

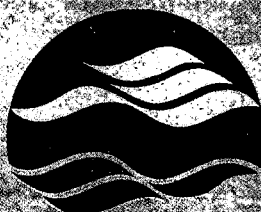
05-174



Environment
Canada

Environnement
Canada

Canada



NATIONAL WATER
RESEARCH INSTITUTE
INSTITUT NATIONAL DE
RECHERCHE SUR LES EAUX

TD
226
N87
No.
05-174

Natural Attenuation of Hydrocarbons
in a cold-climate Fuel Plume in
Ground Water, Northern Ontario

By:

G. Bickerton, D. Van Stempvoort, K. Miller

NWRI Contribution No. 05-174

05-174

NATURAL ATTENUATION OF HYDROCARBONS IN A COLD-CLIMATE FUEL PLUME IN GROUNDWATER, NORTHERN ONTARIO

Greg Bickerton, Dale Van Stempvoort and Kelly Millar
National Water Research Institute, Burlington, Ontario

ABSTRACT

Temperatures in a diesel fuel plume in an aquifer at a tank farm in Moose Factory, Ontario fluctuated seasonally between 0.7 and 10.2°C. The dissolved hydrocarbon plume extended toward the Moose River. Alkyl-benzenes, including ethylbenzene and xylenes are the main contaminants of concern. Monitoring indicated that aerobic respiration and reduction of sulfate and iron were important processes in the plume, presumably linked to biodegradation of hydrocarbons. DNA analyses indicated dominance of beta Proteobacteria, including cold-adapted bacteria and strains previously detected at petroleum-contaminated sites. *Rhodoferax ferrireducens* was detected at one location; it had also been detected in microcosms in which FeOOH was added. Trimethyl-benzene (total) was used as a tracer for conservative estimates of first order biodegradation rate for BTEX. Overall the evidence indicates ongoing biodegradation of hydrocarbons by cold-adapted bacteria; the plume appears to be stable.

NWRI RESEARCH SUMMARY

Plain language title

Field study of degradation of petroleum fuel in cold groundwater by bacteria, Moose Factory, Ontario, Canada

What is the problem and what do scientists already know about it?

The cold climate in Canada's north may prevent or retard the natural recovery of sites affected by petroleum spills. Little is known about the presence of bacteria that break down hydrocarbons in the ground under such conditions, especially in the subsurface when there is a lack of oxygen.

Why did NWRI do this study?

NWRI was asked to assist Health Canada in finding a suitable approach to remediate a petroleum spill at a hospital site in Moose Factory, northern Ontario.

What were the results?

This field study provides strong evidence that cold-adapted bacteria in the contaminated ground at the site are able to degrade hydrocarbons, some using oxygen and others without oxygen. Overall the results suggest that the contaminant plume is stable and that bacteria already present in the subsurface can be utilized to clean up the site.

How will these results be used?

Taking advantage of the positive results may lead to cost-savings at this and other petroleum-contaminated cold-climate sites in Canada. As they are published, the results will be

available to others who are managing and remediating similar sites in Canada.

Who were our main partners in the study?

Health Canada, Moose Cree First Nation

ATTÉNUATION NATURELLE DES HYDROCARBURES DANS UN PANACHE DE CARBURANT EN CLIMAT FROID DANS DES EAUX SOUTERRAINES DU NORD DE L'ONTARIO

Greg Bickerton, Dale Van Stempvoort et Kelly Millar
Institut national de recherche sur les eaux, Burlington (Ontario)

RÉSUMÉ

Les températures dans un panache de carburant diesel dans un aquifère d'un parc de stockage de Moose Factory (Ontario) fluctuaient, selon la saison, entre 0,7 et 10,2 °C. Le panache d'hydrocarbures dissous s'étendait en direction de la rivière Moose. Des alkyl-benzènes, dont l'éthylbenzène et des xylènes, sont les principaux contaminants préoccupants. La surveillance a révélé que la respiration aérobie et la baisse des concentrations de sulfates et de fer étaient des processus importants dans le panache, et qu'ils étaient probablement liés à la biodégradation des hydrocarbures. Des analyses d'ADN ont montré la dominance de bêta-protéobactéries, ainsi que la présence de bactéries et de souches adaptées au froid détectées antérieurement à des sites contaminés par du pétrole. *Rhodoferrax ferrireducens* a été détecté à un endroit; il a également été détecté dans des microcosmes auxquels du FeOOH avait été ajouté. Le triméthyl-benzène (total) a été utilisé comme traceur pour obtenir des estimations prudentes du taux de biodégradation du premier ordre du BTEX. Dans l'ensemble, les observations montrent une biodégradation continue des hydrocarbures par des bactéries adaptées au froid; le panache semble stable.

Sommaire des recherches de l'INRE

Titre en langage clair

Étude sur le terrain de la dégradation par des bactéries d'un carburant pétrolier à base de pétrole dans des eaux souterraines froides à Moose Factory (Ontario) au Canada.

Quel est le problème et que savent les chercheurs à ce sujet?

Le climat froid du Nord canadien peut empêcher ou retarder le rétablissement naturel des sites perturbés par des déversements de pétrole. On connaît peu de choses sur la présence des bactéries qui décomposent les hydrocarbures dans le sol dans ces conditions, particulièrement en dessous de la surface en l'absence d'oxygène.

Pourquoi l'INRE a-t-il effectué cette étude?

On a demandé à l'INRE d'aider Santé Canada à trouver une méthode appropriée pour assainir le site d'un hôpital qui a subi un déversement de pétrole à Moose Factory dans le nord de l'Ontario.

Quels sont les résultats?

Cette étude sur le terrain présente des preuves tangibles que des bactéries adaptées au froid présentes dans le sol contaminé du site peuvent dégrader les hydrocarbures, certaines en utilisant de l'oxygène, et d'autres sans oxygène. Dans l'ensemble, les résultats portent à croire que le panache de contaminants est stable et que les bactéries présentes sous la surface peuvent être utilisées pour nettoyer le site.

Comment ces résultats seront-ils utilisés?

En utilisant ces résultats positifs, on pourrait réaliser des économies à ce site et à d'autres sites de climat froid contaminés par du pétrole au Canada. Avec leur publication, ces résultats seront accessibles à d'autres responsables de la gestion et de l'assainissement de sites semblables au Canada.

Quels étaient nos principaux partenaires dans cette étude?

Santé Canada, Moose Cree First Nation

NATURAL ATTENUATION OF HYDROCARBONS IN A COLD-CLIMATE FUEL PLUME IN GROUNDWATER, NORTHERN ONTARIO

Greg Bickerton, Dale Van Stempvoort and Kelly Millar
National Water Research Institute, Burlington, Ontario

ABSTRACT

Temperatures in a diesel fuel plume in an aquifer at a tank farm in Moose Factory, Ontario fluctuated seasonally between 0.7 and 10.2°C. The dissolved hydrocarbon plume extended toward the Moose River. Alkyl-benzenes, including ethylbenzene and xylenes are the main contaminants of concern. Monitoring indicated that aerobic respiration and reduction of sulfate and iron were important processes in the plume, presumably linked to biodegradation of hydrocarbons. DNA analyses indicated dominance of beta Proteobacteria, including cold-adapted bacteria and strains previously detected at petroleum-contaminated sites. *Rhodoferrax ferrireducens* was detected at one location; it had also been detected in microcosms in which FeOOH was added. Trimethyl-benzene (total) was used as a tracer for conservative estimates of first order biodegradation rate for BTEX. Overall the evidence indicates ongoing biodegradation of hydrocarbons by cold-adapted bacteria; the plume appears to be stable.

INTRODUCTION

Nature has a significant capacity to reduce concentrations of contaminants as they migrate as dissolved plumes in groundwater. Microbial transformations are, in general, the primary natural mechanism for limiting the advance of petroleum hydrocarbon plumes in groundwater (Wiedemeier et al., 1999). This is referred to as intrinsic bioremediation. In recent years, interest has grown rapidly in the use of intrinsic bioremediation as a relatively passive and cost-effective approach to manage hydrocarbon plumes in groundwater. This approach, known as monitored natural attenuation (MNA), has become a preferred choice for many petroleum-contaminated sites (e.g., USA). Technical guidance and protocols are available (e.g. Wiedemeier and Chapelle, 1998; U.S. EPA, 1999). However, doubt persists whether MNA is a viable approach in cold environments.

There is some field evidence that intrinsic bioremediation can be effective in cold climates, for

example in Alaska (e.g. Bradley and Chapelle, 1995; Braddock and McCarthy, 1996; Herrington et al., 1997). More recently, evidence that intrinsic bioremediation is important in groundwater in western Canada has been reported (Armstrong et al., 2002). However, information on effectiveness of intrinsic bioremediation of hydrocarbons in groundwater at cold temperatures (~5°C) is still limited. In particular there is very little information on the role that anaerobic microorganisms may play in the biodegradation of hydrocarbons in groundwater at cold (~5°C) temperatures.

Study Site

In 2002, Health Canada asked our research team to undertake an investigation to help them to manage a diesel fuel plume in groundwater at a tank farm at Weeneebayko Hospital in Moose Factory, northern Ontario (Figure 1). A major spill had occurred at the tank farm in the mid 1970's. Given the cold climate site conditions (annual average air temperature is -1.1°C), some previous advisors were skeptical that bioremediation (intrinsic or enhanced) would be a viable option. Previous investigators, with varying degrees of complexity, attempted to estimate the rate of advance of the hydrocarbon plume and to determine how long it would take to impact the Moose River, which is ~100 m down gradient of the tank farm.

Whether the dissolved hydrocarbon plume at this site is expanding, stable or shrinking is a key question. The nature, cost and aggressiveness of the selected remedial approach for the site will likely be determined by the stability of the plume. As reported in this paper, we conducted a detailed field investigation to determine the significance of intrinsic bioremediation. We used several lines of evidence: (a) historic and spatial trends of contaminant concentrations, (b) patterns of geochemical indicators in the groundwater consistent with the biodegradation of petroleum hydrocarbons, and (c) relevant microbial analyses.

METHODS OF INVESTIGATION

To delineate and monitor the dissolved

hydrocarbon plume, we used a network of 19 existing monitoring wells, and filled in information gaps by installing 19 more wells, largely in the downgradient portion of the plume that extended toward the Moose River. This included one new "nest" of 3 wells with short screens (0.3 m), centered at 1.5, 2.3 and 3 m below ground. This nest provided information on the vertical profile of the plume. Groundwater was sampled from the network 5 times between August 2002 and September 2004. All samples were placed on ice and stored at 4°C prior to analyses, which were conducted within 14 days of collection.

Field acidified samples were analyzed for BTEX (benzene, toluene, ethylbenzene, m/p-xylene and o-xylene), isomers of trimethyl-benzene (TMB) and isomers of tetramethyl-benzene (TeMB) by purge and trap, GC-MSD. Acidification was required to preserve BTEX. TMB and TeMB were analyzed as potential conservative species to trace BTEX degradation (see Results). Total hydrocarbons (C_6 to C_{24}) were extracted in dichloromethane and analyzed by GC-FID.

Solinst LevelLoggers (automated probes with dataloggers) were installed in several wells to monitor water levels and in-situ temperatures. Dissolved concentrations of available electron acceptors were analyzed: dissolved oxygen (DO) in the field using a YSI 556 probe and flow cell; nitrate and sulfate by ion chromatography. Alkalinity (titration) and dissolved (45- μ m field-filtered) iron and manganese (ICP-OES) were analyzed as potential metabolic by-products of degradation. At the near-neutral pH conditions, total iron and manganese were reliable surrogates for iron(II) and manganese(II). Oxidation-reduction potential (ORP) of groundwater was measured on site (YSI 556 multiprobe, flow cell) as a semi-quantitative indicator.

For microbial analyses, total DNA was extracted from 1-L groundwater samples using Ultraclean™ kits (MoBio Labs) and further purification through Sephacryl S400HR columns (Amersham). Eubacterial 16S rDNA was amplified by touchdown PCR using primers 341F-GC and 907R as described by Muyzer et al. (1998). DGGE analyses were conducted at 60°C, 100V, for 15.5 hours, using 7% acrylamide gel with 25-55% denaturing gradient. DGGE bands were excised, reamplified, sequenced, and compared to GenBank databases using the BLAST algorithm (Altschul et al., 1990) and the Ribosomal Database Project (RDP) using

Sequence Match (Cole et al., 2005).

RESULTS AND DISCUSSION

The temperatures in near surface ground water (monitoring wells) fluctuated seasonally between 0.7 and 10.2°C, and were < 5°C for several months each year. As anticipated these data indicated cold conditions considered to be challenging for hydrocarbon-degrading microorganisms. Water levels in the monitoring wells were generally between 0.5 and 2.5 meters below ground surface (mbgs) but fluctuated seasonally by up to 1.5 m.

Hydrocarbon plume

The latest (September, 2004) distribution of the fuel plume in the groundwater is indicated by the contours of total hydrocarbon concentrations in Figure 1. The concentrations typically ranged from about 5-10 mg/L in the source area to < 0.1 mg/L down gradient, near the Moose River

BTEX distribution

The most abundant BTEX compounds in the plume were xylenes (up to 405 μ g/L) and ethylbenzene (Figure 2). Benzene and toluene (not shown) were detected in wells near the tank farm (up to 10 and 5 μ g/L respectively), but were either non-detectable or below regulatory guidelines in all samples from wells within 70 m of Moose River.

The m/p-xylene plume extended to the bank of the Moose River, but concentrations decreased by 3 orders of magnitude from the source area to the Moose River, where concentrations in groundwater seepage were at trace levels (~ 0.1–0.5 μ g/L) below the surface water compliance criterion for m-xylene (Table 1).

In the down gradient portion of the plume near the Moose River, ethylbenzene was generally near or below detection, and below the Ontario objective for surface water quality (Figure 2; Table 1). The downgradient extent of the ethylbenzene plume (Figure 2) was less than that of m/p-xylenes (not shown).

At the multi-level installation of three wells with short screen intervals (0.3 m), no consistent pattern in the vertical distribution of BTEX concentrations could be identified between the sampling events.

Trimethylbenzene (TMB) as Tracer of Degradation

Total TMB concentrations were higher than TeMB concentrations, so TMB was chosen as the most useful tracer. The overall TMB plume geometry (Figure 3) resembled that of total hydrocarbons (Figure 1). TMB was detected in groundwater discharge at the banks of the Moose River but only at trace levels ($\sim 0.1 \mu\text{g/L}$).

The ratio BTEX/TMB declined down gradient of the source region (Figure 4). Following the method described by Wiedemeier et al. (1999), this ratio can be used to estimate a conservative, first-order rate constant for BTEX biodegradation, assuming that TMB is not degraded in the plume. Using an estimated average groundwater velocity (14 m/year) based on a calibrated flow model, we calculated overall first-order biodegradation constants of between 0.0004 and 0.0015 day^{-1} for total BTEX. These rates are consistent with the rate constants (median of 0.0013 day^{-1}) inferred for total BTEX in 25 plumes in western Canada (Armstrong et al., 2002). In an extensive review of field-measured biodegradation rates for BTEX (150 plumes, largely in USA), Suarez and Rifai (1999) reported median first order biodegradation rates for anaerobic conditions that were of the same order in magnitude as our inferred rate. For example, they reported median first order rates for biodegradation of ethylbenzene and xylenes of 0.002 day^{-1} under iron reducing conditions, and 0.001 to 0.003 day^{-1} for methanogenic conditions. This comparison indicates that the rate we inferred for BTEX biodegradation at Moose Factory based on TMB is similar to BTEX biodegradation rates observed elsewhere, including "warm" sites in the USA.

Geochemical Indicators

Geochemical parameters displayed patterns consistent with intrinsic bioremediation at the site. In the hydrocarbon plume, iron (II) and manganese (II) were elevated, and sulfate and oxygen were depleted (Figures 5-6, Table 2). Nitrate (not shown) was generally not detected. Inside the plume, ORP values were generally found to be lower, and alkalinity concentrations higher (data

not shown). Methane was not measured, so the role of methanogenesis at the site is unknown.

Comparison of concentrations of oxygen, sulfate, iron and manganese concentrations in the plume to concentrations in "background" groundwater (Table 2) provides an indicator of relative roles of these electron acceptors in biodegradation of hydrocarbons in the plume. Calculations (Table 2) indicate that oxygen, sulfate and iron appear to be important electron acceptors linked to hydrocarbon degradation. However, calculations for iron and manganese likely underestimate the importance of these electron acceptors, because iron(II) and manganese(II) are reactive species, readily removed from groundwater by mineral precipitation and ion exchange.

The inferred consumption of electron acceptors in the plume is equivalent to mineralization of 1.4 to 8.2 mg/L of hydrocarbons (Table 2). Note that this range is similar to the range of total concentrations of dissolved hydrocarbons observed in the plume (Figure 1), suggesting that intrinsic bioremediation is effective in degrading the hydrocarbons.

At the multi-level nest of three wells (1.5, 2.3, 3.0 mbgs), sulfate decreased significantly with depth but no consistent pattern was observed for the other geochemical indicators. This vertical profile suggests that as water passes through the soil zone to the water table, it replenishes the upper portion of the hydrocarbon plume with sulfate contributing to further degradation of the hydrocarbons along the flow path. Seasonal geochemical data (not shown) supports this hypothesis. Given this evidence, the estimates for hydrocarbon consumption linked to lateral depletions of sulfate (and perhaps oxygen) in Table 2 should be viewed as conservative.

Collectively, the observed geochemical patterns indicate that significant intrinsic bioremediation of fuel oil occurs at the site. Oxygen, sulfate and iron(III) appear to be dominant electron acceptors.

Microbial Analyses

DGGE analysis indicated a predominance of the beta subgroup of Proteobacteria at the site (Table 3). There were close matches to a number of cold-adapted bacteria, many of which have been detected at other hydrocarbon-contaminated sites. Banding patterns generated by DGGE indicate a relatively consistent microbial community in six of

seven samples collected downgradient of the tank farm (ii-iii, v-viii on Figure 7). Bands shared by these six samples are D, G, H, L, and O. Of greatest intensity is band D, having a closest sequence match with an uncultured *Polaromonas* sp. found in arctic sea ice. Bands G and O, are closely related to strains of the genera *Syntrophus* and *Spirochaeta*, both previously detected in a jet fuel-contaminated aquifer in Michigan undergoing intrinsic bioremediation (Dojka et al., 1998). *Acidovorax* and *Chlorobi* strains were best matches for bands L and H, respectively, both of which have been detected in benzene-contaminated groundwater (Cole et al., 2005). Further downgradient of the tank farm at location iv (cf. Figures 1 and 7), a significant change in the microbial community is apparent. The three sequences obtained for this sample returned matches of *Rhodoferax ferrireducens*, an anaerobic, iron-reducing, bacterium. Interestingly, this strain was detected in an earlier microcosm experiment conducted using soil from location vii (Figure 1), to which iron (FeOOH) was added as a terminal electron acceptor (Van Stempvoort et al. 2004). FeOOH amendments resulted in the greatest losses of hydrocarbons, suggesting that iron-reducing bacteria may play an important role in the intrinsic bioremediation of the plume at this site. Absence of this strain from the DGGE profile at location vii and others within the plume (cf. Figures 1,7), indicates that conditions, iron availability for example, were unfavourable for *Rhodoferax ferrireducens* to be a dominant member of the microbial community.

Plume Stability

Wells currently outside the plume have consistently had no detectable hydrocarbons since first samples were collected in 2001-2002. Seasonal variations in total hydrocarbon concentrations in selected wells within the plume are shown in Figure 8. In general, concentrations in summer were elevated relative to the fall. We suspect that this apparent seasonality may be largely an artifact of water level fluctuations, which affect the portion of the saturated profile that is sampled, determined by the relationship between the well screen interval and the water table.

Using the Mann-Kendall Test (Gilbert, 1987) for the total hydrocarbon (Figure 8) and BTEX concentration data (not shown), no statistically significant trend could be established. This analysis suggests that the plume is currently

stable.

The evidence of plume stability is consistent with recent research (Rice et al., 1995; Mace et al., 1997) indicating that most petroleum plumes of this age (~ 30 years) are stable. These earlier studies demonstrated that dissolved hydrocarbon plumes typically change slowly and they stabilize with down gradient extents typically less than 100 m.

Our suggestion that the plume is stable is contrary to findings and opinions expressed in previous assessments of the site, which attempted to estimate the rate of advance of the hydrocarbon plume and to determine how long it will take to impact the Moose River. Based on our results, the plume has already reached the Moose River, albeit at concentrations below regulatory guidelines.

Considerable evidence in this study suggests that natural attenuation has been, and may continue to be, effective for treating the existing plume. This finding expands the possible treatment technologies and management strategies that may be considered for remediation of dissolved-phase contamination at this cold climate site.

Based on geochemical indicators, microbial analyses, and evidence for stability of this hydrocarbon plume, it appears that the cold climate at Moose Factory is not a major hindrance to intrinsic bioremediation. This implies that either MNA or technologies based on enhancing biodegradation may be considered for application at this and similar cold climate sites.

ACKNOWLEDGEMENTS

Technical assistance was provided by John Voralek (sampling), Susan Brown (organic analyses), Helena Steer (DGGE), and Jerry Rajkumar (metals). The Wastewater Technology Centre (Environment Canada, Burlington) conducted the large majority of chemical analyses.

REFERENCES

- Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J. 1990. Basic local alignment search tool. *J. Mol. Biol.* 215:403-410.
- Armstrong, J.E., Biggar, K., Staudt, W., Cross, K., Moore, B.J., Greene, A. 2002. Assessment of Monitored Natural Attenuation at Upstream Oil and Gas Facilities in Alberta: Final Report, Canadian

Association of Petroleum Producers Research Report 2001-0010, Komex International Ltd., Calgary, AB.

Braddock, J.F., McCarthy, K.A. 1996. Hydrologic and microbiological factors affecting persistence and migration of petroleum hydrocarbons spilled in a continuous-permafrost region, *Environ. Sci. Technol.* 30(8):2626-2633

Bradley, P.M., Chapelle, F.H. 1995. Rapid toluene mineralization by microorganisms at Adak, Alaska: Implications for intrinsic bioremediation in cold environments, *Environ. Sci. Technol.*, 29:2778-2781.

Canadian Council of Ministers of the Environment (CCME). 2003. Canadian Environmental Quality Guidelines: Summary Table, December 2003.

Cole, J.R., Chai, B., Farris, R.J., Wang, Q., Kulam, S.A., McGarrell, D.M., Garrity, G.M., Tiedje, J.M. 2005. The Ribosomal Database Project (RDP-II): sequences and tools for high-throughput rRNA analysis. *Nucleic Acids Res.* Jan 1;33 (Database Issue):D294-D296. doi: 10.1093/nar/gki038.

Dojka, M.A., Hugenholtz, P., Haack, S.K., Pace, N.R. 1998. Microbial diversity in a hydrocarbon- and chlorinated-solvent-contaminated aquifer undergoing intrinsic bioremediation. *Appl. Environ. Microbiol.* 64:3869-3877.

Gilbert, R.O. 1987. *Statistical Methods for Environmental Pollution Monitoring*, Van Nostrand Reinhold.

Herrington, R.T., Benson, L., Downey, D., Hansen, J. 1997. Validation of fuel hydrocarbon attenuation in low-temperature groundwater environments. *In* *In Situ and On-Site Bioremediation* 4(1), Battelle Press, 303-308.

Mace, R.E., Fisher, R.S., Welch, D.M., Parra, S.P. 1997. Extent, Mass and Duration of Hydrocarbon Plumes from Leaking Petroleum Storage Tank Sites in Texas, Geological Circular 97-1, Bureau of Economic Geology, University of Texas.

Muyzer, G., Brinkhoff, T., Nübel, U. Santegoeds, C., Schäfer, Wawer, C. 1998. Denaturing gradient gel electrophoresis (DGGE) in microbial ecology, p. 1-27. *In* (A.D.L. Akkermans, J. D. van Elsas, and F. J. de Bruijn, eds.) *Molecular microbial ecology manual*, vol. 3.4.4. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Rice, D.W., Grose, R.D., Michaelson, J.C., Doohar, B.P., MacQueen, D.H., Cullen, S.J., Kastenber, W.E., Everett, L.G., Marino, M.A. 1995. California Leaking Underground Fuel Tank (LUFT) Historical Case Analysis, California State Water Resources Control Board.

Suarez, M.P., Rifai, H.S. 1999. Biodegradation rates for fuel hydrocarbons and chlorinated solvents in groundwater. *Bioremediation J.* 3(4):337-362.

United States Environmental Protection Agency (U.S. EPA). 1999. Use of Monitored Natural Attenuation at Superfund, RCBA Corrective Action, and Underground Storage Tank Site Sites, OSWER Directive 9200.4-17P, April 21, 1999.

Van Stempvoort, D.R., Bickerton, G., Lesage, S., Millar, K. 2004. Cold-climate, in situ biodegradation of petroleum fuel in ground water, Moose Factory, Ontario, Canada. *Proceedings, NGWA & API Petroleum Hydrocarbons and Organic Chemicals in Ground Water: Prevention, Assessment, and Remediation Conference*, August 17-18, 2004, Baltimore, Maryland, USA, pp. 131-138.

Wiedemeier, T.H., Chapelle, F.H. 1998. *Technical Guidance for Evaluating Monitored Natural Attenuation of Petroleum Hydrocarbons and Chlorinated Solvents in Groundwater at Naval and Marine Corps Facilities*, Naval Facilities, Engineering Command, Alternative Restoration Technology Team.

Wiedemeier, T.H., Rifai, H.S., Newell, C.J., Wilson, J.T. 1999. *Natural Attenuation of Fuels and Chlorinated Solvents in the Subsurface*, John Wiley and Sons, 1999.

Table 1.

Hydrocarbon Species	Community/ potable water guidelines (MOE, 1997; CCME, 2003) (µg/L)	Ontario (1994) surface water quality objectives ¹ (µg/L)	Groundwater near Moose R. (3 Wells) 2002-2004 (µg/L) ²	Groundwater Seepage at Moose R. (1 Seep) 2004 (µg/L) ²
benzene	5	100	< 0.1 – 2	trace
toluene	24	8	< 0.1 – 0.5	trace
ethylbenzene	2.4	0.8	< 0.1 – 5	trace
m-xylene	-	2		
o-xylene	-	40	< 0.1 – 3	< 0.1
p-xylene	-	30		
m/p-xylenes	-	-	< 0.1 – 33	trace
total xylenes	300	-		
trimethylbenzenes	-	3	trace – 104	trace

¹comparable Canada-wide (CCME) guidelines for protection of freshwater aquatic life are higher

²trace concentrations are near the method detection limit of 0.1 µg/L

Table 2. Hypothetical amounts of hydrocarbons mineralized in the plume linked to aerobic respiration, and reduction of manganese, iron and sulfate, based on 2002-2004 monitoring data.

Dissolved Indicator Species	Background Concentration ¹ (mg/L)	Plume Concentration ² (mg/L)	Hydrocarbon Utilization Factor ³ (mg/mg)	Inferred Amt. Hydrocarbons Degraded ⁴ (mg/L)
Oxygen	2.6 to 7.6	< 0.2 to 3.6	3.14	0.0 to 2.4
Manganese	< 0.1 to 0.5	0.6 to 0.8	21.1	0.0 to 0.2
Iron	< 0.1 to 6	19 to 38	21.8	0.1 to 1.7
Sulfate	10 to 18	< 0.1 to 0.2	4.7	1.3 to 3.8
Total				1.4 to 8.2

¹based on samples from wells located outside plume; ²based on samples from wells at locations iv and v in plume (Figure 1); ³mass ratio of electron acceptors removed or metabolic by-products produced to hydrocarbons degraded (based on stoichiometry for mineralization of BTEX: Table 5.2 in Wiedemeier et al., 1999); ⁴based on differences between background and plume concentrations, and the hydrocarbon utilization factors indicated.

Table 3. Database (GenBank/RDP-II) matches for sequenced DGGE bands.

Band	Putative division	Database match	Source	%similarity
A	Comamonadaceae	<i>Rhodoferrax ferrireducens</i>	U.S. lake sediment, Fe(III) enrichment	98 %
B	Comamonadaceae	glacier bacterium FJS31	subglacial sediments and ice	100 %
C	Rhodocyclaceae	uncultured bacterium O6	oil well	100 %
D	Comamonadaceae	uncultured bacterium ARKMP-16	Arctic sea ice	99 %
E	Proteobacteria (α)	uncultured α-proteobacterium	Adirondack mountain lakes	100 %
F	Rhodocyclaceae	uncultured α-proteobacterium 8-5	coal-tar-waste contaminated aquifer	97 %
G	Syntrophaceae	uncultured eubacterium WCHB1-12	hydrocarbon-contaminated aquifer	99 %
H	Bacteroidetes	uncultured bacterium SJA-28	trichlorobenzene-degrading consortium	97%
I	Bacteroidetes	uncultured bacterium FukuN63	German lake	94 %
J	Bacteroidetes	uncultured eubacterium OCG6	oil-contaminated groundwater	99%
K	Comamonadaceae	uncultured bacterium GKS2-122	Austrian lake	99 %
L	Comamonadaceae	uncultured bacterium LJ2	Korean lake sediment	99 %
M	Comamonadaceae	<i>Variovorax paradoxus</i>	glacial ice core, anaerobic enrichment	98 %
N	Novel	uncultured eubacterium WCHB1-02	hydrocarbon-contaminated aquifer	67 %
O	Spirochaetes	uncultured eubacterium WCHB1-40	hydrocarbon-contaminated aquifer	97 %

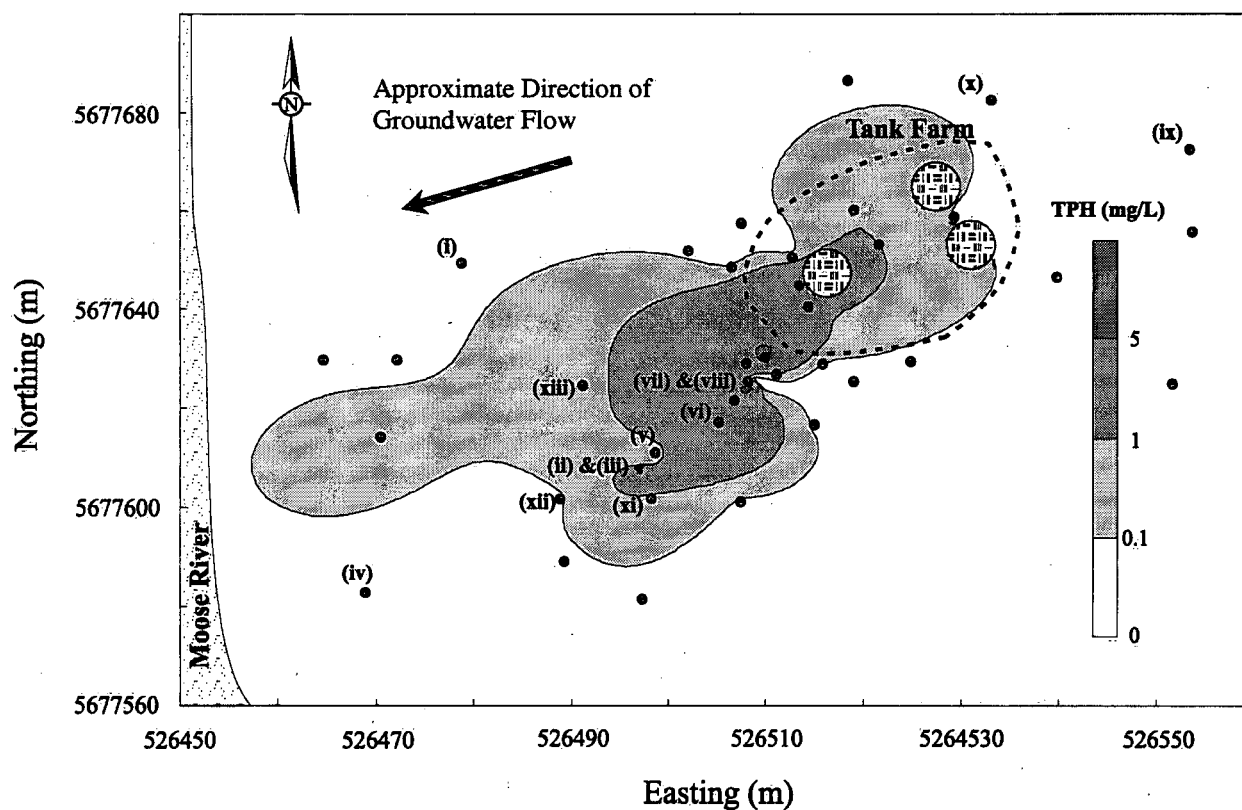


Figure 1: Site plan, showing plume of total petroleum hydrocarbons (TPH) in September 2004. Sampling points (i.e. wells) are shown as black circles. See text for discussion of points identified with (i) – (xiii).

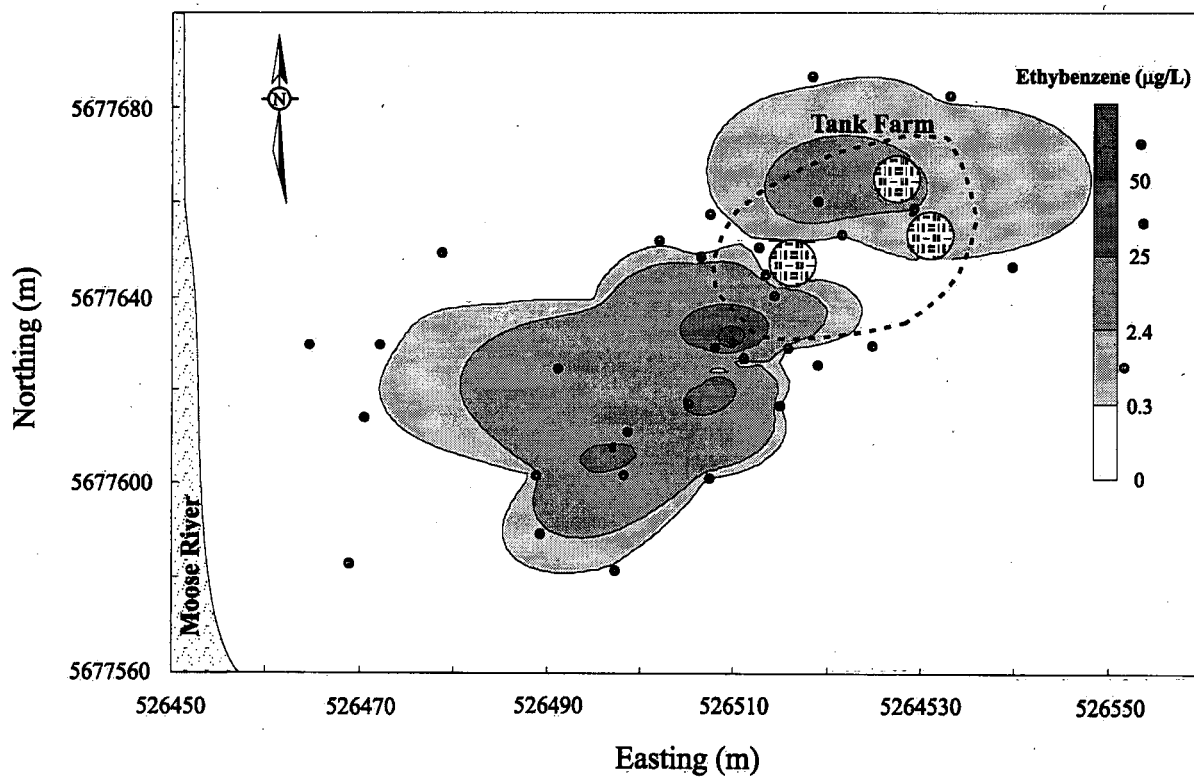


Figure 2: Ethylbenzene plume in September 2004.

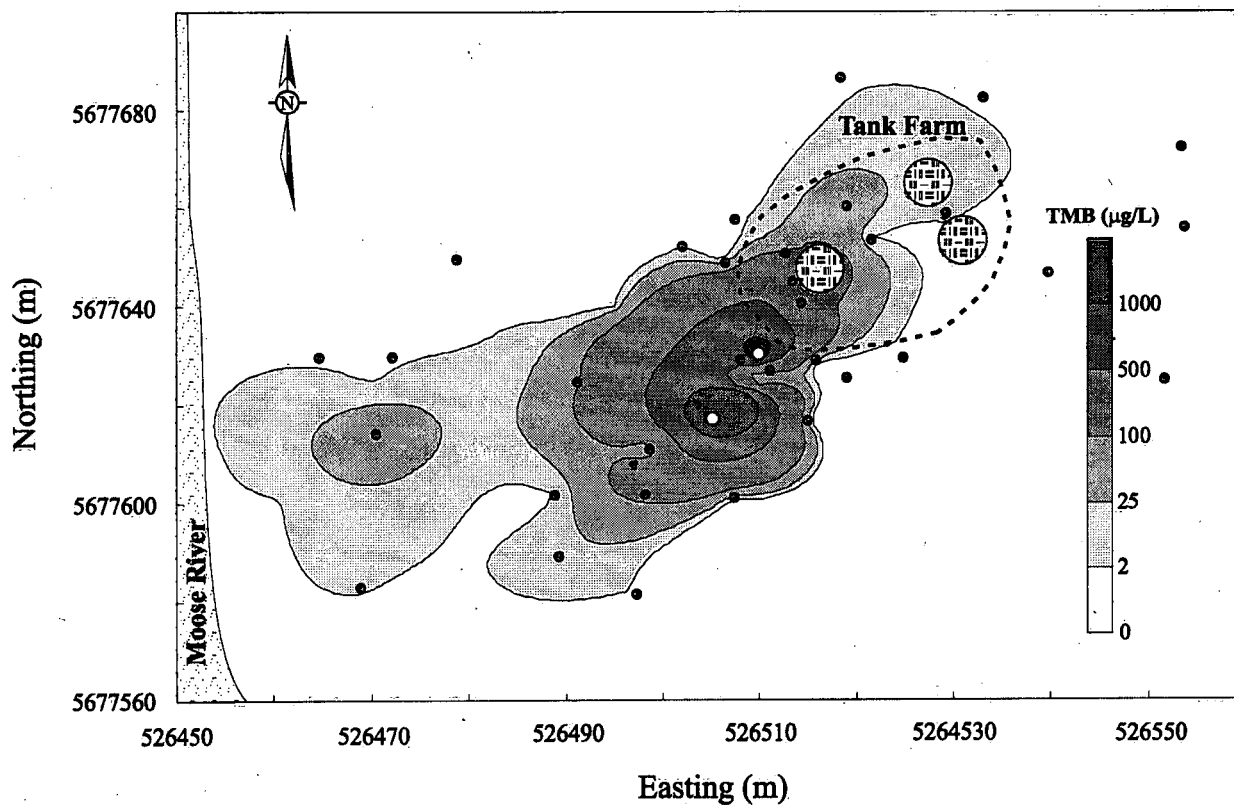


Figure 3: TMB plume in September 2004.

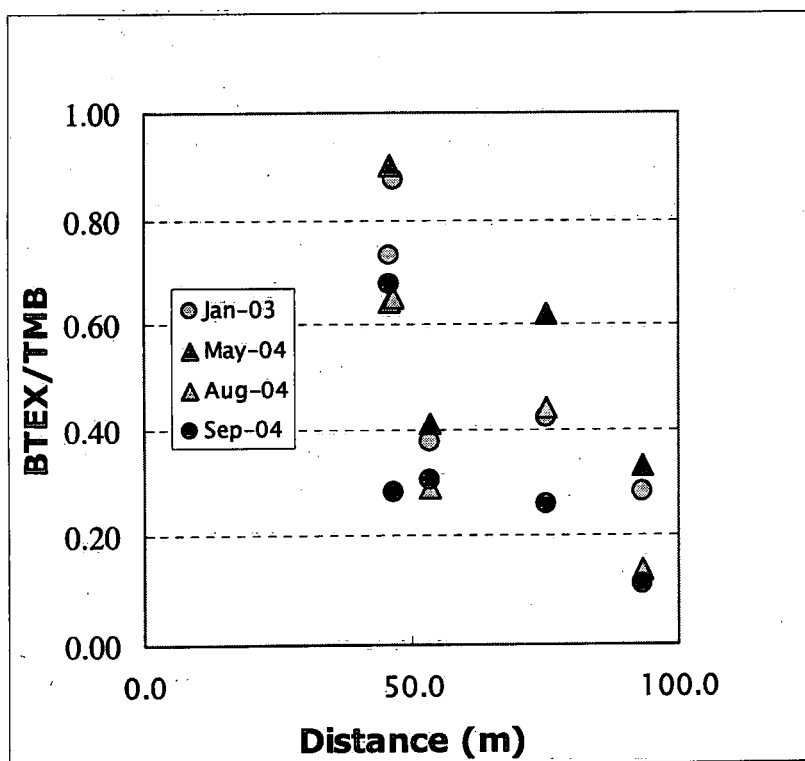


Figure 4: Figure showing ratio of total BTEX/TMB versus distance from source area. This relationship can be used to give a conservative estimate of the first order biodegradation rate for BTEX (see text).

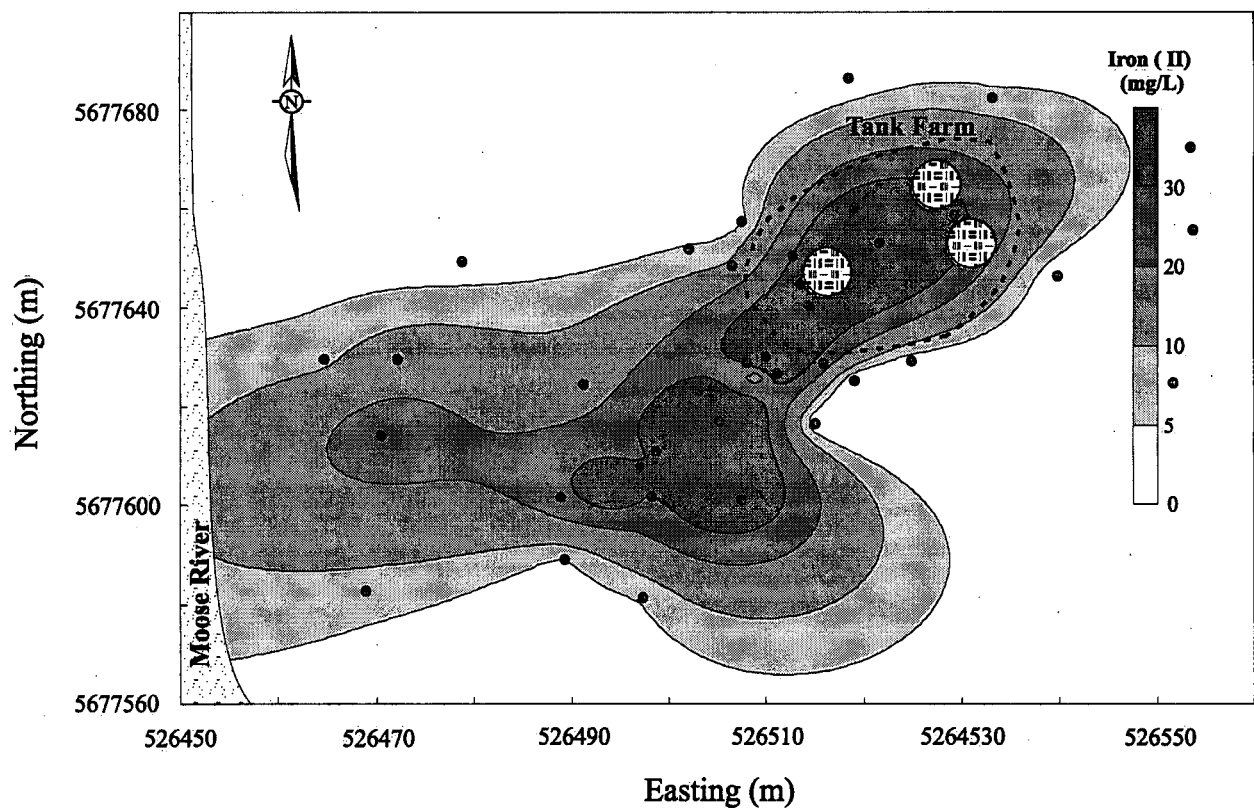


Figure 5. Dissolved iron distribution in September 2004.

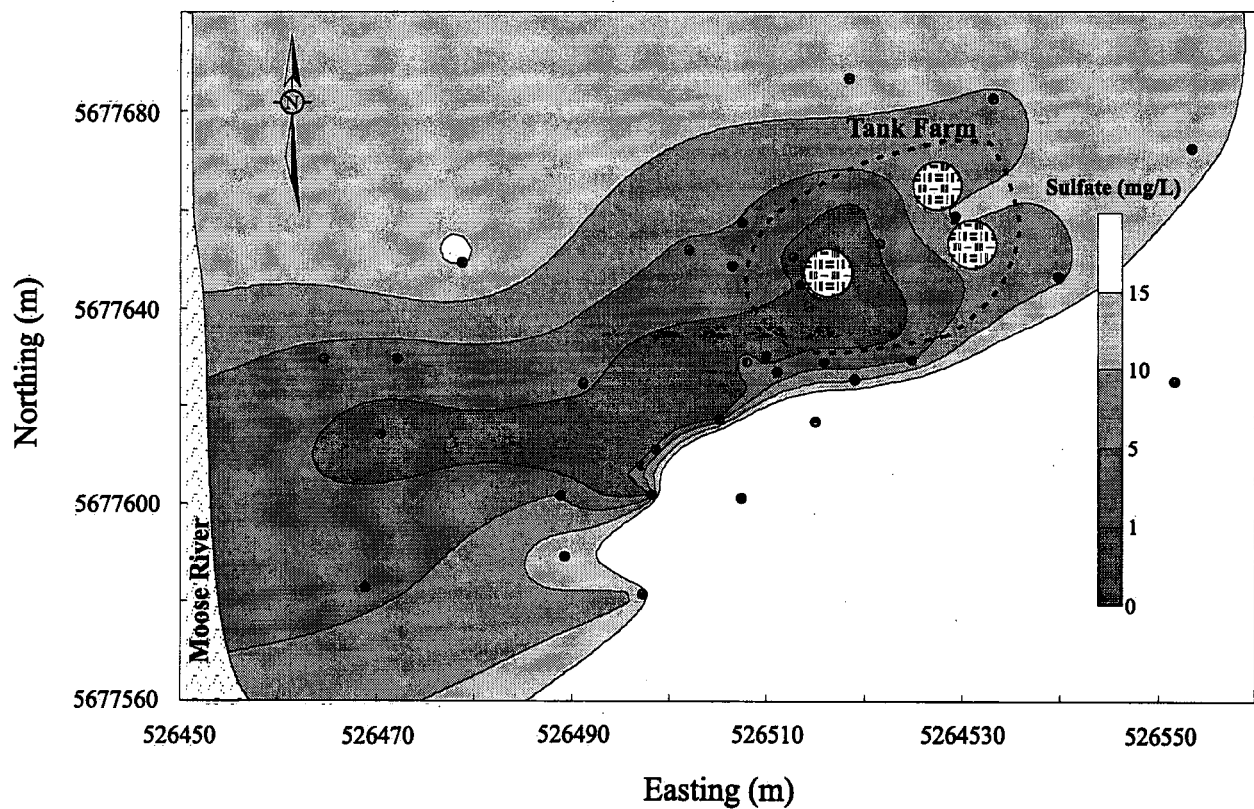
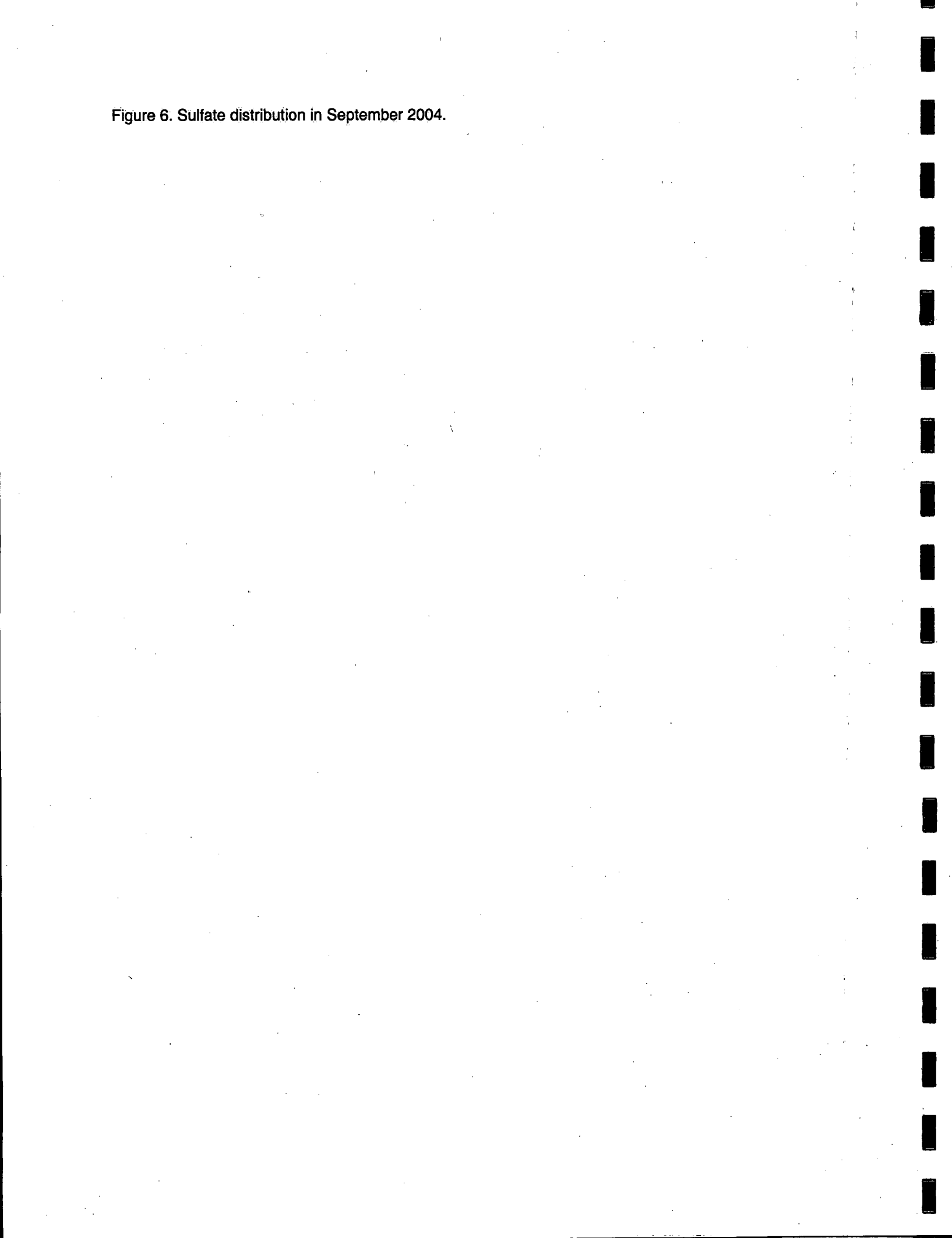


Figure 6. Sulfate distribution in September 2004.



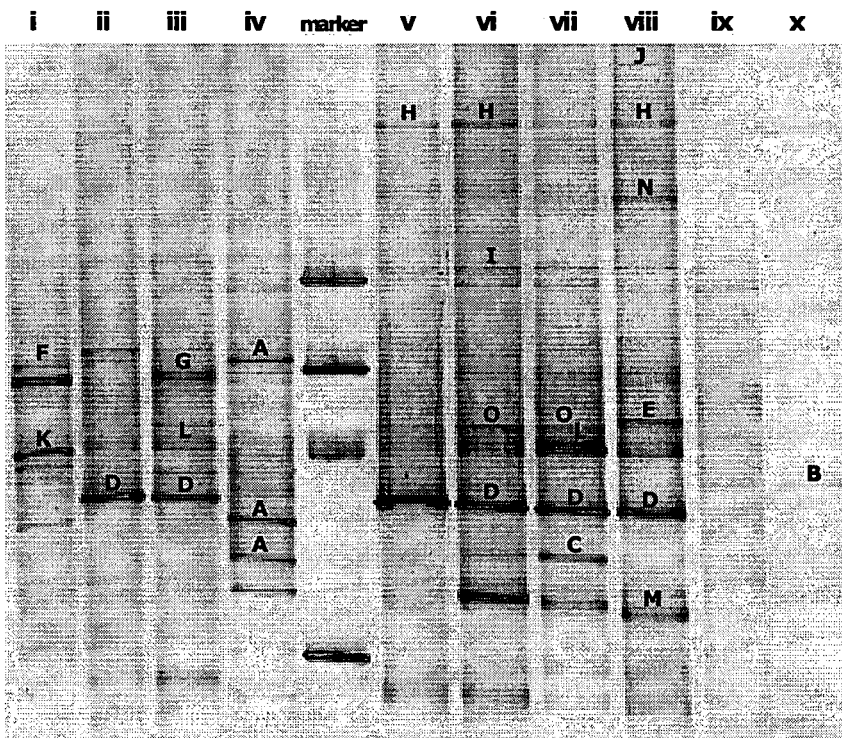


Figure 7. Photograph of DGGE gel used to separate 16S rDNA fragments. Columns i through x refer to groundwater sample locations shown on Figure 1. Labeled bands (A to O) were successfully reamplified and sequenced (Table 3). Bands with same label had identical sequences.

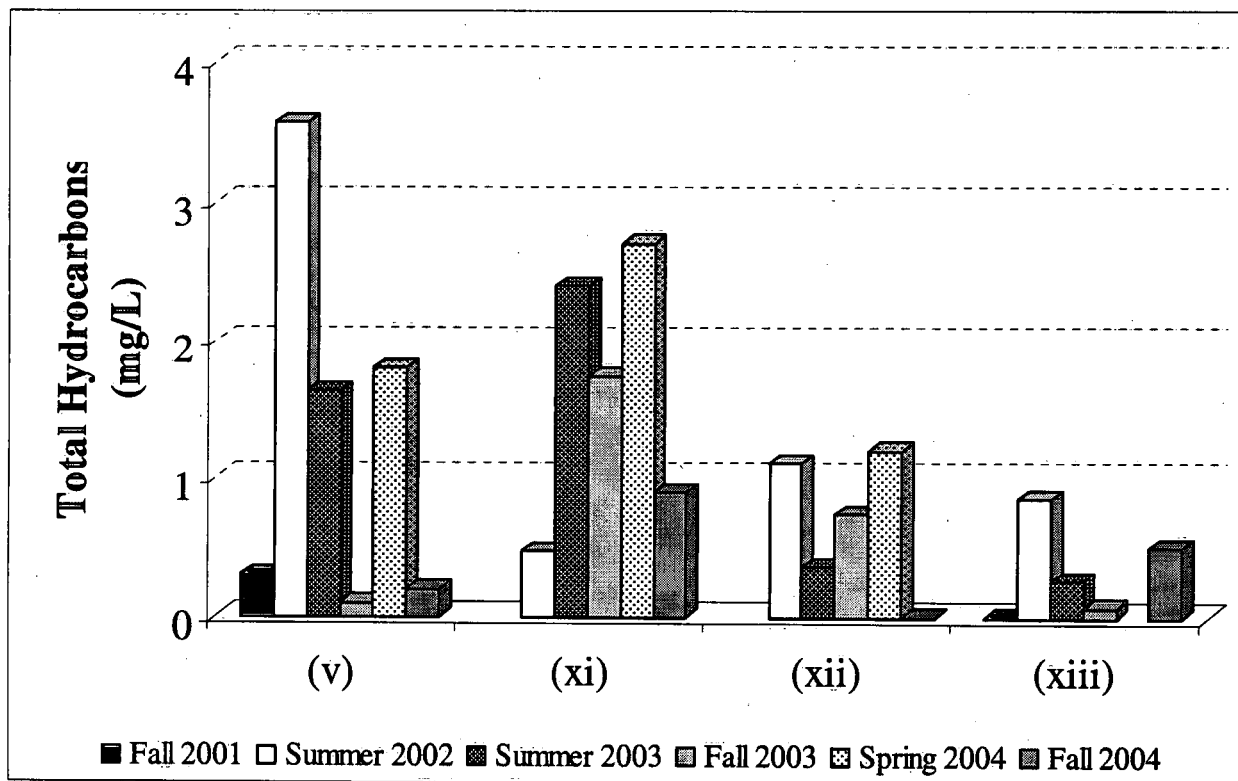


Figure 8. Seasonal variations in total hydrocarbon concentrations in selected wells, v, xi, xii and xiii (see locations on Figure 1). Previous data from Fall 2001 also included.

Environment Canada Library, Burlington



3 9055 1017 5678 0

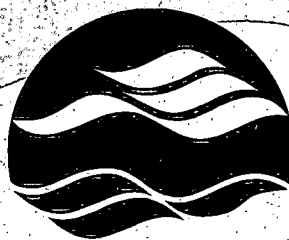
PRINTED IN CANADA
IMPRIMÉ AU CANADA



ON RECYCLED PAPER
SUR DU PAPIER RECYCLÉ

National Water Research Institute
Environment Canada
Canada Centre for Inland Waters
P.O. Box 5050
867 Lakeshore Road
Burlington, Ontario
L7R 4A6 Canada

National Hydrology Research Centre
11 Innovation Boulevard
Saskatoon, Saskatchewan
S7N 3H5 Canada



**NATIONAL WATER
RESEARCH INSTITUTE**
**INSTITUT NATIONAL DE
RECHERCHE SUR LES EAUX**

Institut national de recherche sur les eaux
Environnement Canada
Centre canadien des eaux intérieures
Case postale 5050
867, chemin Lakeshore
Burlington, Ontario
L7R 4A6 Canada

Centre national de recherche en hydrologie
11, boul. Innovation
Saskatoon, Saskatchewan
S7N 3H5 Canada



Environment
Canada

Environnement
Canada

Canada