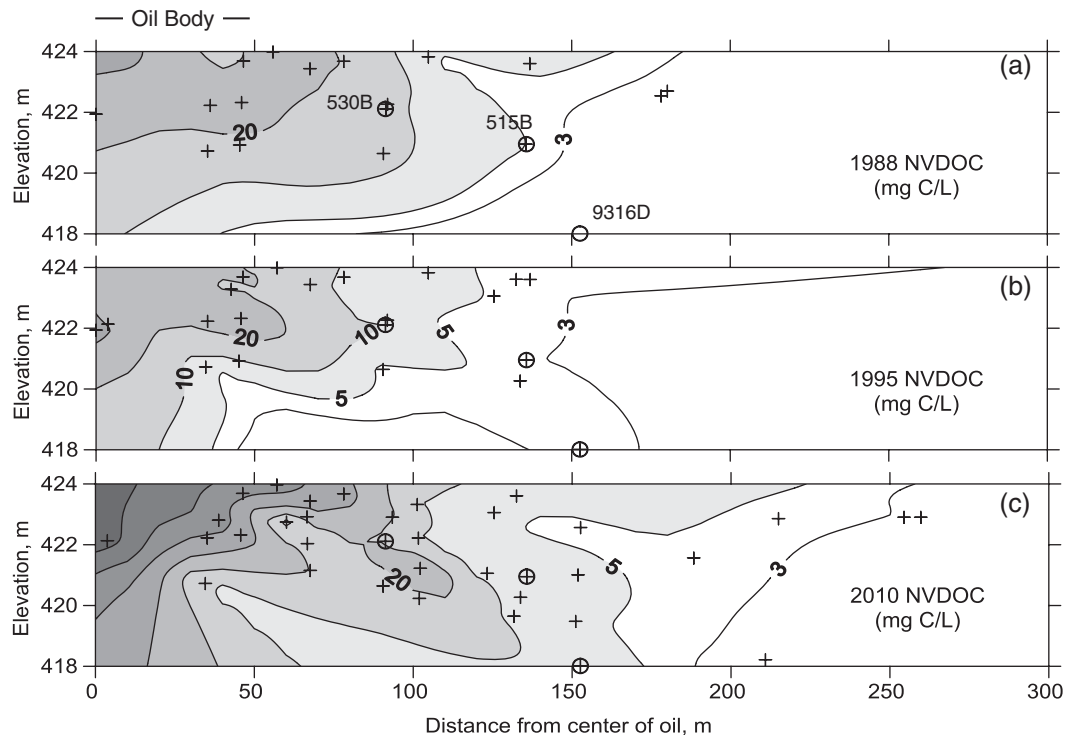


Figure 4. Contour plots of nonvolatile dissolved organic carbon (NVDOC) concentrations in milligrams C per liter along section line A-A' at the Bemidji site (Figure 1) for (a) 1988, (b) 1995, and (c) 2010. Crosses are centers of sampled monitoring well screens. Circled crosses with well numbers are locations of wells plotted in Figure 5. Data indicate NVDOC concentrations in the plume have increased between 0 and 200 m downgradient during the 22-year interval 1988 to 2010.

at well 310E (Figure 1). The low concentration indicates that other techniques are needed to distinguish the natural NVDOC in the aquifer from the plume of metabolites at this downgradient location. Results in Table 1 provide concentrations of NVDOC fractions from 1987 well samples isolated using the techniques described by Thorn and Aiken (1998). The data show that the proportions of the three fractions of the DOC from the wells in the plume (522, 532B, 530B, 515A) differed from those in the background (310E) and the spray zone (603). The HPO-A and HPI-A fractions decreased with distance downgradient but both were present above background levels at well 515A located 136-m downgradient.

The pattern of expansion of the plume can be illustrated by examining temporal changes of NVDOC concentrations at selected wells. Figure 5 shows data from three wells located 91 (well 530B), 135 (well 515B), and 153 m (well 9316D) from the center of the oil. In the well closest to the oil body at 91 m, NVDOC concentrations have always been higher than in the downgradient wells since the start of data collection in 1986. In the wells for which concentrations were available in the 1980s, values were higher and then dropped in the 1990s. Values in all wells increased between 1995 and 2010. In 2013, at the wells located at 135 and 153 m concentrations were the highest ever recorded. In 2015 values dropped at the 135 and 153 m wells, but were still higher than concentrations measured at those same wells in 1995 by 61% and 93%, respectively. The overall trend is of high

values in the first decade after the spill followed by decreases in the 1990s and increases since 1995. This overall pattern is consistent with previously published mass balance and modeling results (Essaid et al. 1995; Amos et al. 2012; Ng et al. 2015). Essaid et al. (1995) noted that the plume initially expands during the period before peak microbial concentrations are established. Once microbial populations reached peak values, by the end of the 1980s biodegradation rates exceeded advection rates and NVDOC concentrations declined by 1992. During the next phase, the large electron-accepting capacity originally present in the iron oxy-hydroxides is slowly depleted (Tuccillo et al. 1999). Amos et al. (2012) showed that the observed depletion of iron oxy-hydroxides in the period between 1995 and 2010 matches the flux of reduced carbon from the source zone including BTEX, NVDOC, and methane. Ng et al. (2015) showed that iron oxy-hydroxide supplies on the aquifer sediments are depleted with time resulting in expansion of the plume toward the downgradient lake.

The observation that the plume is expanding indicates that there is potential for discharge of metabolites into a lake located approximately 335-m downgradient of the oil source (Figure 1). Data to assess whether the NVDOC plume has reached the lake were collected in 2015. Table 2 shows the values of NVDOC and DRO collected from background wells 310B and 310E and wells 1217C and 1217B adjacent to the lake shore (Figure 1). The two background wells and the deep

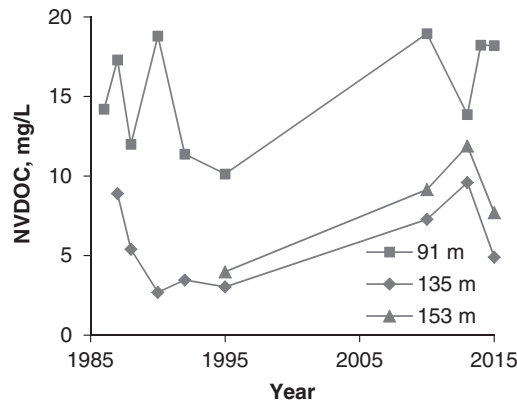


Figure 5. Time series of NVDOC concentrations for monitoring wells at the Bemidji site located 91 (well 530B), 135 (well 515B), and 153 m (well 9316D) downgradient from the center of the oil. Data are suggestive of increasing trends since 1995.

well adjacent to the lake had NVDOC concentrations of 1.4 to 1.9 mg/L, which are comparable to background NVDOC values measured in 2010. Values of DRO at these same wells were below detection. In contrast, NVDOC in the shallow well 1217C adjacent to the lake was 2.8 mg/L and DRO was above detection. The 2015 NVDOC value at well 1217C is similar to the value of 2.9 mg/L measured at the 235 m well in 2010. Together these data suggest that there may be a small concentration of refractory petroleum metabolites of approximately 1 mg/L migrating beyond 235 m and that this plume may have reached the lake at the Bemidji site. This will need to be confirmed by ^{14}C dating or other lines of molecular evidence.

Cass Lake Site

At the Cass Lake site the NVDOC concentrations in 2008 were also much greater than the benzene concentrations (Figure 6). Values for NVDOC ranged from 21 mg/L near the crude oil source to 1.3 mg/L at the background site. Concentrations of NVDOC decreased with distance but values at the most downgradient well were greater than 5 mg/L. Compared to benzene concentrations, the NVDOC concentrations at the same wells ranged from six to thousands of times greater.

The final point to be addressed concerns the relationship between NVDOC concentrations and the standard regulatory analyses, known as either TPHd or DRO. Figure 7 shows a comparison of these data types together with linear regressions for both the Bemidji and Cass Lake sites. The slope of the regression line at Bemidji shows that the DRO values are just 33% of the NVDOC values. Across the entire range of concentrations, the DRO analytical results quantify only about a third of the organic carbon compounds in the plume. At the Cass Lake site, fewer data points are available, but ERDRO values appear to be 45% of the NVDOC concentrations. The background ERDRO value at the Cass Lake site in 2009 was 0.09 mg/L compared to an NVDOC concentration of

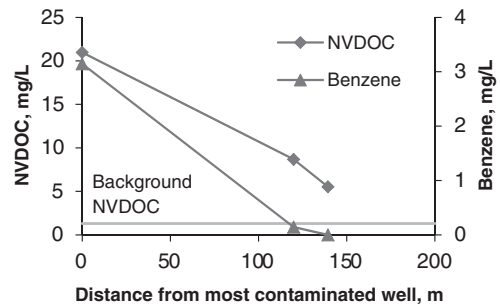


Figure 6. Maximum concentrations of benzene and NVDOC versus distance at the Cass Lake site showing that NVDOC concentrations exceed benzene. Data are from wells MW-10, USGS-3B and USGS-4B (Figure 2).

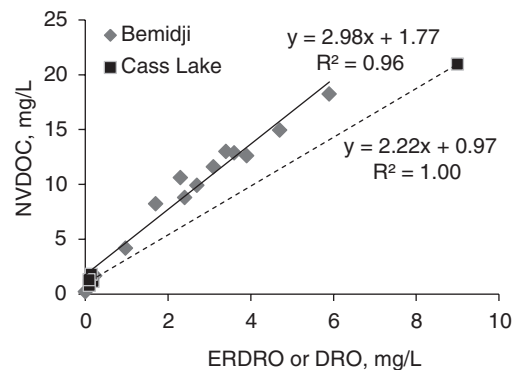


Figure 7. Comparison plot showing that NVDOC is almost three times DRO at the Bemidji site (diamonds and solid line) and more than twice ERDRO at the Cass Lake site (squares and dashed line).

1.3 mg/L. The higher value of 45% associated with the Cass Lake ERDRO data may be partly due to the extended range of the ERDRO analysis compared to DRO. However, there are no NVDOC data values between 2 and 20 mg/L for Cass Lake because monitoring wells could not be installed in the railroad right-of-way that crosses the plume (Figure 2). The small number of data points and limited concentration range also may contribute the difference in the regression lines between the two sites.

Discussion

We have shown that at two sites with residual crude oil sources, plumes of NVDOC extend well beyond the BTEX plumes, and NVDOC concentrations are more than two times greater than concentrations from the regulatory DRO and ERDRO analyses.

Metabolite Plume Fate

Although studies indicate that the molecular structures of some metabolite compounds are highly branched, which may convey resistance to biodegradation (Booth et al. 2007; Melbye et al. 2009), the Bemidji results suggest that much of the NVDOC is biodegraded in the iron-reducing zone (Baedecker et al. 1993; Bennett et al.

1993; Amos et al. 2012; Ng et al. 2014). Evidence for biodegradation includes mass balance of the NVDOC with decreases in the sediment iron oxy-hydroxide and also with increases in DIC. Zemo et al. (2013) also noted that at five petroleum-contaminated sites, both concentrations of metabolites and complexity of molecular structures decreased with distance and suggested that toxicity concerns from metabolites are greatest near residual sources.

The NVDOC plume at Bemidji is expanding toward a small lake located 335 m from the oil spill center. NVDOC concentrations measured in 2015 in a well nest adjacent to the lake are slightly above background levels (Table 2). At the Cass Lake site no temporal data are available, but the same pattern of depletion of iron oxy-hydroxides near the source was present (Drennan et al. 2010) suggesting anaerobic biodegradation of NVDOC was coupled to iron reduction. Thus, the same ongoing depletion of iron oxy-hydroxides that drives the expansion of the NVDOC plume at the Bemidji site also could result in plume expansion at the Cass Lake site.

Multiple lines of evidence indicate that groundwater from the Bemidji plume is discharging into the lake (Mason 2014). These lines of evidence include higher heads in the groundwater and near-shore sediment pore water compared to the lake water level; seepage meter measurements of flow from the sediments into the lake; stable isotope signatures of groundwater discharging into the lake; and groundwater temperature profiles below the lake. Under the oil the specific conductance (SpC) increases from a background value of 417 uS/cm to over 900 uS/cm. The data collected by Mason (2014) show that this plume of increased SpC originating under the oil extends to the lake shore and lake sediment pore water. Evidence supporting discharge of the water from the plume into the lake includes elevated values of SpC ranging from 540 to 585 uS/cm in a well nest adjacent to the lake and values exceeding 500 uS/cm in lake sediment pore water. The NVDOC plume advances more slowly than the elevated SpC plume because the migration of NVDOC compounds that degrade by iron reduction is prevented where iron oxy-hydroxide supplies remain in the aquifer. The NVDOC plume may also grow more slowly due to retardation by sorption. Baedecker et al. (1993) calculated an average retardation for BTEX of 1.5 using a mean sediment organic carbon content of 0.09% and K_{oc} of 150 cm³/g.

Metabolite Potential for Toxicity

Thorn and Aiken (1998) concluded that the NVDOC plume at Bemidji consists mainly of metabolites of compounds from the residual crude oil source. Petroleum-derived oxidation products are one of the least well-understood components of petroleum releases (Thorn and Aiken 1998; Booth et al. 2007; Lundstedt et al. 2007). This is partly due to limitations with conventional GC in which these compounds form an unresolved complex mixture (UCM). Studies that have attempted to use GC to resolve or identify the individual compounds present in the UCM portion of analyses of extracted

TPH compounds report that thousands of co-eluting compounds are present and only some can be matched to mass spectral libraries (Melbye et al. 2009; Mohler et al. 2013). Mohler et al. (2013) were able to match 750 compounds to known standards but this represented only 23% of the total ion chromatogram. Some of these compounds have been identified as alcohols, organic acids (Cozzarelli et al. 1994), aldehydes, ketones, and esters (Zemo et al. 2013; O'Reilly et al. 2015), but Mao et al. (2009) identified complex structures with multiple oxygen molecules that are not readily placed into these categories. The majority of the compounds identified in these studies have carbon numbers less than 18 carbons whereas at Bemidji, the partial degradation products recovered have carbon numbers per average molecule of 18 and higher (Thorn and Aiken 1998), so metabolites in crude oil plumes may differ from those in gasoline plumes.

Studies of mammalian and aquatic toxicity of petroleum oxidation products provide insight into some compounds that may be of most concern from an environmental and human health perspective. Rogers et al. (2002) found that chronic exposure of rats to environmentally relevant concentrations of naphthenic acids in Athabasca oil sands tailings pond water had adverse effects on liver function. Naphthenic acids were not specifically analyzed for in the Bemidji crude oil, but are most likely present, as these appear to occur universally in crude oil (Jones et al. 2001; Saab et al. 2005). In addition, Hostettler and Kvenvolden (2002) provided evidence for microbial degradation of *n*-alkylated cyclohexanes at Bemidji. Formation of carboxylic acids from terminal oxidation of the *n*-alkyl chains of the alkylcyclohexanes would result in metabolites that would fall within the classification of naphthenic acids. Zemo et al. (2013) reviewed the toxicity literature for possible petroleum metabolites and presented a summary of human toxicity rankings for five compound classes represented by suspected polar metabolites. Toxicity of these classes were ranked from moderate to low with alkylphenols being the highest followed by ketones, alcohols, aldehydes, and organic acids. The NVDOC fractions isolated all contain compounds with ketone, alcohol, and carboxylic acid functional groups (Thorn and Aiken 1998). Alkylphenols would result from microbially catalyzed hydroxylation on the aromatic rings of the naphthenoaromatic compounds. Naphthenoaromatic, and methyl substituted aromatic compounds in particular, were identified as one class of parent compounds in the crude oil for the partial degradation products (Thorn and Aiken 1998).

A number of aquatic toxicity studies have inferred or directly implicated the petroleum-derived polar metabolites as a significant contributor to overall toxicity (Neff et al. 2000; Melbye et al. 2009). Mao et al. (2009) used soil microcosms to study the toxicity of metabolites during aerobic biodegradation of fresh and weathered diesel. They found that the mass and oxygen content of metabolites increased as the experiment progressed. The toxicity of metabolites to bacteria peaked at 8 weeks but then decreased,

while a toxicity effect on cress seed germination persisted throughout the 20-week experiment. Melbye et al. (2009) studied the water-soluble fraction of an artificially weathered Norwegian Sea crude oil by separating it into 14 increasingly polar fractions and found that the more polar fraction dominated the toxicity because of its relatively large concentration of 1.5 mg/L. For comparison, Bemidji data show that the most polar fraction, HPI-A, was present in 1987 at concentrations of 8.5 to 10.5 mg/L near the source (well 522) and 1.6 mg/L at a distance of 136-m downgradient (well 515; Table 1). In both locations the HPI-A fraction constituted 20% of the NVDOC (Table 1). In the past, concerns about toxicity at petroleum hydrocarbon contaminated sites have been driven by benzene, for which the US EPA aquatic toxicity standard is 46 µg/L (U.S. Environmental Protection Agency 1996a). At Bemidji, benzene concentrations in 2010 were below this value at 130- to 160-m downgradient (Figure 3) whereas NVDOC concentrations are 5 to 10 mg/L over this same interval (Figure 4). Subtracting the 2015 background NVDOC (well 310E; Table 2) and assuming 20% HPI-A gives an estimated HPI-A concentration of 0.6 to 1.6 mg/L, suggesting the metabolite aquatic toxicity concern is potentially as significant as benzene at this site. Until these compounds and their toxicities in combination are better understood, it is important to note that, where residual sources are present, they may form expanding plumes and monitoring of these plumes would be prudent.

Existing Regulations and Future Directions

An important aspect of the toxicity literature results is that they examined only those compounds that were captured with a solvent extraction. In this study, we have presented data showing that the extracted DRO and ERDRO compounds represent one-third to one-half of the total concentration of petroleum oxidation products found in groundwater at two crude oil spill sites. The differences result from three main aspects of the analytical procedures. The first is the concentration step, which involves extraction into an organic solvent. Polar compounds do not extract well with methylene chloride or hexane (Weisman 1998), the solvents typically used for EPA method 3510C (U.S. Environmental Protection Agency 1996b). For quantitative isolation and recovery of NVDOC from both contaminated and uncontaminated water, including those constituents of NVDOC containing high concentrations of ketone, quinone, carboxylic acid and alcohol groups, resin adsorption techniques and electro dialysis/reverse osmosis procedures are generally required (Aiken et al. 1992; Green et al. 2015). The second reason is the GC analysis. Many polar compounds do not readily pass through the GC column without derivitization (Weisman 1998), thus, important compounds of interest may be retained in the column. The third reason is a restriction in the range of the DRO and ERDRO analytes, which are C₁₀ to C₂₈ and C₁₀ to C₃₂, respectively. Many metabolites of crude oil are present above and below this range (Weisman 1998). These method-dependent issues suggest that

ratios of GRO to NVDOC in plumes from gasoline spills are likely to differ from the values we found for crude oil.

The large number of metabolites and their complex structures present challenges for making progress in assessing their importance. One approach is to analyze the toxicity of the whole water (e.g., Neff et al. 2000). If toxicity is determined, better understanding of the toxic compounds will require extraction techniques targeting a range of polarities and volatilities. Analyses with liquid chromatography on targeted extractions can be used to quantify and identify semi-volatile and nonvolatile constituents (Weisman 1998). Also new analytical methods such as Fourier Transform Ion Cyclotron Resonance Mass Spectroscopy (Cho et al. 2015), and petroleomics (Marshall and Rodgers 2008), could be applied to understand the properties of larger polar compounds. Until these metabolites are better understood, a simple analysis for NVDOC as described in the Methods section of this paper is less costly than the standard DRO analysis and may be more useful for characterizing the extent of these plumes.

Summary and Conclusions

The existing literature suggests that some polar metabolites from oil may have aquatic and mammalian toxicity effects. At two north-central Minnesota sites with residual crude oil at the water table, the oil bodies are ongoing sources of NVDOC plumes containing polar compounds that are partial oxidation products of the oil. At Bemidji, the partial oxidation products appear to derive in part from the aromatic, resin and asphaltene fractions of the oil. Peak NVDOC concentrations are 20 to 50 mg/L, and NVDOC levels are two to three times greater than concentrations measured using the regulatory methods for DRO or ERDRO in the same wells. The data from these two sites indicate that the concentrations of petroleum metabolites in groundwater contaminant plumes are not fully quantified by the standard regulatory DRO and ERDRO extraction and analyses. At both sites, the most important electron acceptor is iron oxy-hydroxide coatings on the aquifer sediments. The high concentrations and continuous supply of NVDOC from the source zones have depleted the iron oxy-hydroxide supplies within and downgradient from the source zones at both sites. Data from the last 27 years at the Bemidji site show the NVDOC plume is expanding downgradient as the electron iron oxy-hydroxide accepting capacity is exhausted. A small lake is located approximately 335 m downgradient from the Bemidji source zone. Several lines of evidence demonstrate that groundwater migrating from the oil source zone discharges through the sediments to this lake. The data from these two sites suggest that, because of the large concentrations and continuous supply of metabolites associated with residual petroleum sources in soil and groundwater, more research is needed on the generation, fate, and monitoring of petroleum metabolites in groundwater.

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