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APPLICATION OF A MODIFIED ANALYTICAL DERIVATIZATION
METHOD TO THE DETERMINATION OF MICROBIAL
METABOLITES IN AQUIFER SYSTEMS

by

Jie Lu

A Thesis
Submitted to the
Faculty of The Graduate College
in partial fulfillment of the
requirements for the
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Jie Lu

APPLICATION OF A MODIFIED ANALYTICAL DERIVATIZATION METHOD TO THE DETERMINATION OF MICROBIAL METABOLITES IN AQUIFER SYSTEMS

Jie Lu, M.A.

Western Michigan University, 1994

The geochemical evolution of Low Molecular Weight Fatty Acids (LMWFA's) in various ground water samples and aquifer solid samples from both contaminated and uncontaminated sites has been studied. An improved analytical method has been established for both qualitative and quantitative determination of LMWFA's. The low molecular weight (LMW) organic acids are metabolic intermediates of the oxidative biodegradation of hydrocarbon contaminants. The hydrocarbons are believed to be the electron donors in these redox reactions. Formic and acetic acids were found to be the major LMWFA's in these ground water samples and aquifer solid samples. Their concentrations increase as the redox condition of sample sites changed from oxidizing condition to reducing condition. Increased concentrations of formic and acetic acids were also observed as dissolved ferrous iron concentration increased. Aquifer solids were found to contain higher concentration of LMWFA's than in ground water. The data indicate that LMWFA's in both ground water and aquifer solid are very important to a better understanding of the details of biodegradation processes.

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CHAPTER I

INTRODUCTION

Background

Ground water has been a source of fresh water since ancient times. It is the source of drinking water for approximately 50 % of the population of the United States. (Ground Water, ACS, 1989) It also is a major source of water for irrigation and industry. Usually, ground water is defined as the water that is found in saturated zones or aquifers beneath the earth's surface. From a few meters underground to many thousands, ground water slowly creeps, often moving at rates from a few centimeters to several meters per year. It is one of our most valuable natural resources. Due to the extensive use of ground water, its supply and quality are critical concerns facing all of us. Resolving these concerns will continue to be heavily dependent on improved understanding of subsurface chemistry and biology.

Ground water inadvertently serves as a sink for wastes. Wastes from accidental spills; runoff of agricultural and domestic fertilizers, insecticides and pesticides; leaky sewers; urban and highway storm water runoff; landfills and waste water from residential septic tanks; and industrial impoundments may migrate into ground water.

Contamination of groundwater with petroleum products is a widely recognized

problem which has been studied for years. Spills occurring on the surface, leaking underground storage tanks and leaking pipelines lead to accumulations of free-phase and subsurface contaminant plumes containing significant amounts of hydrocarbons such as benzene, toluene, ethyl benzene, and xylene (BTEX). This group of compounds ranks second only to trichloroethylene in frequency of occurrence as groundwater contaminants. Serious risks to public and environmental health can occur when the hydrocarbons like BTEX, which are relatively soluble in water, form a plume of contaminated ground water spreading downgradient from the original site of contamination. Remedial action may not be effective unless it is based on an understanding of the groundwater flow system and the biogeochemical reactions that control the movement and fate of the contaminants.

The concentrations of contaminants that enter the ground may decrease as they move away from their source. Changes in concentration can occur because of physical, chemical, and biological processes that take place more readily in oxic zones. These processes, such as sorption, dispersion, hydrolysis, and biodegradation, can influence contaminant transport and reduce concentrations of contaminants from those near the source. For some contaminants, the subsurface solids serve as a mechanism for water treatment, reducing mobility and toxicity, and thereby altering the contaminants' potential impact. When this natural capacity for contaminant attenuation is exceeded, ground water may be affected. Once contaminated, an aquifer is extremely expensive to clean up by conventional means. (Ground Water, ACS, 1989; Goodier, et al., 1983)

Various cleanup techniques are being used and have proven successful in certain cases. These efforts have ranged from containment to destruction of the contaminants, either in their original position in the aquifer system or by withdrawing the contaminated ground water. Examples of these remedial methods include installing pumping wells to remove contaminated water, building trenches to arrest the contaminated flow, and stimulating biodegradation of ground water contaminants. (Myller, et al., 1993; Albuquerque, et al., 1993)

Among the cleanup techniques, in situ biodegradation of toxic organic compounds has received increasing attention in recent years as a site cleanup option. It appears to be a cost-effective alternative to conventional "pump and treat" methods. Moreover, biodegradation can result in the complete degradation of hazardous substances, while technologies such as carbon adsorption and air-stripping simply transfer the pollutants to a different medium.

The chemical evolution of ground water is characterized by increasing concentrations of dissolved ionic constituents as water flows along hydrological gradients. Many of the most important dissolved constituents added to ground water, in term of chemical reactivity and mass balance, are the result of microbial processes. For example, elevated concentrations of dissolved ferrous iron, hydrogen sulfide and methane in aquifers can result from microbial mediated iron reduction, sulfate reduction and methanogenesis, respectively. (Cozzarelli, et al., 1992; Godsy, et al., 1992) Proof of biotransformation requires knowledge of the occurrence and pathways of biodegradation under specific geochemical conditions.

Low molecular weight fatty acids (C1 to C5) have been reported as intermediates of microbial degradation in ground water environments. (Berry, et al., 1987; Cozzarelli, et al., 1990) These acids have been frequently reported in oxygen-restricted environments because of the microbial decomposition of natural matter. (Barcelona, et al., 1980, 1989; Cozzarelli, et al., 1980, 1992) Formic and acetic acids are present in various samples in high concentrations attributed to bacterial decomposition of particulate and dissolved organic carbon. Propionic, butyric and pentanoic acids are generally present in detectable concentrations under only strong reducing conditions.

The fate of organic contaminants in natural environments is of interest to researchers in geochemistry, hydrogeology and microbiology as well as engineers involved in subsurface clean up efforts. Little work has been done in determining the distributions of low molecular volatile organic acids in selected ground water samples from contaminated and uncontaminated environments of varying oxidation-reduction conditions. The information about the metabolites of regulated compounds may provide evidence for biodegradation, transformation pathways, potential mass balances, and more realistic health risk assessments. In this way, bioremediation treatment techniques may be engineered and carried out more efficiently.

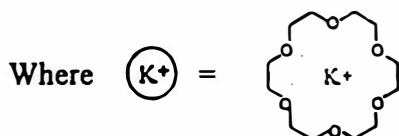
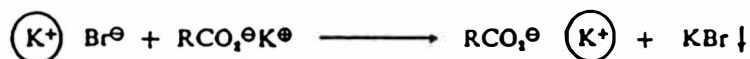
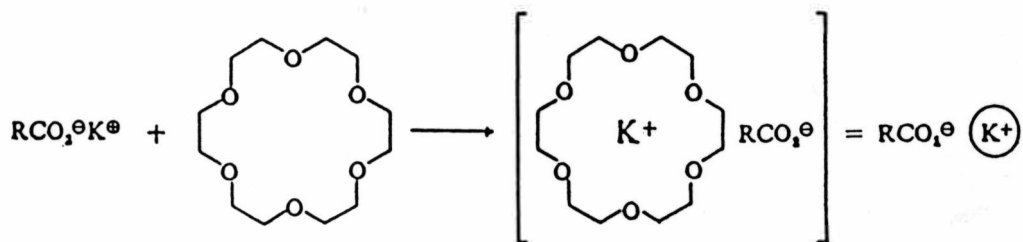
Methodology

The determination of Low Molecular Weight Volatile Fatty acids (C1-C7) in aqueous samples has been very difficult due to their aqueous solubility, volatility, and

self-association. Their solubility makes it particular difficult to extract them by liquid:liquid extraction or solid-phase concentration techniques. The matrix of water samples is another important factor which analysts have to contend with since dissolved inorganic and organic constituents often interfere with analytical methods. (Koyama, et al., 1964)

There are various analytical methods available to determine LMWFA's. Generally, these methods can be divided into two groups: determination of underivatized acids (free acid form); and determination of derivatized acids (esters of these acids). Methods for underivatized acids are fast and easy to perform. But the percent recoveries are poor for these methods mainly because of the high volatility and poor solvent extractability of these acids. (Narkis, et al., 1978; Keene, et al., 1983) Poor resolution via gas chromatography (GC) or liquid chromatography (LC) are other major problems since the acid molecules tend to form H-bonding between each other. Other disadvantages are poor flame-ionization detector (FID) sensitivity (especially for formic and acetic acids), the appearance of ghost peaks, etc..

The method used in this study is based on a derivatization reaction between the carboxylic acid and a very reactive alkylating reagent, α ,p-dibromoacetophenone. (Durst, et al., 1974, 1975) Carboxylate anions are relatively poor nucleophiles because of their weak basicity (charge delocalization between the two oxygen atoms) and their tendency to be efficiently solvated by most protic solvents. The nucleophilicity of the carboxylate anion is also compromised by coulombic interaction with its companion cation. The rate of the reaction between an anion (carboxylate)



and neutral molecules (alkyl halide) increases by several orders of magnitude going from a protic solvent (e.g., water, alcohol, etc.) to polar aprotic solvents (e.g., acetonitrile, acetone, and dimethylsulfoxide). In 1974, Durst and his co-workers found that a crown ether compound (dicyclo-hexyl-18-crown-6) has catalytic properties to form p-bromophenacyl ester derivatives of carboxylic acids. The crown ether serves as a phase transfer agent from aqueous or solid matrices to an organic solvent. It selectively complexes potassium ion, as a companion cation of carboxylate anion, to create a "naked anion" which is extremely reactive. Reaction with an alkyl halide such as α , p-dibromo-acetophenone then affords the corresponding ester in high yield. The resultant phenacyl esters exhibit good chromatographic properties for either liquid or gas/solid separation and identification.

The experimental procedures used in this study were mainly based on Kawamura's method (Kawamura, K.; Kaplan, I.R., 1984) and Barcelona's (Barcelona, et al., 1980) with the inclusion of an isotope dilution technique as a quantitation tool for better quality control. The authenticity of the product ester identifications was verified by gas chromatography/mass spectrometry (GC/MS, Varian).

CHAPTER II

METHODS AND MATERIALS

Reagents and Standard Solutions

LMWFA Standard Solution

A volatile acid mixture (C1 - C7, 10 mM each in water) was obtained from Supelco, Inc. (Bellefonte, PA)

Internal Standard (Potassium Acetate- d_3 solution, 10 mM)

850.6 mg of acetate- d_3 , Na⁺ salt was weighed and dissolved in about 5 mL of deionized water. The solution was transferred onto an ion-exchange column which contains 20 ml of cation-exchange resin (K⁺ form). The column was washed with 5 bed volumes of deionized water, and all eluate was collected in an 100 mL volumetric flask. The solution was diluted to volume with deionized water. (Solution A, 100.0 mM)

1 ml of solution A was diluted to 10 ml with deionized water. (Solution B, 10 mM)

α ,p-Dibromoacetophenone

Obtained from Janssen Chimica through Spectrum Chemical Manufacturing

Corp.(Gardena, CA). 0.2219 g of α ,p-dibromoacetophenone was weighed and dissolved in 4 ml acetonitrile. (0.2 M)

Dicyclo-hexyl-18-Crown-6

0.0298 g of dicyclo-hexyl-18-crown-6 (obtained from Aldrich Chemical Co.) was dissolved in 4 ml of acetonitrile. (0.02 M)

Ion-exchange Resins and Columns

About 50 ml of ion-exchange resin (AG W-X4, 100-200 mesh, hydrogen form, from BIO-RAD) was first cleaned as follow: the resin was rinsed with two bed volumes each of 10% aqueous KOH (w/v), deionized water, 10% HCl (v/v), deionized water, 10% aqueous methanol (v/v) and deionized water.

The resin was regenerated as follows: 3 bed volumes of 1 N HCl, 2 bed volumes of deionized water, 3 bed volumes of saturated KCl at pH 8, and deionized water until the eluate was neutral.

Silicic acid Column

About 250 g of silicic acid (BIO-SIL A, 200-400 mesh) was activated at 400°C for 3 hr. It was then stored in hexane until use. The silicic acid was slurried with hexane and packed into glass-wool packed pasteur pipette for cleanup of the reaction mixtures.

Experimental Procedures

Sampling Procedures

Water samples were collected from established wells by bailing or pumping with dedicated bladder pumps at a number of sites in shallow unconfined aquifers. (Barcelona et al, 1989) Samples were preserved by the addition of KOH and refrigeration at 4°C until analysis. Aquifer solid samples were collected by either hand-augering or split-spoon sampling techniques adjacent to the screens of monitoring wells. Solid samples were packed in clean glass jars and refrigerated at 4°C prior to analysis.

Determination Procedures

Ion-Exchange Processes

50 ml of groundwater sample was adjusted to pH 8-9 with 1 N aqueous KOH. The volume of the sample was first reduced to about 5 ml by using rotary evaporator. Then it was loaded on a cation exchange column (K^+ form) filled with about 10 ml of ion-exchange resin. The acids were eluted with 4 bed volumes of deionized water and the eluate was collected and combined in a 50 ml pear-shaped flask. Finally the solution was dried by rotary evaporation.

Derivatization Reaction

4 ml of acetonitrile was added to the dried water sample (K^+ salts) in the pear-shaped flask, followed by 100 μ l of α ,p-dibromoacetophenone solution (0.2 mM) and 200 μ l of dicyclo-hexyl-18-crown-6 solution (0.02 mM). The flask was then gently heated at the bottom in a sand bath at about 80°C to effect a gentle reflux inside the flask for about 2 hours under vigorous stirring. The reaction mixture was cooled and dried with a rotary evaporator.

Purification Processes

The products of derivatization reaction in the pear-shaped flask were transferred onto the SiO_2 column (about 1 ml of SiO_2 was packed in a pasteur pipette) with 2 ml of hexane/methylene chloride (2:1). Excess reactants were washed off by adding 10 ml of hexane/methylene chloride (2:1) to the column. The p-bromophenacyl esters of volatile acids were then eluted into a 4 ml vial with 2 ml of methylene chloride/methanol (95:5). The esters were dried in the vial under a stream of N_2 and then dissolved in 500 μ l hexane for GC/MS analysis.

GC/MASS Analyses

A Varian ion-trap mass spectrometer coupled to a 3400 gas chromatograph and a Saturn data system was used for all measurements in this research. Derivatives of both calibration standards and groundwater samples were injected automatically

by a Varian autosampler. Compound separations were performed on a fused silica capillary column (0.32 mm i.d. x 30 m, 0.25 μ m film thickness, Alltech Econo-Cap SE-54). A 1077 split/splitless injector was used in splitless mode.

Gas chromatograph parameters were: injector temperature 230°C; column temperature programming: 40°C (1 min) to 140°C at 20°C/min, then to 220°C at 5°C/min; transfer line temperature at 220°C; carrier gas (helium, ultra pure) at 10 psi pressure.

Mass spectrometer parameters included: manifold temperature at 220°C; background mass at 35 amu; scan rate at 1 scan/sec, scan range 35-400 amu; mass defect of 100 mmu/100amu; ionization process in electron-impact (EI) mode; and selected ion monitoring mode for ions at m/z 43 and 46.

One microliter of each sample was injected onto the GC column. Figure 1 depicts a typical gas chromatogram for standard acids from C₁ to C₇. Sharp and well resolved peaks were easily obtained. Figures 2, and 3 show typical mass spectra for p-bromophenacyl acetate ester and p-bromophenacyl acetate-*d*₃ ester.

Quantitation

Compound quantitations were achieved by use of the isotope dilution method, comparing standard acid derivatives for acetate and *d*₃-acetate. In each mass chromatogram, the peak areas for the m/z 43 (for *d*-0 acetate) and m/z 46 (for *d*-3 acetate) were measured to quantitate the total acetate in the sample (Figure 4). The

Comment: JL0191, 40(1)-140020-220(5)05, INJ230, TL220, 1077
 Scan No: 2000 Retention Time: 33:20 RIC: 9959 Mass Range: 35 - 35
 Plotted: 400 to 2000 Range: 1 to 2100 100% = 227444%

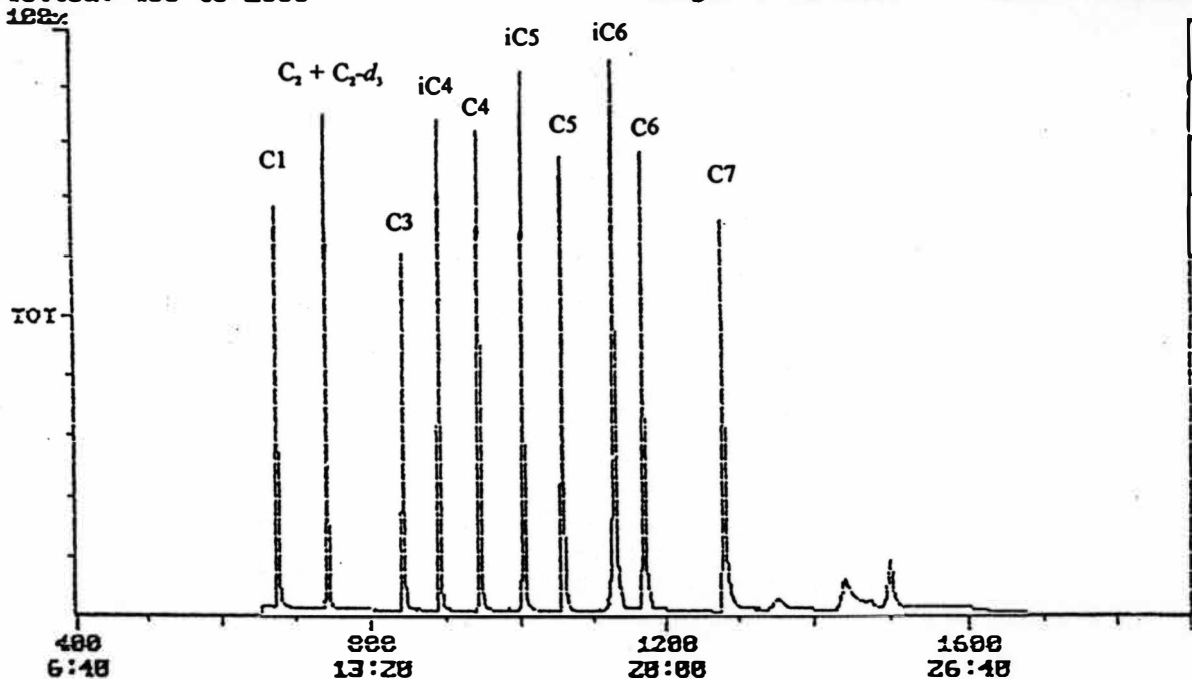


Figure 1. Gas Chromatogram of p-bromophenacyl Esters for Volatile Acid Standards (C1 to C7). One microliter (each ester: 0.2 nmol/ μ L in hexane) was injected onto the GC column.

concentration of *d*-0 acetate was calculated according to the concentration of *d*-3 acetate and the peak area of *d*-3 acetate.

$$C = \frac{A_{sam}}{A_{i,s}} \frac{n_{i,s}}{RF} \frac{1}{V_{sam}}$$

Where A_{sam} and $A_{i,s}$ are peak areas of sample and internal standard.

$n_{i,s}$ is number of micromoles of internal standard

RF is response factor

V_{sam} is sample volume

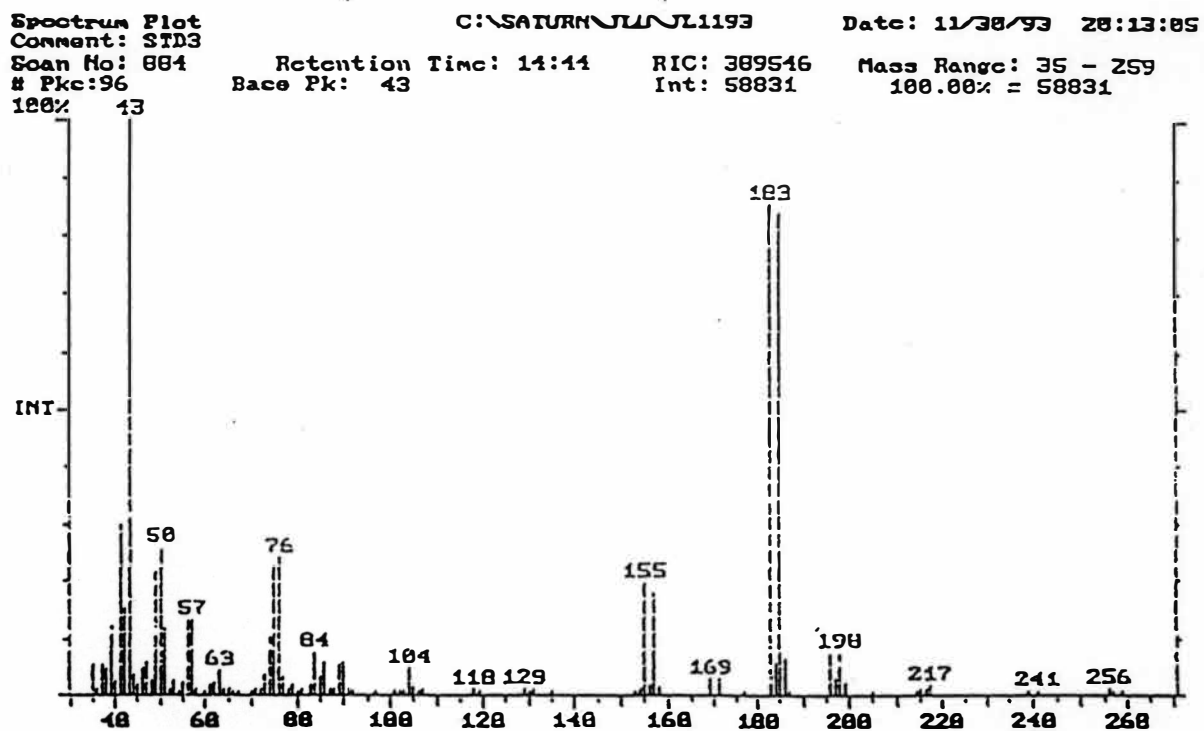


Figure 2. Electron Impact Mass Spectrum of p-bromophenacyl Acetate Ester.

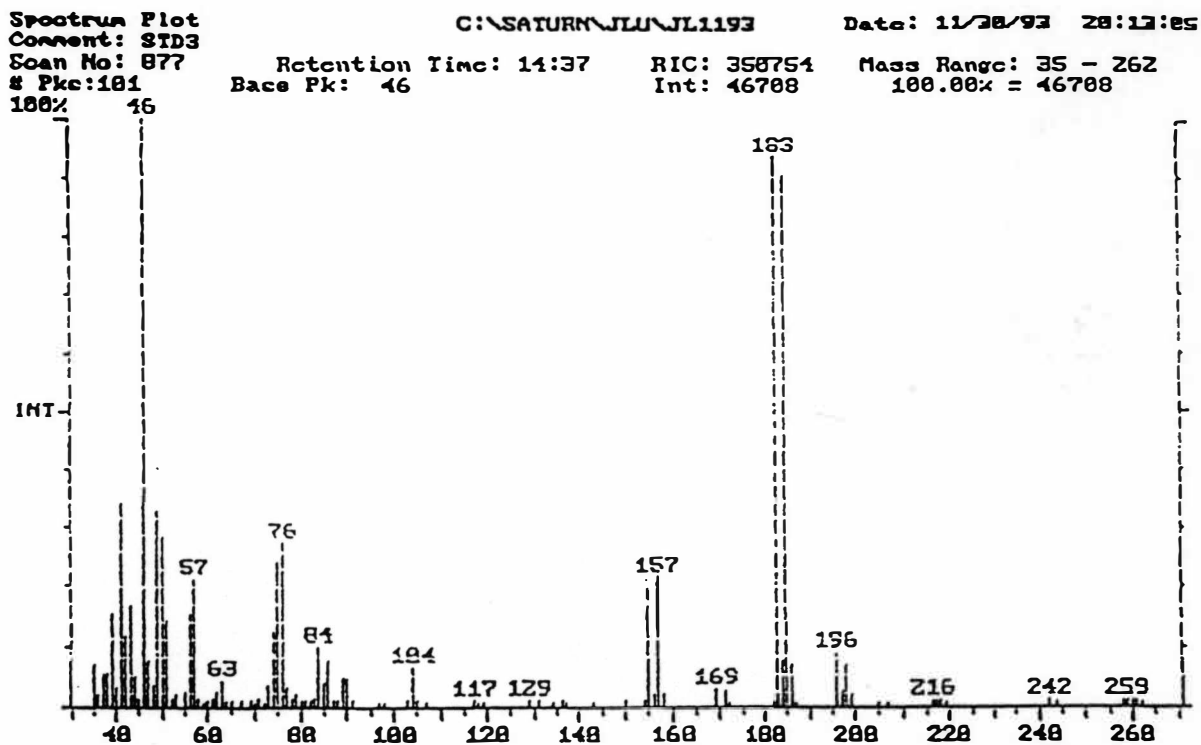


Figure 3. Electron Impact Mass Spectrum of p-bromophenacyl d3-acetate Ester.

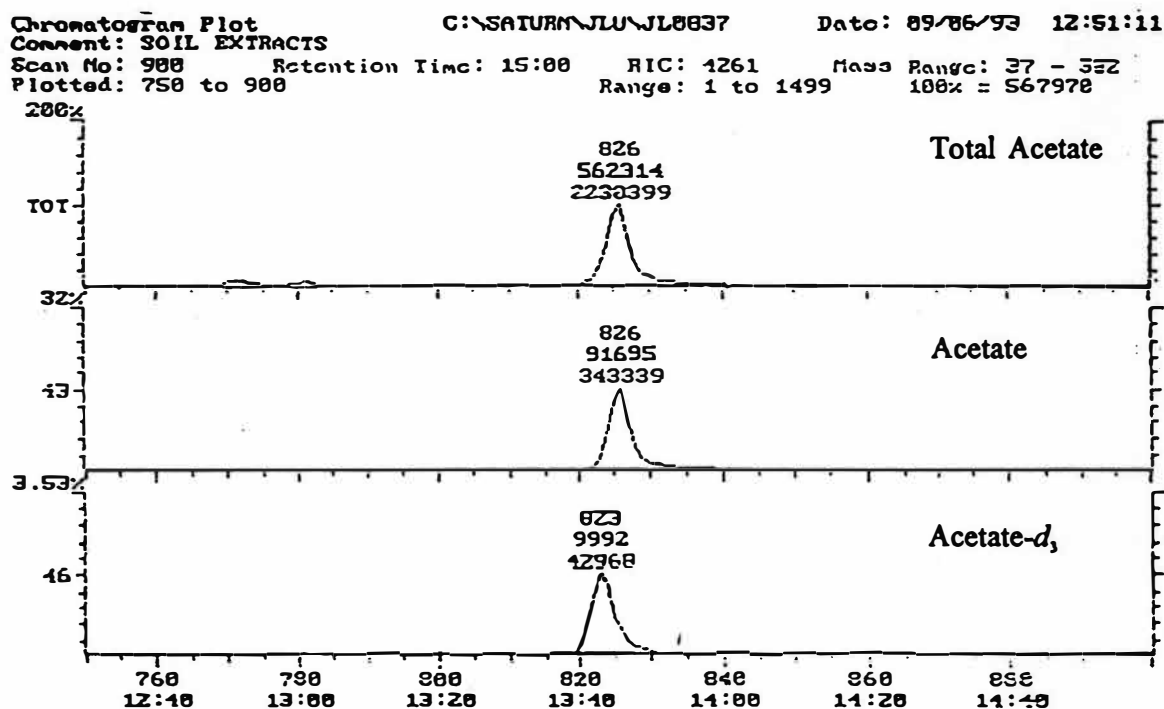


Figure 4. Selected Ion Monitoring Mass Chromatogram for p-bromophenacyl Acetate(m/z 43), p-bromophenacyl d_3 -acetate (m/z 46), and Total Acetate.

The concentrations of other organic acids were calculated with the same equation above. The response factors of the organic acids were predetermined by derivatizing standard acids assuming identical recoveries for all acids.

CHAPTER III

RESULTS AND DISCUSSION

Method Performance

Table 1 shows the precision of the modified method. Ten aliquots of volatile acid standards solution (C1-C7: 10 mM each in water) were taken (10 μ l) and transferred to ten 50 ml pear-shaped flasks, respectively. The standards were derivatized and purified with the same experimental procedures as for samples. The esters were analyzed by GC/MS. The standard deviation (SD) and relative standard deviation (RSD) were calculated. The method precision given as relative standard deviation was better than 8 % for C1 to C5 acids. Average recovery of C2 in 5 different ground water samples was 79.01 %. As shown in Figure 1, each peak contained 0.2 nmol of phenacyl esters, indicating that this method is adequately sensitive for environmental investigations (GC detection limit is ca. 0.2 nmol). When the concentration of acids in specific samples is low, it is possible to use larger volumes of water samples to compensate. Reagent blanks were run for the procedure using deionized water. No acids were found in the blanks.

Figures 5 and 6 are two gas chromatograms for ground water sample and soil extract collected from the Sleeping Bear Dune Sites, Empire, Michigan. The C1 to C4 volatile acids were identified by comparing retention time of the peaks with that

of authentic standards and by their characteristic mass spectra.

Figures 2 and 3 show mass spectra of *p*-bromophenacyl acetate ester and d_3 -acetate ester respectively, as examples. The *p*-bromophenacyl esters of each volatile acid gave characteristic base peaks at m/z 183 and 185, corresponding to the fragment ion $\text{BrC}_6\text{H}_4\text{CO}^+$. This isotopic pattern results from a relative abundance of ^{79}Br (50.52%) and ^{81}Br (49.48%). The C_2 and $d_3\text{-C}_2$ acid *p*-bromophenacyl ester show molecular ions at m/z 256 and 259, respectively, as shown in Figures 2 and 3. Other esters besides C_2 also gave their own molecular peaks as well as a base peak (m/e 183, 185).

Table 1
Analytical Performance of the Method on Standards

Parameter	Concentrations of LMWFA's, μM						
	C1	C2	C3	C4	C5	C6	C7
Mean(10)	7.46	10.4	9.18	10.8	9.13	7.99	4.44
S.D.	0.496	0.414	0.385	0.737	1.32	1.70	2.08
R.S.D.	6.65	3.97	4.19	6.80	14.5	21.2	46.9

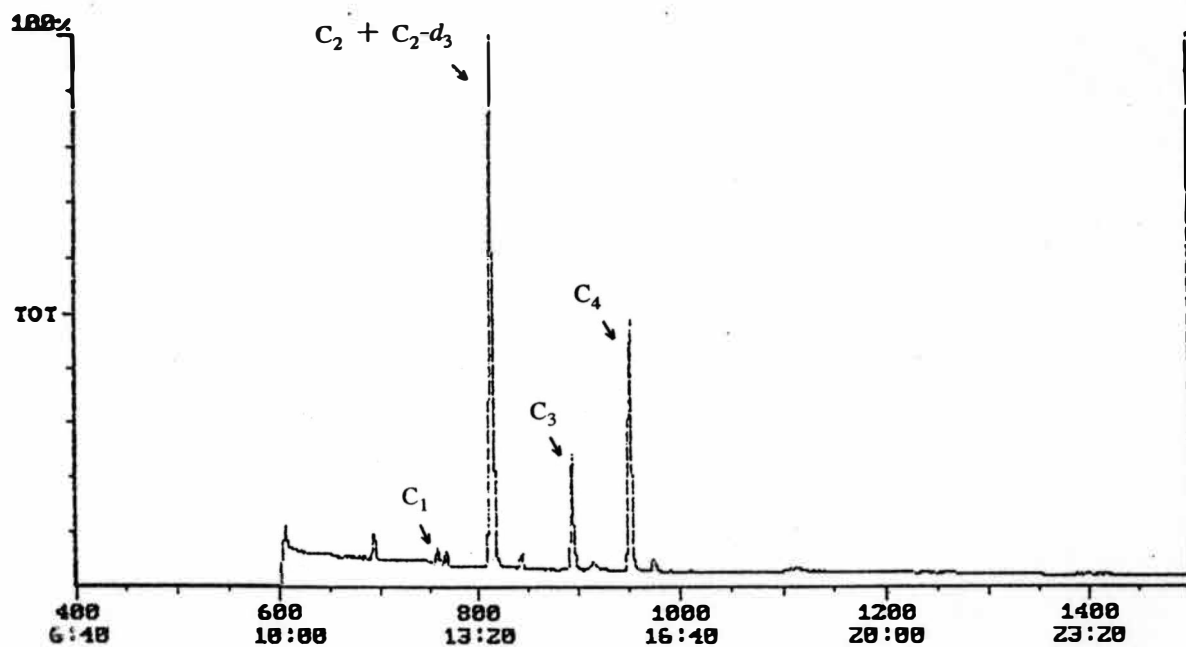


Figure 5. Gas Chromatogram of *p*-bromophenacyl Esters for LMWFA's in a Solid Sample Extract From the Sleeping Bear Dune Site, Empire, MI.

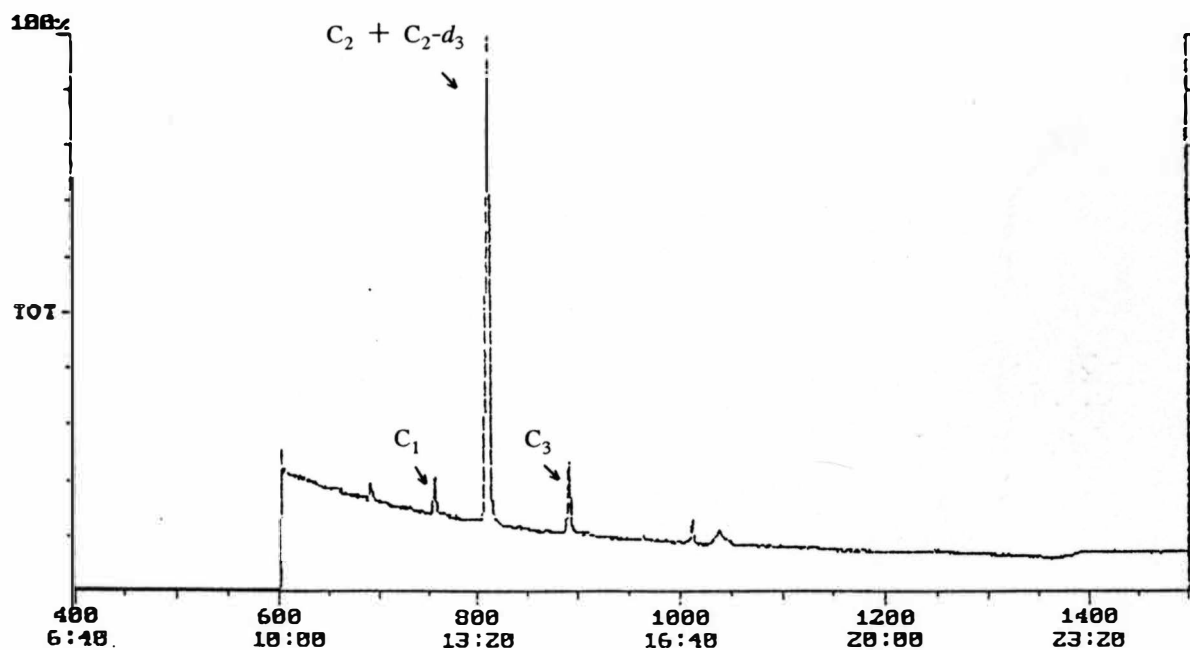


Figure 6. Gas Chromatogram of *p*-bromophenacyl Esters for LMWFA's in a Ground Water Sample From the Sleeping Bear Dune Site, Empire, MI.

Method Application

Previous studies of the distribution of LMWFA's in marine sediments and subsurface environments have suggested that biological transformations of hydrocarbons to organic acid intermediates may be important attenuation processes in anoxic environments. They may contribute significantly to the total organic carbon and provide at least qualitative indications of the geochemical conditions under which transformations may occur. (Baedecker et al., 1979, 1992; Barcelona, 1980). In general, concentrations of total dissolved acids have been found to be significantly higher under reducing or contaminated conditions than under oxidizing or uncontaminated conditions.

Samples from 3 different sites have been studied: a shallow sand and gravel, ground-water system contaminated with anaerobic impoundment leachate (Beardstown, IL) (Barcelona et al., 1989), a dune-sand shallow ground-water site at which several petroleum product releases occurred (Sleeping Bear Dunes State Park, Empire, MI), and three underground storage tank sites in low permeability clay settings (Houston, TX). (Samples from the tank sites were provided by Dr. Joseph Salanitro of the Shell Development Company)

General Leachate Contamination/Reducing Conditions

Inorganic indicators of biotransformation processes under various redox condition, such as Fe^{2+} , SO_4^{2-} , NO_3^- , and NH_4^+ , have been studied for years.

(Barcelona et al., 1989; Pedersen et al., 1991) Microcosm studies in laboratory have also shown that hydrocarbons are important electron donors in anoxic environments (Berry, et al., 1987). They have been proven to be involved in the processes such as iron reduction, sulfate reduction, and nitrate reduction. It was logical to pursue corresponding organic indicators of biogeochemical conditions in organic-rich systems.

Figure 7 shows the average concentrations of dissolved formate and acetate in ground water from the Beardstown site (Illinois) which included suboxic (i.e. dissolved oxygen < 0.5 mg/L, CH_4 levels below 0.05 mg/L) and reducing zones (i.e. no detectable O_2 , CH_4 levels > 0.05 mg/L). Generally, low concentrations of organic acids were found at high Eh levels (relative to the standard hydrogen electrode), Table 2. Eh, the redox potential of the system, is a measure of the activities of species at equilibrium that participate in oxidation-reduction reactions. As Eh value decreases (the reducing zone), acids concentration increased significantly. Formic acid, in particular, increased from 162 ppb to 547 ppb in the redox transition. This result is coincident with the previous studies that LMWFA's are intermediate compounds of biodegradation processes in anaerobic aquifers. (Godsy, et al., 1992; Cozzarelli, et al., 1993) In their study, both formic and acetic acids were found to increase in concentration in ground water from the anoxic zone. Formic acid concentrations increased from non-detectable (< 0.10 ppb), at oxic or suboxic zones, to 0.97 ppb at anoxic zone. Increased acetic acid concentration also was observed, from non-detectable to 45.14 ppb.

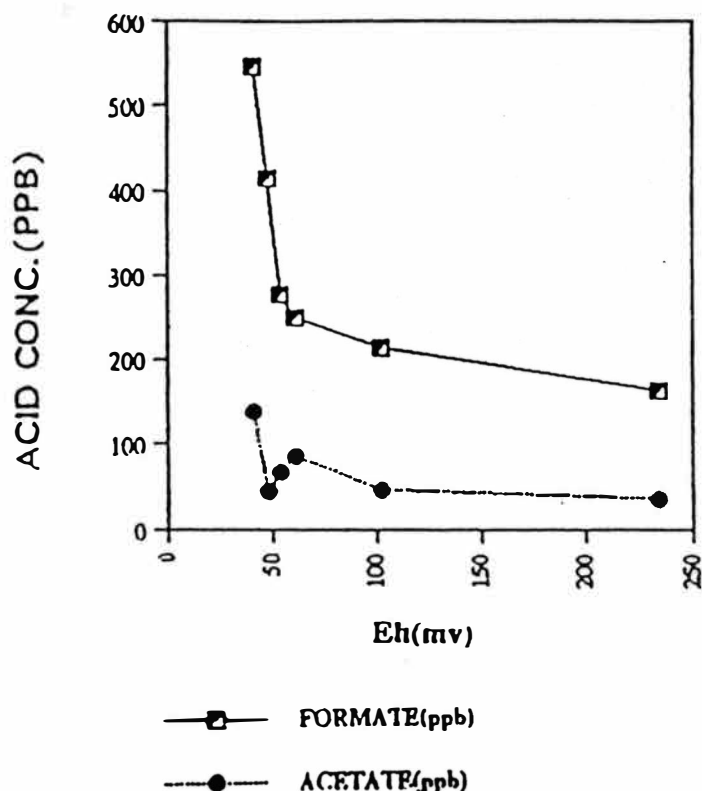


Figure 7. Concentrations of Formate and Acetate vs. Measured Eh(mv) (Samples Were Collected From Beardstown, IL).

There were no observations of propionic or butyric acids which have been reported in high concentrations in marine sediments (strong reducing conditions). (Barcelona, 1980) Microcosm studies have shown that C_3 to C_6 acids usually can be degraded down to C_2 and C_1 . (Godsy, et al 1992) In this study, these levels of organic acid are probably most indicative of the microbial activity of fermenters, proton reducers and methanogens characteristic of anoxic ecosystems, without light, low in electron acceptors other than CO_2 . (Berry, et al., 1987; Godsy, et al., 1992) Formic acid has been reported far less frequently due to analytical sensitivity

Table 2
Results of Geochemical Analyses of Ground Water
at Beardstown, IL (General Leachate
Contamination/Reducing Condition)

Well #	Formate (ppb)	Acetate (ppb)	Eh* (mv)	Methane (ppm)	TOC (ppmC)
MW-6	162	35	234	0.00	2.32
MW-8	214	46	102	1.33	6.78
MW-10	413	44	48	0.40	9.19
MW-11	276	67	54	2.54	7.21
MW-12	547	136	41	4.22	8.57
MW-13	248	86	61	1.31	6.87

* Redox potential measured by Pt electrode/Ag/AgCl reference corrected to standard hydrogen electrode.

limitations, if not derivatized, and to the fact that under active methanogenesis it is present at trace levels. The aquifer site in this study was not organic rich since DOC (dissolved organic carbon) concentration is from 0.4 to 1 mg/g similar to the range measured in uncontaminated samples from the site. It is likely that the relatively high formic acid concentrations resulted from the discharge of a relatively polished effluent from the anaerobic treatment impoundments and its persistence under mildly reducing conditions.

Underground Storage Tanks(UST) Sites

Results of chemical analyses of selected water samples from three Underground Storage Tanks (UST) sites (Houston, TX) are shown in Table 3. They provide further evidence for the value of organic geochemical indicators of fossil fuel transformation in the subsurface. Figure 8 shows the average concentrations of C_1 , C_2 as a function of dissolved ferrous iron (Fe^{2+}) concentrations in ground water from monitoring wells. Both formic and acetic acids concentrations increased as dissolved ferrous iron concentration increased by a factor of 2. The study done by

Table 3

Results of Chemical Analyses of Ground Water at Service
Station Sites (Underground Storage
Tanks, Houston, TX)

Site	Well #	BTEX (ppb)	DO (ppm)	Fe^{2+} (ppm)	Formate (ppb)	Acetate (ppb)
DP	MW-1	2051	1.5	2.6	103	250
	MW-3	2	1.7	0.2	39	127
NMF	MW-4	3343	0.5	1.25	51	187
	MW-5	3648	0.0	1.6	42	148
HV	MW-13S	31379	2.3	0.0	60	110
	MW-13D	1892	0.8	0.0	34	125

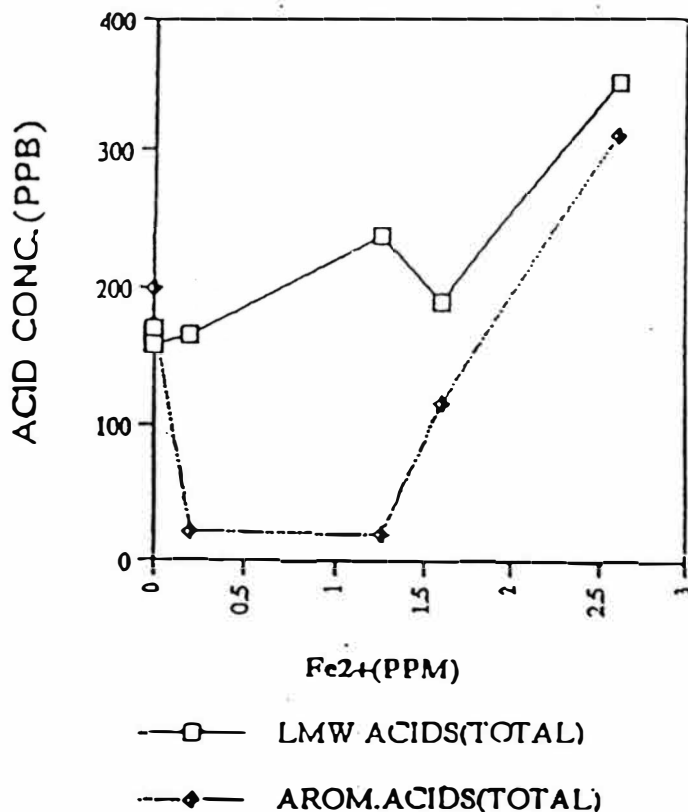


Figure 8. Concentrations of Total LMWFA's vs. Dissolved Iron(II) (Samples Were Collected From Underground Storage Tank Sites, Houston, TX).

Cozzarelli, Baedecker, et al. (1993) showed that acetic acid concentration increased from 127.8 ppb to 505.2 ppb in ground water from an anoxic zone, where a significant increase in concentration of ferrous iron (by a factor of 14) was observed over the same time period. Their previous studies (1992) had shown that ferrous iron has concentration of 19.9 ppb in the contaminated water. Concentration of ferrous iron was below detection limit in the background water.

In this study, formic acid concentrations showed better overall correlation with apparently more reducing conditions than did acetate, but it is likely that acetate is more closely associated with the aquifer solids. (Cozzarelli, et al., 1993) At best,

these concentrations are only symptomatic of the total mass of these intermediates in the subsurface.

Multiple Fuel Spill Sites

Table 4 shows the results of chemical analyses of ground water samples from Sleeping Bear Dunes, Empire, Michigan. Formic and acetic acids were still the dominant LMWFA's in these samples. Figures 9, 10 and 11 show concentrations of dissolved total BTEX, formic and acetic acids respectively at source, 12 m and 27 m downgradient locations within the contaminant plume (the work was done by Kampbell, Cook, and Wilson). The BTEX curves show the decreased concentration of these compounds in transport from the source region to downgradient locations (in depth). The formic and acetic acid concentration profiles show the fluctuation of their concentrations with depth. They reflect both production in transport (i.e. an increase with depth and distance from the source) and tendency to "lead" the plume given their solubility and mobility. In this study, acetic acid was observed to be a major intermediate which support some other researchers' observation of active methanogenesis. Formic acid persisted at the 27 m downgradient location. It may be attributed to the infiltration and reoxygenation of the ground water which had increased dissolved oxygen levels from <0.5 mg/L in source zone to values in the range of 1.1 to 2 mg/L. Previous studies have shown similar levels of the formic and acetic acids in petroleum-contaminated plumes. (Cozzarelli and Baedecker, 1992; and Cozzarelli et al. 1990) They found that aliphatic acids are the most prevalent of the

Table 4

Results of Chemical Analyses of Ground Water Samples
From the Sleeping Bear Dunes, Empire, MI

Location	Well #	Depth (m)	Fe-Total (ppm)	TOC (ppm)	BTEX* (ppm)	Formate (ppb)	Acetate (ppb)
Source	AQ	1	13.0	170	56704	213	1168
	AP	2	17.5	100	51067	157	902
	AR	3	10.0	54	11543	35	173
	AM	4	7.0	26	1440	192	248
	AO	4.7	2.5	5	2	107	139
12m Down Gradient	AJ	1	12.0	220	85	88	1455
	AI	2	7.0	110	3338	273	466
	AK	3	8.0	30	456	246	573
	AF	4	6.0	15	1411	22.5	551
	AH	4.7	3.5	11	0	54	319
27m Down Gradient	AC	1	12.0	110	24.6	91	293
	AB	2	6.0	40	3373	36	343
	AD	3	4.0	11	0.5	47	912
	AZ	4	3.5	0	0.4	155	257
	AE	4.7	0.4	0	0	43	530

* Sum of concentrations of benzenes, toluenes, ethylbenzenes & xylenes.

Sleeping Bear Total BTEX Profiles

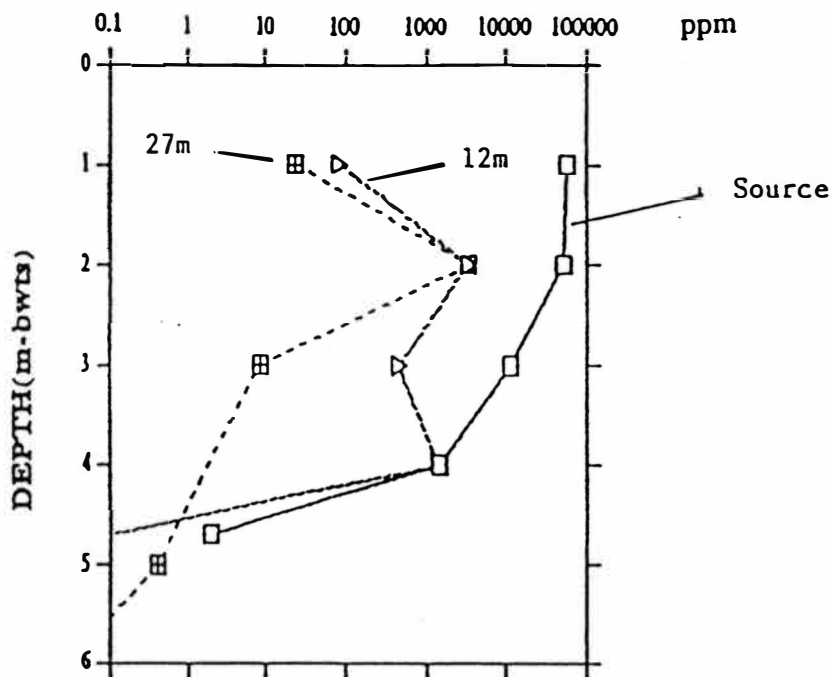


Figure 9. Analyses of Total BTEX in the Sleeping Bear Dune Sites, MI.

low-molecular-weight organic acids near oil edge but disappear within the oxygen-restricted zones. Acetic acid was the dominant aliphatic acid, and its concentration reached 3 ppb in ground water near the oil lens. Due to fact that their method was not capable of determining formic acid, no information can be used as reference at this time. They noted further that degradation of these intermediates was evident in increasingly oxic regions of the subsurface. The persistence of the formic and acetic acids we observed demonstrates that local geochemical effects of fuel transformations may extend beyond the plume defined by determinations of source compounds.

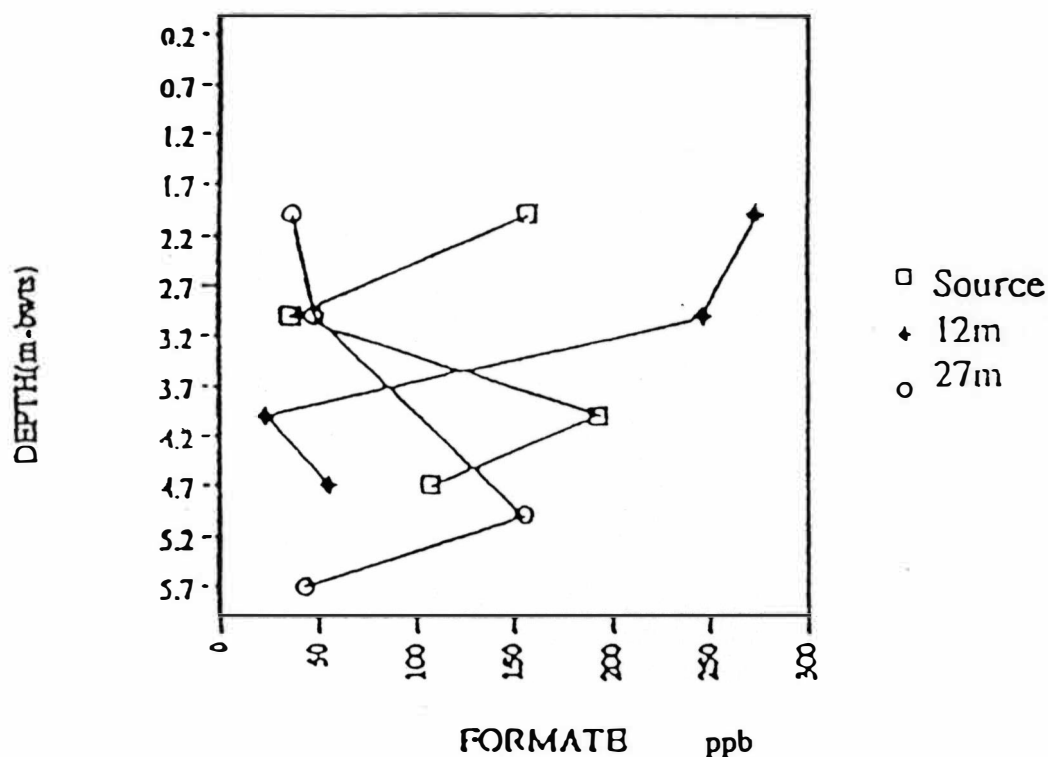


Figure 10. Distribution of Formate in Various Groundwater Sample Sites(Sleeping Bear Dune, MI): Source, 12 m Downgradient, 27 m Downgradient.

Leachability of Solid-Associated Organic Matter

Table 5 presents the dissolved concentrations of C_1 and C_2 acids in solid samples which were collected at the Sleeping Bear Dunes Site. These samples were extracted with four different solvents or solvent mixtures which include H_2O , KCl aqueous solution, Methanol, and Methanol,KOH(0.01 N)/Toluene. C_1 and C_2 acids were observed in relatively high concentrations in these solid samples compare to their concentrations in ground samples. Comparisons of the masses of DOC, formate and acetate in a one liter volume of aquifer show that less than 1 % of these species

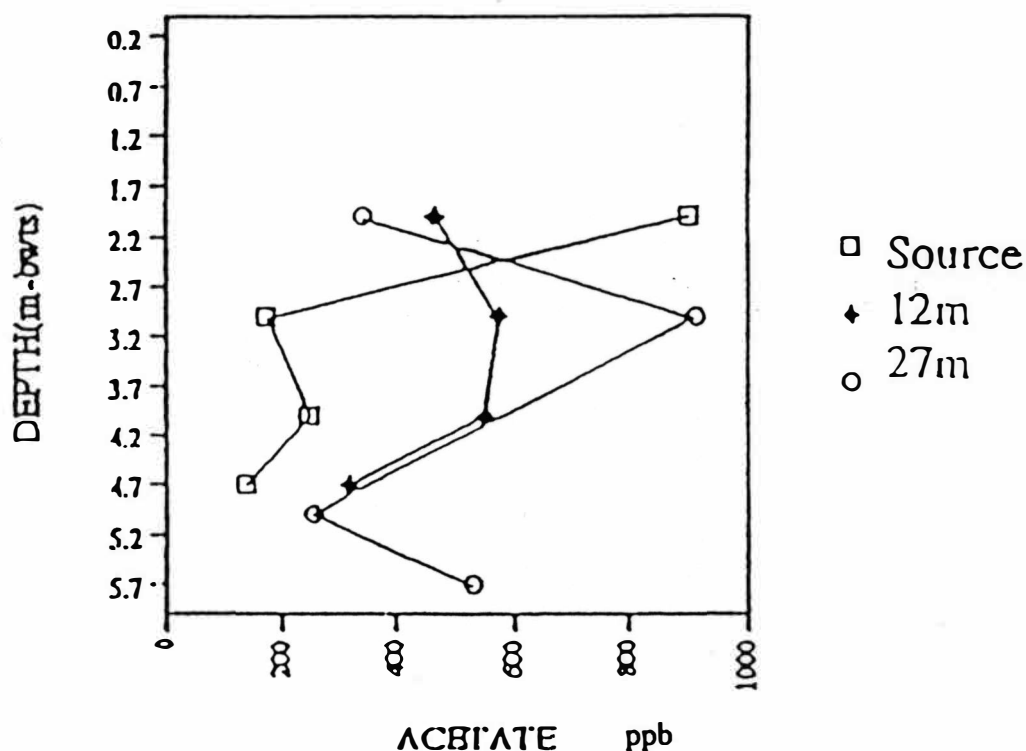


Figure 11. Distribution of Acetate in Various Groundwater Sample Sites(Sleeping Bear Dune, MI): Source, 12 m Downgradient, 27 m Downgradient.

resided in the ground water phase. Significant amounts of these species were extractible with the mild solvent treatments (i.e. H_2O , KCl and Methanol). It is likely that the bulk of the material may continually leach into the aqueous phase over time. This observation partly explains the persistence of contaminant and intermediate product distributions despite extended pump and treat efforts. From the results, we can conclude that contaminant plumes do not represent the total source since transformations can occur on and within aquifer solids. Obviously, the solid cannot be ignored in efforts to achieve mass-balances on net contaminant removal under active or passive remediation schemes. In this study, formate and acetate were also

Table 5

Results of Organic Analyses of Soil Extracts From
the Sleeping Bear Dune Sites, Empire, MI

Sample #	Location	Depth (m)	LMWFA's (ppb)			
			C1	C2	C3	C4
1001	BKGRD	0.1	105.4	1365.2	--*	--
9	Source	1.0	277.8	3385.4	--	--
8		1.8	120.9	2156.9	--	--
7	Down Gradient	12-0.4	33.7	553.9	--	--
6		12-1.3	36.6	1009.8	106.0	167.0
2		12-1.5	30.9	978.8	--	--
5		27-0.5	117.1	1046.7	143.0	297.0
4		27-2.0	272.6	1023.8	--	--

* -- means not available, or insufficient sample.

present in 'background' aquifer solid materials and methanol/toluene extraction removed less than 60 % of the total organic carbon.

CHAPTER IV

CONCLUSIONS

LMWFA's in various ground water and aquifer solid samples have been studied. Volatile fatty acids are qualitative indicators of suboxic to reducing redox zones. Solid extract samples collected under reducing condition were found to contain higher concentration of the acids on a volumetric basis. Other acids (C_3 and C_4) were only detected in extracts of solid samples from contaminated sites since their concentration were generally low in the natural water.

Derivatization reactions coupled with capillary gas chromatography and ion-trap mass spectrometry represent a very powerful analytical technique for the determination of LMWFA's applicable to wide variety of samples. Although there may be additional products from the sample preparation procedure, specifically brominated products, these products did not interfere (co-elute on GC) with the acids of interest.

BIBLIOGRAPHY

- Baedecker, M.J., & Cozzarelli, I.M., (1990). The Determination and Fate of Unstable Constituents of Contaminated Groundwater. Groundwater Contamination and Analysis at Hazardous Waste Sites, 425-461.
- Baedecker, M.J., Cozzarelli, I.M., et al (1992). Authigenic mineral formation in aquifers rich in organic material. Proceedings of the 7th International Symposium on Water-Rock Interaction, 257-262.
- Barcelona, M.J. (1980). Dissolved organic carbon and volatile fatty acids in marine sediment pore waters. Geochimica et Cosmochimica Acta, 44, 1977-1984.
- Barcelona, M.J., Liljestrand, H.M., & Morgan, J.J. (1980). Determination of low molecular weight volatile fatty acids in aqueous samples. Analytical Chemistry, 52, 321-325.
- Barcelona, M.J., et al (1989). Spatial and Temporal Gradients in Aquifer Oxidation-Reduction Conditions. Water Resources Research, 25(5), 991-1003.
- Berry, D.F., Francis, A.J., & Bollag, J.M. (1987). Microbial Metabolism of Homocyclic and Heterocyclic Aromatic Compounds under Anaerobic Conditions. Microbiological Reviews, 43-59.
- Chen, C.T. (1992). Understanding the Fate of Petroleum Hydrocarbons in the Subsurface Environment. Journal of Chemical Education, 69(5), 357-361.
- Cozzarelli, I.M., et al (1980). Determination of low molecular weight volatile fatty acids in aqueous samples, 1980. 321-324.
- Cozzarelli, I.M., Eganhouse, R.P., & Baedecker, M.J. (1990). Transformation of Monoaromatic Hydrocarbons to Organic Acids in Anoxic Groundwater Environment. Environmental Geological Water Science, 16(2), 135-141
- Cozzarelli, I.M., Baedecker, M.J. (1992). Oxidation of hydrocarbons coupled to reduction of inorganic species in groundwater. Proceedings of the 7th international symposium on Water-Rock Interaction, 275-278.
- Cozzarelli, I.M., & Baedecker, M.J., et al (1992). The geochemical evolution of low-molecular-weight organic acids derived from the degradation of petroleum

- contaminants in groundwater. Geochimica et Cosmochimica Acta, 57, 000-000.
- Devlin, J.F. (1990). Field Evidence for the Effect of Acetate on Leachate Alkalinity. Ground Water, 28(6), 863-867.
- Durst, H.D. (1974). Tetrahedron Letter, 2421-2424.
- Durst, H.D., et al (1975). Analytical Chemistry, 47, 1797-1801.
- Eglinton, G., Murphy, M.T.J.(1969). Organic Geochemistry, 245-251.
- Fogel, S., Findlay, M., & Moore, A. (1988). Enhanced Bioremediation Techniques for In Situ and Onsite Treatment of Petroleum Contaminated Soils and Groundwater. Petroleum Contaminated Soils, 2, 201-209.
- Gersberg, R.M., Dawsey, W.J., & Ridgway, H.F. (1988). Biodegradation of Dissolved Aromatic Hydrocarbons in Gasoline-Contaminated Groundwaters Using Denitrification. Petroleum Contaminated Soils, 2, 211-217.
- Godsy, E.M., Goerlitz, D.F., & Grbic-Galic, D. (1992). Methanogenic Biodegradation of Creosote Contaminants in Natural and Simulated Ground-Water Ecosystems. Ground Water, 30(2), 232-242.
- Gron, C., et al (1992). Biodegradability of dissolved organic carbon in ground water from an unconfined aquifer. The Science of the Total environment.
- Ground Water, information pamphlet, ACS, 1989.
- Kawamura, K., & Kaplan, I.R. (1984). Capillary gas chromatography determination of volatile organic acids in rain and fog samples. Analytical chemistry, 56, 1616-1620.
- Keene, W.C., et al (1983). Geophysics Resouces, 88, 5122-5130.
- Koyama, T., & Thompson, T.G. (1964). Journal of Oceanogr. Soc. Jpn., 20(5), 7-18.
- L'emeillat, Y., Menez, J.F., Berthou, F., & Bardou, L.(1980). Quantitative gas chromatographic determination of low-molecular-weight straight-chain carboxylic acids as their p-bromophenacyl esters after extractive alkylation in acidic medium. Journal of Chromatography, 206, 89-100.
- Lopez-Avila, V., Hirata, P., et al (1985). Determination of Atrazine, Lindane,

- Pentachlorophenol, and Diazinon in Water and Soil by Isotope Dilution Gas Chromatography/Mass Spectrometry. Analytical Chemistry, 57, 2797-2801.
- McMahon, P.B., & Chapelle, F.H. (1991). Microbial production of organic acids in aquitard sediments and its role in aquifer geochemistry. Letters to Nature, 349, 233-235.
- Mikesell, M.D., Kukor, J.J., & Olsen, R.H. (1993). Fundamentals of Bacterial Degradation of BTEX. Manuscript submitted for publication.
- Narkis, N.; & Henfeld-Furie, S. (1978). Water Resources, 12, 437-446.
- Peter, B. M., et al (1991). Microbial production of organic acids in aquitard sediments and its role in aquifer geochemistry. Nature, 349, 233-235.
- Salanitro, J.P., (1993). The Role of Bioattenuation in the Management of Aromatic Hydrocarbon Plumes in Aquifers. Ground Water Monitoring & Remediation, 150-161.
- Sternson, L.A. (1979). General Aspects of Precolumn Derivatization with Emphasis on Pharmaceutical Analysis. Chemical Derivatization in Analytical Chemistry, 1, 173-175.
- Wilson, B.H., et al (1986). Environmental Science Technology, 20, 997 - 1002.
- Zeyer, J., et al (1990). Anaerobic Degradation of Aromatic Hydrocarbons. Biotechnology and Biodegradation, 33-40.