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**IDENTIFICATION OF AROMATIC ACIDS AS MICROBIAL METABOLITES
OF FOSSIL-FUEL COMPOUNDS PRESENT IN AQUIFER SYSTEMS**

by

Daniel Michael Tomczak

**A Thesis
Submitted to the
Faculty of The Graduate College
in partial fulfillment of the
requirements for the
Degree of Master of Arts
Department of Chemistry**

**Western Michigan University
Kalamazoo, Michigan
April 1994**

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Ironically, the shortest section of this thesis would be the last to be written. Acknowledging all those who helped in one way or another in the completion of this project is difficult, but an attempt shall be made.

First I would like to thank my advisor, Dr. Michael J. Barcelona, for his guidance, patience, and support. In addition, I would like to thank my colleagues for their advice and recommendations. The input from all of them proved to be most valuable.

Gratitude must also be expressed to my family and friends. Their advice and support was always important to me.

Finally, to those who say it can't be done, I dedicate this to you.

Daniel Michael Tomczak

IDENTIFICATION OF AROMATIC ACIDS AS MICROBIAL METABOLITES OF FOSSIL-FUEL COMPOUNDS PRESENT IN AQUIFER SYSTEMS

Daniel Michael Tomczak, M.A.

Western Michigan University, 1994

The identification and quantitation of aromatic acids as transformation products present in contaminated aquifer systems was studied from selected sites. These acids are formed as microbial metabolites in the oxidative degradation of aromatic compounds. The microbial activity in the subsurface can be coupled to the oxidation of the aromatic compounds and the reduction of specific inorganic indicators. Inorganic indicators identified from the sample sites include the reduced forms of iron, nitrate, sulfate, and dissolved oxygen.

The highest concentrations of the aromatic acids were found in the plumes of the contaminated sites. These concentrations also correlated with specific changes in concentrations of selected inorganic indicators. Salicylic acid, previously unidentified in contaminated aquifer systems, was found in high concentrations relative to the other aromatic acids. Decreasing concentrations of the aromatic acids were observed with an increase in distance from the plume.

This study was done to further understand the transformation and geochemical fate of specific aromatic contaminants once they enter a subsurface environment.

TABLE OF CONTENTS

ACKNOWLEDGMENTS.....	ii
LIST OF TABLES.....	v
LIST OF FIGURES.....	vi
CHAPTER	
I. INTRODUCTION.....	1
II. METHODOLOGY.....	8
Materials.....	8
Aromatic Acid Standard Solution.....	8
Internal Standard Solution.....	8
Derivatizing Reagent.....	8
Derivatization Using BF_3 /Methanol.....	9
Experimental Procedures.....	12
Sampling.....	12
Freeze-Drying Concentration.....	12
Derivatization.....	13
Gas Chromatography/Mass Spectrometry (GC/MS) Analysis.....	14
Quantitation.....	15
III. RESULTS.....	19

Table of Contents - Continued

CHAPTER

Method Performance.....	19
Method Application.....	21
IV. DISCUSSION.....	30
Underground Storage Tank (UST) Sites.....	30
Sleeping Bear Dunes Site.....	33
Transformation Pathways.....	42
V. CONCLUSIONS.....	46
BIBLIOGRAPHY.....	47

LIST OF TABLES

1.	Analytical Accuracy and Precision Data for the Derivatization of the Aromatic Acids.....	20
2.	Precision in the Acid Determinations From the Underground Storage Tank (UST) Samples.....	22
3.	Precision in the Acid Determinations From the Sleeping Bear Dunes Samples: Source Region.....	23
4.	Precision in the Acid Determinations From the Sleeping Bear Dunes Samples: 12 m Downgradient.....	24
5.	Precision in the Acid Determinations From the Sleeping Bear Dunes Samples: 27 m Downgradient.....	24
6.	Characterization of the Underground Storage Tank (UST) Sites.....	31
7.	Aromatic Acid Concentrations From the Underground Storage Tank (UST) Sites.....	31
8.	Characterization of the Sleeping Bear Dunes Site.....	34
9.	Aromatic Acid Concentrations From the Sleeping Bear Dunes Site.....	35

LIST OF FIGURES

1.	Mechanistic Pathway of an Acid-Catalyzed Esterification.....	11
2.	Chromatogram of the Derivatized Aromatic Acid Standard.....	16
3.	Mass Spectrum of Methyl Benzoate.....	17
4.	Mass Spectrum of Methyl Toluate.....	17
5.	Mass Spectrum of Methyl Hydroxybenzoate.....	18
6.	Mass Spectrum of Methyl p-Chlorobenzoate.....	18
7.	Chromatogram of a UST Sample.....	25
8.	Chromatogram of a Sleeping Bear Dunes Sample.....	25
9.	Aerial Layout of the Sleeping Bear Dunes Site.....	28
10.	Vertical Profile of the Sleeping Bear Dunes Site.....	29
11.	Plot of Total Aromatic Acid Concentrations as a Function of Dissolved Iron(II) Concentrations From UST Sites.....	32
12.	Depth Profile of Total Dissolved BTEX Concentrations at Sleeping Bear Dunes.....	36
13.	Correlation Between Sulfate Concentrations and Aromatic Acid Concentrations.....	37
14.	Correlation Between Methane Concentrations and Aromatic Acid Concentrations.....	38

List of Figures - Continued

15.	Correlation Between Ammonia Concentrations and Aromatic Acid Concentrations.....	39
16.	Oxidation of Selected BTEX Compounds.....	43
17.	Detailed Degradation Pathways of Benzene and Toluene.....	45

CHAPTER I

INTRODUCTION

Ground water contamination has been a major environmental problem for many years. Considering the fact that approximately 50% of all the people in the United States rely on ground water as their source of drinking water, this environmental problem needs to be addressed more seriously (USEPA, 1980). As the twenty first century approaches, factors such as industrialization, urbanization, and increasing population could make efforts to protect ground water even more difficult than what it may seem to be today.

Ground water use varies depending upon land needs and demographics. Rural communities tend to be more dependent on ground water for their drinking water than urban communities. It is estimated that approximately 96% of all rural drinking water comes from ground water, whereas only about 20% is used in urban areas (USEPA, 1980). Ground water use can also vary for each of the states. States such as Massachusetts and New Jersey use large quantities of ground water for urban water supplies, whereas states including Texas and Arizona will use larger quantities of ground water for irrigation rather than for public consumption (Burmaster, 1984).

Ground water consumption has also been changing with time. In 1950

approximately 34 billion gallons of ground water were used by the United States per day. By 1970 the consumption rate had increased to 68 billion gallons per day and by 1980 the rate had increased to 88.5 billion gallons of ground water per day (Pye et al., 1983). This continued increase in the consumption rate by the United States indicates the importance of ground water for society.

Even though ground water has been shown to be a vital natural resource, the contamination of ground water systems has become a common discovery. Petroleum hydrocarbons are among the most frequent sources in the contamination of ground water (Nyer and Skladany, 1989). Petroleum hydrocarbons can enter an environmental system from many sources. Some examples may include leaking underground storage tanks (UST) at gasoline stations, oil terminal spills, and fuel pipeline leaks. Since there are more than two million underground storage tank systems that are used for petroleum products (Chen, 1992), a better understanding of the fate of the petroleum hydrocarbons in subsurface environments needs to be obtained.

Some common petroleum products which frequently contaminate soils and ground waters include gasoline, diesel, and fuel-oils. Although petroleum products are mixtures of many organic constituents, the aromatic hydrocarbons are of particular interest due to their toxicity. The aromatic compounds which include benzene, toluene, ethylbenzene, and xylenes (BTEX) are of concern because they are confirmed or suspected carcinogens, even at low concentrations (Dean, 1985). Due to the high solubility of the BTEX compounds in water it is

very feasible for these compounds to migrate through and contaminate the ground water. Because of these characteristics the BTEX compounds have received much attention in studies on the fate of petroleum products in subsurface environments.

Various remediation techniques have been implemented at sites which were contaminated with petroleum hydrocarbons. Techniques for soil remediation have included excavation and off-site disposal, in-situ soil venting, in-situ biodegradation, and above-ground or in-situ chemical oxidation. Aquifer remediation may require techniques such as pump and treat in above-ground systems and in situ bioremediation (Nyer and Skladany, 1989). But there are important factors which need to be addressed before remedial techniques can be effective. There needs to be an understanding of the specific ground water flow system and how the transport and fate of the contaminants are controlled by biogeochemical reactions (Cozzarelli et al., 1990).

This research project was done to further understand the fate of specific petroleum hydrocarbons (BTEX) as a result of biogeochemical reactions in subsurface environments. Microbial, physical, and chemical processes will influence the transportation, transformation, and overall fate of the petroleum hydrocarbons. Specific examples of these processes may include adsorption, chemical degradation, diffusion, volatilization, and biodegradation (Nyer and Skladany, 1989).

Biodegradation processes are gaining more attention because of the role

that microorganisms may have in the breakdown of environmental contaminants (Madsen, 1991). Microbial activity has been coupled to the aerobic and anaerobic degradation of BTEX compounds in contaminated ground water systems (Cozzarelli et al., 1990; Madsen et al., 1991). The microbial degradation of aromatic hydrocarbons in subsurface systems will depend on the availability of molecular oxygen and specific redox conditions. Since there is usually a lack of molecular oxygen within a contaminated source, recent studies have begun to look at other microbial mediated redox reactions linked to the degradation of aromatic hydrocarbons.

Until recent times, the fate of aromatic hydrocarbons in the subsurface under anaerobic conditions was uncertain. Aromatic compounds (BTEX) were generally believed to be recalcitrant to anaerobic degradation until the mid-1980's (Edwards et al., 1992). The first documented paper on the anaerobic degradation of benzene and toluene did not appear until 1980 (Ward et al., 1980). Since then others have begun to seriously look at the transformation processes involved in anaerobic degradation of contaminant compounds.

Many laboratory studies have demonstrated that different electron acceptors (inorganic indicators) could replace oxygen in the anaerobic degradation of aromatic compounds. Examples of electron acceptors which may be present in subsurface environments include Fe^{3+} , SO_4^{2-} , NO_3^- , CO_2 , and Mn^{4+} . These inorganic indicators have been shown to have a major influence on the oxidation-reduction intensity of subsurface systems (Barcelona et al., 1989;

Pederson et al., 1991). Degradation processes incorporating these inorganic indicators include nitrate reduction (Kuhn et al., 1988), sulfate reduction (Edwards and Grbic-Galic, 1992), iron reduction (Lovley et al., 1989), and methanogenesis (Wilson et al., 1986).

The importance of organic indicators is also seen when studying the redox conditions of aquifer systems. Laboratory studies have shown that aromatic hydrocarbons serve as important electron donors under anaerobic conditions (Berry et al., 1987). A contaminant plume, particularly one of fossil-fuel origin, would be characterized as an organic-rich system. The organic matter present in such a system would serve as an electron donor and contribute to the reduction capacity of the aquifer (Barcelona and Holm, 1991). This reduction capacity influences the stability of the source organic contaminants and the accumulation of intermediates and end products. By monitoring the changes in the components of a contaminated site, a better understanding can be reached on the nature and extent of organic removal, processes, major controlling factors, and mass balances of a specific aquifer.

The identification of intermediates will aid in understanding the overall transformation pathways for contaminant compounds. Microcosm studies have shown that certain aromatic acid intermediates can form due to anaerobic degradation of aromatic compounds (Grbic-Galic and Vogel, 1987; Lovley and Lonergan, 1990). The correlation of the parent contaminant compounds, intermediates, inorganic indicators, and end-products to their spatial distribution

within an aquifer system will give a better understanding of net bioremediation performance.

In addition, the formation of degradation intermediates can effect the geochemistry of an aquifer system. Bennett and Siegel (1987) reported that dissolved organic acids, produced by the biodegradation of petroleum in the subsurface, can increase the solubility of quartz in natural waters.

Degradation intermediates can also be important in relation to environmental health issues. Intermediates which form may prove to be more toxic, mobile, and persistent than the parent contaminant compounds (Cozzarelli and Baedecker, 1992). Chemical changes to the intermediate, such as chlorination, could also convert an otherwise nonhazardous compound into a potentially mutagenic or toxic compound (Coleman et al., 1989).

The accumulation of aromatic acid intermediates under anaerobic conditions can be coupled to other microbially mediated electron-transfer reactions (Grbic-Galic and Vogel, 1987; Lovley and Lonergan, 1990). Co-metabolic biotransformation processes consist of mechanisms by which refractory contaminants (e.g. chlorinated alkene) may be degraded by an organism or extracellular enzyme adapted to degrade aromatic hydrocarbons.

This research project helps to describe the geochemical evolution of aromatic acids in anaerobic ground water coupled to various electron accepting processes. The results from this project will hopefully provide further evidence

in the association of redox reactions with degradation processes in subsurface environments.

CHAPTER II

METHODOLOGY

Materials

Aromatic Acid Standard Solution

Benzoic acid (Fischer Scientific, Itasca, IL), o-, m-, p-toluic acids (Aldrich Chemical Company, Milwaukee, WI), and o-, m-, p-hydroxybenzoic acids (Sigma Chemical Company, St. Louis, MO) were concentrated to 10 mM in methanol (pesticide grade, Fischer Scientific, Itasca, IL)

Internal Standard Solution

p-Chlorobenzoic acid (Aldrich Chemical Company, Milwaukee, WI) was concentrated to 10 mM in methanol (pesticide grade, Fischer Scientific, Itasca, IL)

Derivatizing Reagent

Boron trifluoride/methanol complex in excess methanol (~50 wt % BF_3 , Aldrich Chemical Company, Milwaukee, WI)

Derivatization Using BF_3 /Methanol

The employment of analytical derivatization for gas chromatographic analysis can be done for various reasons. One reason is to impart volatility to otherwise nonvolatile compounds. The aromatic acids under study are of low volatility with boiling points ranging from 249-274°C. By derivatizing the aromatic acids to methyl esters the compounds become more volatile. The boiling points for the methyl esters of the aromatic acids range from 199.6-223.3°C. This increase in volatility allows the compounds to be more suitable for chromatographic analysis.

The increase in volatility of the methyl esters of the acids is largely due to the masking of polar groups in the compounds. The polar groups within the aromatic acid compounds undergo hydrogen bonding which contributes significantly to intermolecular attraction (Knapp, 1979). This strong intermolecular attraction accounts for the lower volatility of the acids. Masking of the polar groups can be done by replacing the hydrogen in the polar group through alkylation. In this study the replacement of the active hydrogen on the acid was done with a methyl group to form the methyl ester.

Improving chromatographic resolution is another reason for employing analytical derivatization. The polar groups within the acids may adsorb on the active surfaces of both the column walls and the solid support (Knapp, 1979). These adsorption effects could result in unsymmetrical peaks in the chromatogram. The derivatization of the aromatic acids to methyl esters aids in

the reduction of the adsorption effects and therefore helps in the resolution of the chromatographic peaks.

The derivatizing reagent used in this study for converting the aromatic acids to methyl esters was boron trifluoride in methanol (BF_3 /methanol). This reagent has been found to be suitable for the esterification of a variety of organic acids (Mitchell et al., 1940; Metcalfe and Schmitz, 1961; Kawamura and Gagosian, 1987). Early studies showed that the percent yield of the ester increases as the amount of boron trifluoride in the methanol increases (Nieuwland and Sowa, 1936). Nieuwland and Sowa (1936) also showed that a maximum yield is reached where the increase in boron trifluoride dissolved in methanol after this point does not have an effect on the yield of the esters.

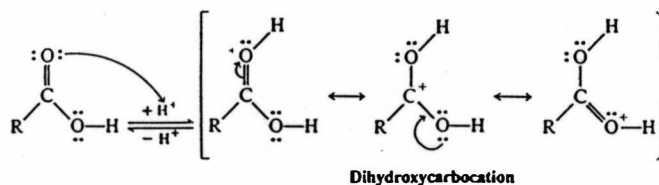
The BF_3 /methanol derivatization method was later applied to specific aromatic acids. One study showed that high yields of the esters could be produced by using two equivalents of a commercial boron trifluoride-methanol complex in excess methanol (Hallas, 1965). Identification of the methyl esters in this study was done by comparison of melting or boiling points of the esters with the reported values. The percent recoveries for the esters ranged from 89% for the methyl ester of p-toluic acid to 96% for the methyl esters of benzoic and m-hydroxybenzoic acids. These recoveries helped to support the reason for using the BF_3 /methanol as a derivatizing reagent in this quantitative study.

The mechanism in the derivatization of the acids to methyl esters involves the action of a Lewis acid as a catalyst. The effectiveness of BF_3 to serve as a

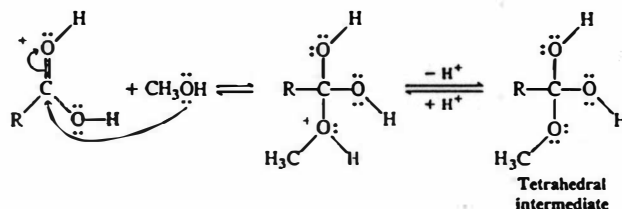
strong Lewis acid is aided by its ability to become only partially hydrolyzed by water. The BF_3 forms an adduct with the acid and therefore lowers the activation energy of the rate determining step in which the water molecule is eliminated from the acid structure (Cotton et al., 1987). The formation of the adduct occurs by the donation of an electron pair from the carbonyl oxygen in the acid to the boron. Figure 1 illustrates the mechanistic pathway of an acid-catalyzed esterification.

Mechanism of Acid-Catalyzed Esterification and Ester Hydrolysis:

STEP 1: Protonation of the carboxy group



STEP 2: Attack by methanol



STEP 3: Elimination of water

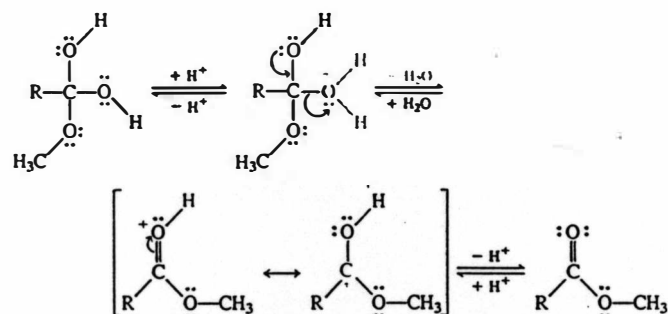


Figure 1. Mechanistic Pathway of an Acid-Catalyzed Esterification.

Source: Vollhardt, K.P.C., Organic Chemistry, W.H. Freeman and Company, New York, NY, 1987.

The Lewis acid-catalyzed esterification can be viewed in the same manner as the pathway in figure 1. The formation of an adduct by the BF_3 renders the carbonyl carbon in the acid susceptible to nucleophilic attack by the methanol. The proton loss from the methanol group results in the tetrahedral intermediate in step 2. Step 3 shows how the protonation of the hydroxy oxygen can result in the loss of the water group and the production of the methyl ester (Vollhardt, 1987).

Experimental Procedures

Sampling

Ground water samples were collected from established wells by bailing or pumping with bladder pumps at a number of sites in shallow unconfined aquifers (Barcelona et al., 1989). Samples were preserved by the addition of KOH and refrigerated 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.

Freeze-Drying Concentration

100 mL aliquots of the ground water samples were each placed in a 250 mL flat-bottom flask and adjusted to approximately pH 4 with 1 N HCl. 10 μL of the 10 mM p-chlorobenzoic acid internal standard were added to each sample. The samples were then frozen using a dry ice/isopropanol bath and freeze-dry

concentrated using a Labconco Freeze Dry System (Labconco Corporation, Kansas City, MO).

The dried residues were then taken up with three 4 mL washings of methanol and transferred into a 50 mL pear-shaped flask. The washings were dried by rotary evaporation. The dried samples were then taken up with two 1 mL washings of methanol and transferred into a 3.7 mL vial. The washing volume was reduced to 500 μ L using a nitrogen blow-down system.

Derivatization

Once the sample volumes were reduced to 500 μ L, 10 μ L of the BF_3 /methanol derivatizing reagent (~50 wt %, Aldrich Chemical Company, Milwaukee, WI) were added to each vial. The vials were sealed with a Teflon coated cap and placed in a water bath (~90°C) for 90 minutes. This reaction time seem to provide the most adequate recoveries of the methyl esters.

After the 90 minute reaction time, the vials were removed from the water bath and rinsed under cool water to quench the reaction. Once cooled, the reaction mixtures were transferred into 1.8 mL autosampler vials and the volumes were adjusted if necessary with methanol to 500 μ L. The vials were then capped in preparation for GC/MS analysis.

Gas Chromatography/Mass Spectrometry (GC/MS) Analysis

The methyl esters of the aromatic acids were determined using a Varian Saturn 3 GC/MS system (Varian Associates, Sunnyvale, CA) with a Varian 8100 autosampler. The 1077 split/splitless injector was set in the splitless mode and the injector temperature was set at 260°C. One microliter of each sample was injected into the GC/MS system via the autosampler. The data from the samples was collected using the Star Integrator data acquisition and interpretation system.

The chromatography was done using a 0.32 mm i.d. x 30 m DB-5 fused silica capillary column (J&W Scientific, Folsom, CA). The column temperature was programmed from 60°C to 90°C (3 min) at 10°C/min, ramped to 120°C at 5°C/min, and ramped to 250°C (10 min) at 30°C/min.

Figure 2 depicts a typical chromatogram for the derivatized aromatic acid standard. The chromatogram shows how all of the compounds in the standard were able to produce sharp and well resolved peaks.

The parameters for the ion trap mass spectrometer included electron impact (EI) ionization mode, manifold temperature of 260°C, transfer line temperature of 260°C, scan range of 35-250 amu, and mass defect of 100 mmu/100 amu.

Figures 3 through 6 display the mass spectra for the methyl ester forms of benzoic acid, toluic acids, hydroxybenzoic acids, and p-chlorobenzoic acid respectively. The m/z ratios and their relative intensities in each of the spectra correlated well with the literature values.

Quantitation

Quantitation of the aromatic acids was done by comparing the peak area measurements from the sample chromatograms to those in the methyl ester standard. The p-chlorobenzoic acid internal standard was used to calculate the response factor for each of the compounds in the derivatized acid standard.

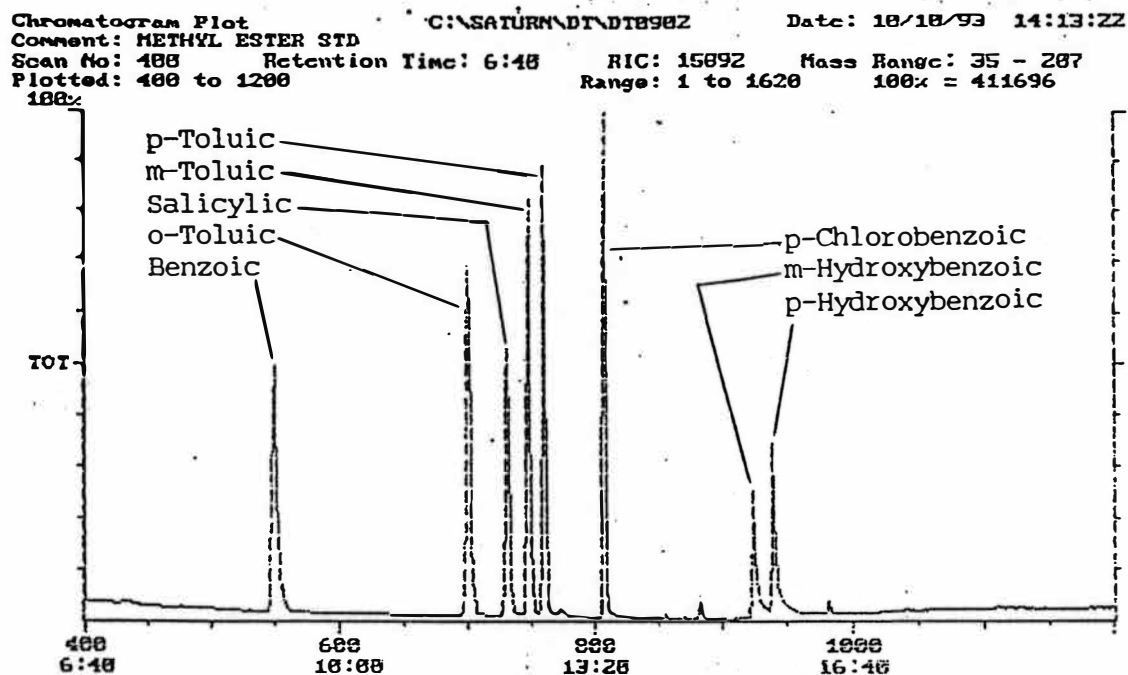


Figure 2. Chromatogram of the Derivatized Aromatic Acid Standard.

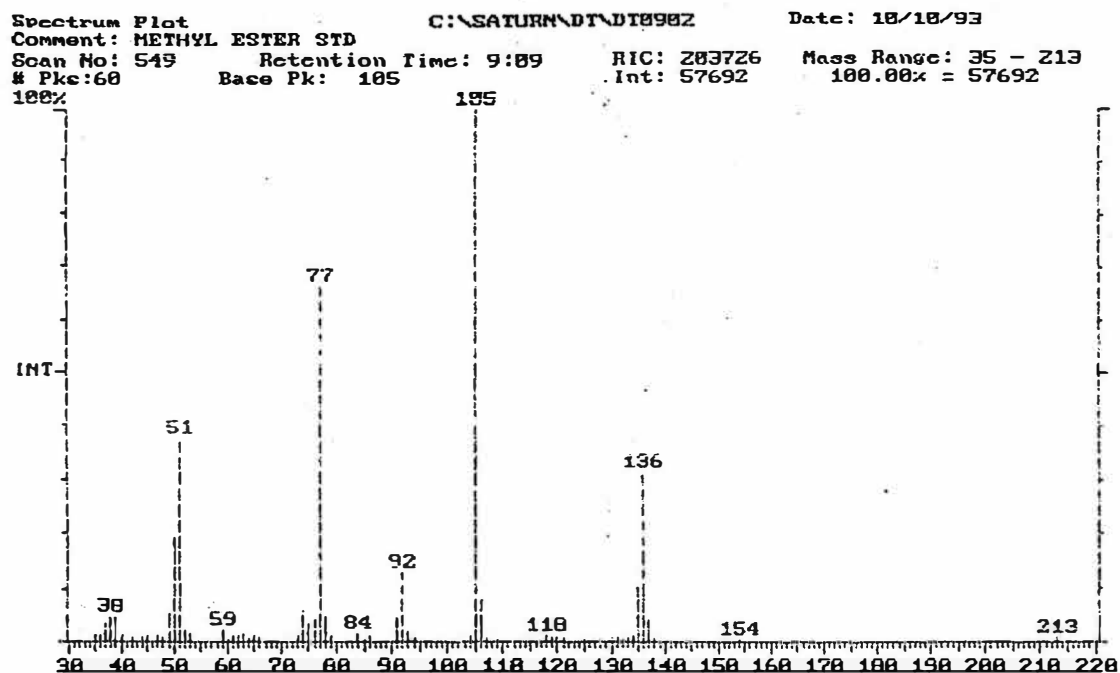


Figure 3. Mass Spectrum of Methyl Benzoate (m/z at 77, 105, 136).

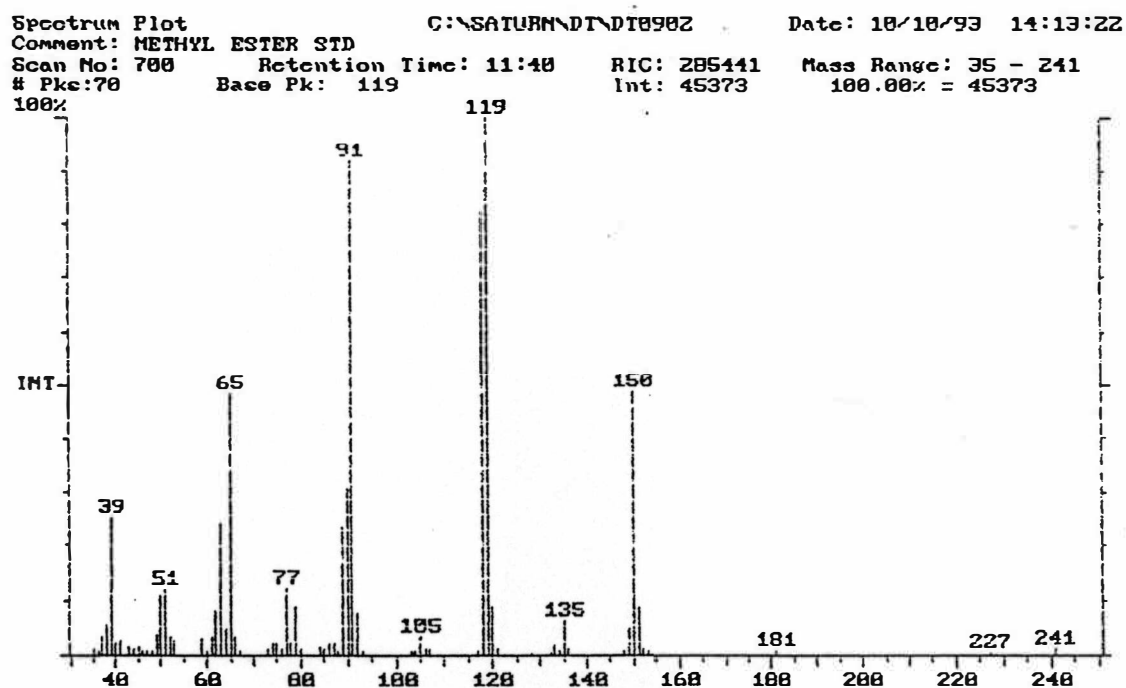


Figure 4. Mass Spectrum of Methyl Toluate (m/z at 91, 119, 150).

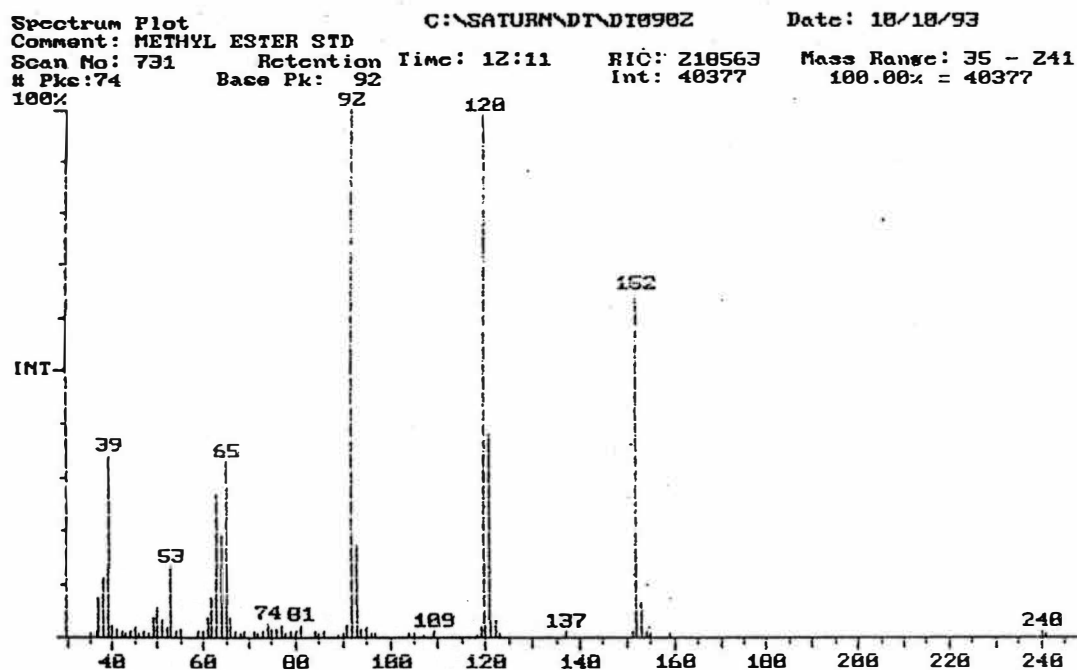


Figure 5. Mass Spectrum of Methyl Hydroxybenzoate (m/z at 92, 120, 152).

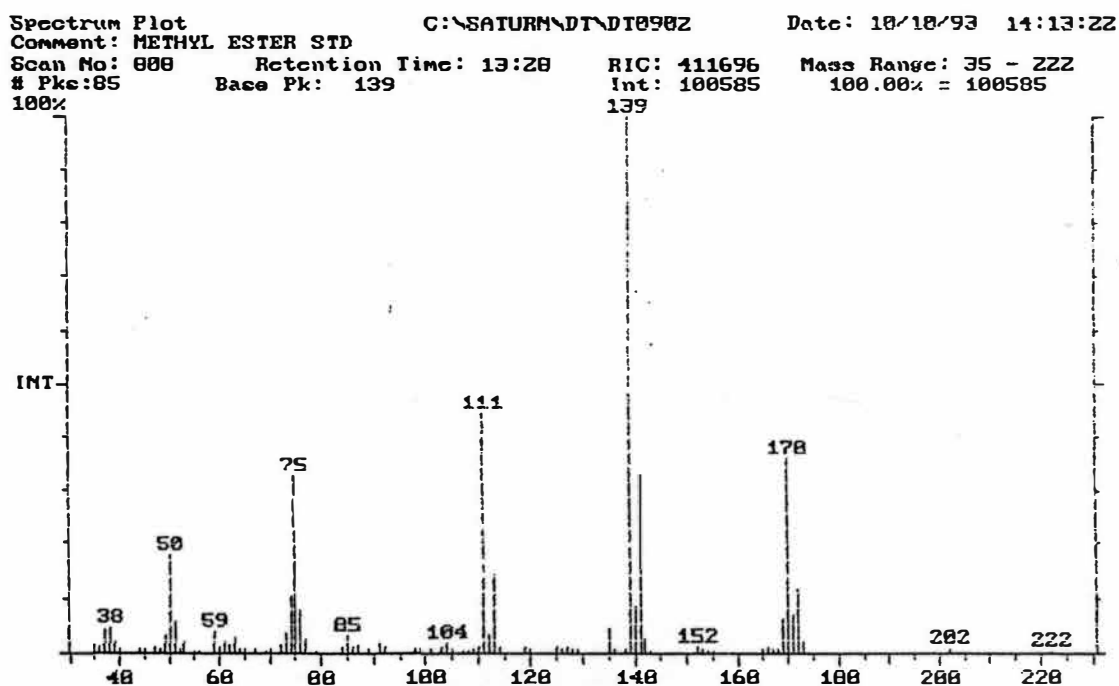


Figure 6. Mass Spectrum of Methyl p-Chlorobenzoate (m/z at 111, 139, 170).

CHAPTER III

RESULTS

Method Performance

Analytical accuracy and precision data for the derivatization method used in this study is shown in Table 1. This information was collected by running five replicate determinations of an aromatic acid standard solution (10 mM each acid) through the same method used on the ground water samples. 10 μ L aliquots of the standard solution and the internal standard solution were used for each analysis. The derivatized forms of the aromatic acids (methyl esters) were then analyzed by GC/MS and quantified in order to determine the percent recovery.

The standard deviation and the relative standard deviation for the percent recoveries of each acid were calculated. The precision in the recoveries of the acids is given by their relative standard deviations. The average of the percent recoveries for benzoic acid, o-, m-, and p-toluic acids was 82.5%. The precision for these same four acids was better than 8.3%. The results for salicylic acid showed a much lower precision. Lower precision may indicate incomplete esterification of the acid, thereby causing a large range in the recoveries from the different standards. The recoveries for the methyl esters were not as high as

Table 1

Analytical Accuracy and Precision Data for the
Derivatization of the Aromatic Acids

AROMATIC ACID	% RECOVERY MEAN	STD DEV	R.S.D. (%)
BENZOIC	81.4	6.72	8.26
o-TOLUIC	70	4.23	6.04
m-TOLUIC	93.5	5.47	5.85
p-TOLUIC	85.2	4.99	5.85
SALICYLIC	85.9	18.2	21.2

previous studies (Hallas, 1965), but the results show that this method is adequate for quantitative work on aromatic acid identification in environmental samples.

Cozzarelli, Baedecker, Eganhouse, and Goerlitz (1993) conducted a series of analyses for specific aromatic acids present in samples from contaminated ground water systems. The authors used an ether, liquid-liquid extraction method in order to isolate the free acids for determination by gas chromatography with a flame ionization detector (FID). The acids were not derivatized prior to analysis. The average of the percent recoveries for benzoic acid, o- and p-toluic acids in this method was 69.3%. The precision for these same acids was only slightly better than 11.5%. The lower recoveries by the authors may be due poor solvent extractability of the acids. Although these results are adequate for quantitative environmental analysis, the method used for this research project

appears to give more accurate, precise, and sensitive (detection limit is ca. 0.2 nmol) results.

Tables 2 through 5 provide information on the precision of the actual ground water samples at the two sites. Since each sample was analyzed in duplicate the tables provide the average concentration, standard deviation, and relative standard deviation for each identified aromatic acid. In samples where the acid was only found in one of the two duplicate analyses, only that one concentration was reported in the table. The results show a broad range in the precision of the samples.

Figures 7 and 8 are two gas chromatograms of ground water samples collected from the underground storage tank (UST) sites (Houston, TX) and Sleeping Bear Dunes State Park (Empire, MI) respectively. Identifications of aromatic acids were done by comparing relative (to the internal standard) retention times of the peaks in the sample chromatogram with those of the standard chromatogram. The mass spectra patterns for the derivatized acids were also compared to literature spectra for additional confirmation purposes.

Method Application

The identification of aromatic acid intermediates in the subsurface degradation of aromatic hydrocarbons may lead to both mechanistic and conceptual improvements in understanding more about the transformation pathways. Laboratory microcosm studies have shown that the aromatic acids can

Table 2

Precision in the Acid Determinations From the Underground Storage Tank (UST) Samples

SAMPLE	AROMATIC ACID	SAMPLE 1 (μ M)	SAMPLE 2 (μ M)	AVERAGE (μ M)	STD DEV	R.S.D. (%)
DP-1	BENZOIC	0.269				
	o-TOLUIC	0.303				
	SALICYLIC	1.92	0.86	1.39	0.75	54
	m-TOLUIC	0.26	0.052	0.156	0.147	94.3
	p-TOLUIC	0.337	0.05	0.194	0.203	105
DP-3	SALICYLIC	0.161				
NWF-4	SALICYLIC	0.076				
	m-TOLUIC	0.02	0.013	0.016	0.005	30
	p-TOLUIC	0.051	0.05	0.05	0.001	1.4
NWF-5	BENZOIC	0.138	0.215	0.176	0.054	31
	o-TOLUIC	0.054	0.265	0.16	0.149	93.5
	p-TOLUIC	0.715	0.376	0.546	0.24	43.9
HV-13S	BENZOIC	0.311	0.068	0.19	0.172	90.7
	o-TOLUIC	0.384	0.476	0.43	0.065	15
	p-TOLUIC	0.812	0.925	0.868	0.08	9.2
HV-13D	BENZOIC	0.63	0.49	0.56	0.099	18
	o-TOLUIC	0.576	0.982	0.779	0.287	37

Table 3

Precision in the Acid Determinations From the Sleeping
Bear Dunes Samples: Source Region

SAMPLE	AROMATIC ACID	SAMPLE 1 (μM)	SAMPLE 2 (μM)	AVERAGE (μM)	STD DEV	R.S.D. (%)
54AQ	o-TOLUIC	3.27	2.6	2.93	0.47	16
	SALICYLIC	0.859	0.648	0.754	0.149	19.8
	m-TOLUIC	0.098	0.066	0.082	0.023	28
	p-TOLUIC	0.097	0.12	0.108	0.016	15
AP	o-TOLUIC	2.3	2.61	2.46	0.21	8.7
	SALICYLIC	0.837	0.712	0.774	0.088	11
	m-TOLUIC	0.26	0.614	0.437	0.25	57.3
	p-TOLUIC	0.233	0.341	0.287	0.076	27
54AR	BENZOIC	0.316	0.039	0.178	0.196	110
	o-TOLUIC	1.7	0.841	1.27	0.61	48
	SALICYLIC	2.39	1.31	1.85	0.76	41
	m-TOLUIC	1.41	0.798	1.1	0.43	39
	p-TOLUIC	0.984	0.306	0.645	0.479	74.3
AM						
54AO	SALICYLIC	1.18	0.063	0.623	0.792	127
	p-TOLUIC	0.043	0.063	0.053	0.014	27

Table 4

Precision in the Acid Determinations From the Sleeping
Bear Dunes Samples: 12 m Downgradient

SAMPLE	AROMATIC ACID	SAMPLE 1 (μM)	SAMPLE 2 (μM)	AVERAGE (μM)	STD DEV	R.S.D. (%)
54AJ	SALICYLIC	0.093	0.337	0.215	0.172	80.2
AI	o-TOLUIC	0.133				
	m-TOLUIC	0.784				
	p-TOLUIC	0.232				
54AK	SALICYLIC	0.524	0.284	0.404	0.17	42
	m-TOLUIC	0.015	0.015	0.015	0	0
	p-TOLUIC	0.013	0.004	0.008	0.006	75
AF						
54AH						

Table 5

Precision in the Acid Determinations From the Sleeping
Bear Dunes Samples: 27 m Downgradient

SAMPLE	AROMATIC ACID	SAMPLE 1 (μM)	SAMPLE 2 (μM)	AVERAGE (μM)	STD DEV	R.S.D. (%)
54AC	o-TOLUIC	0.094				
	SALICYLIC	0.022	0.031	0.026	0.006	24
	p-TOLUIC	0.107	0.032	0.07	0.053	76
AB						
54AD	SALICYLIC	0.179	0.254	0.216	0.053	24
54Z						
AE	o-TOLUIC		0.315			

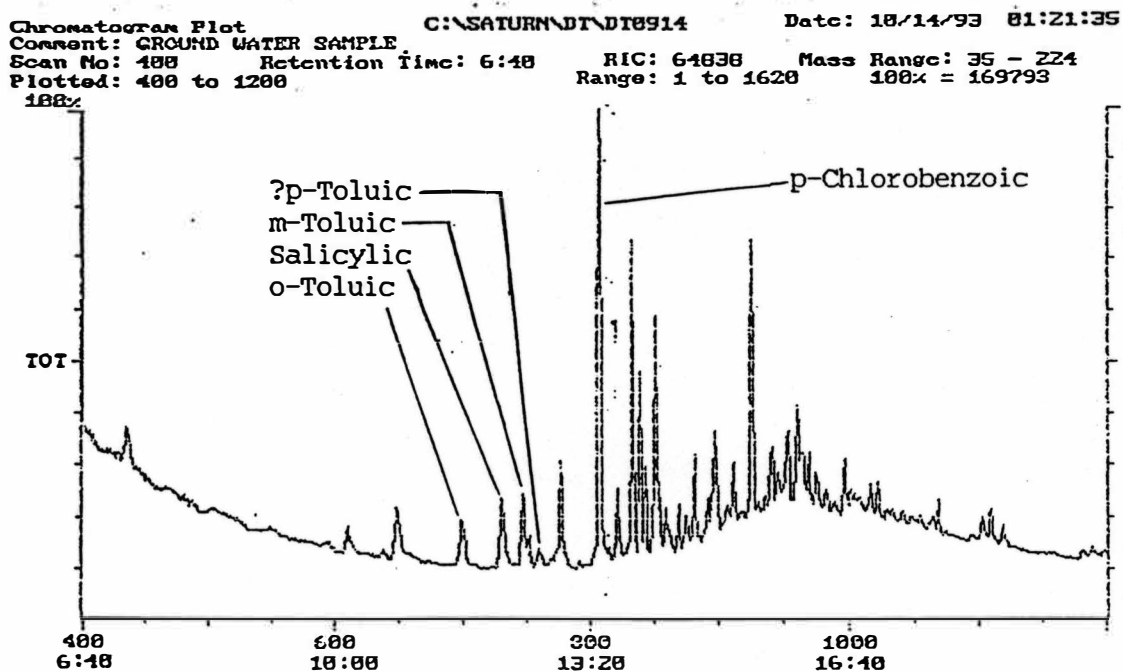


Figure 7. Chromatogram of an Underground Storage Tank (UST) Sample.

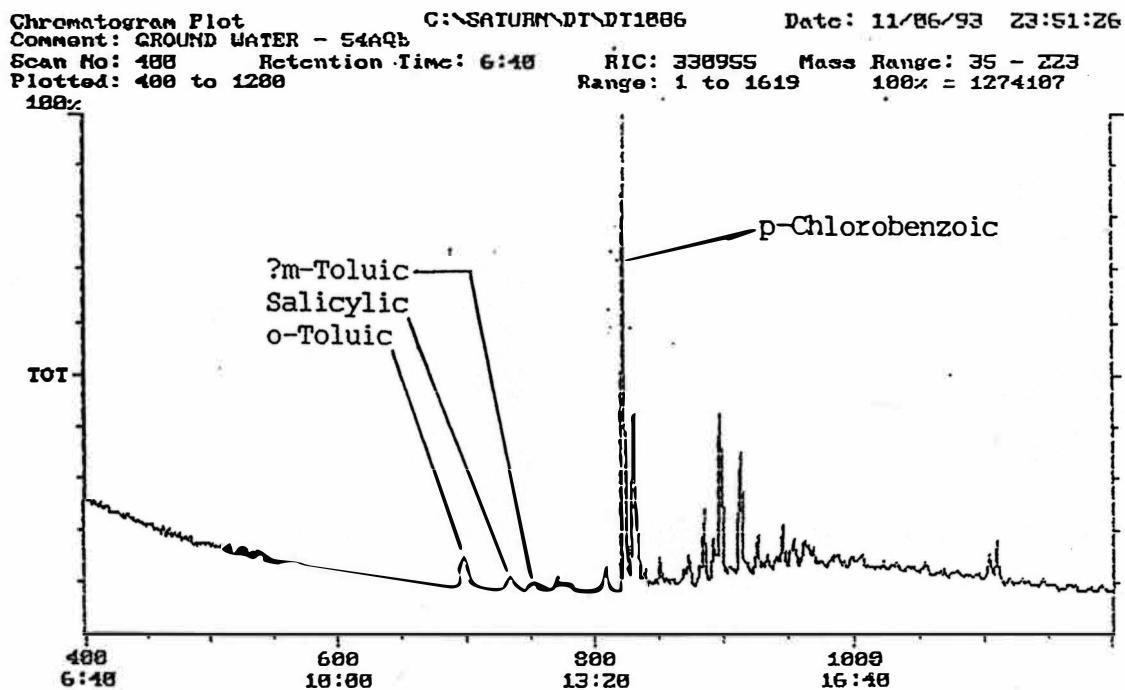


Figure 8. Chromatogram of a Sleeping Bear Dunes Sample.

form in both aerobic and anaerobic environments (Kuhn et al., 1988; Lovley and Lonergan, 1990; Cozzarelli et al., 1990). The aromatic acids may contribute significantly to the total organic carbon (TOC) and alkalinity of a specific site, but little is known of the long-term fate of the acids once they form.

The samples which were analyzed for this study had been collected from two different locations. One location was near Sleeping Bear Dunes State Park in Empire, Michigan. This site had experienced several fuel/heating oil and gasoline spills into a dune-sand shallow ground water environment. Sampling wells had been installed within the source of contamination, 12 meters downgradient from the source, and 27 meters downgradient from the source. Figures 9 and 10 display an aerial view and a vertical profile of the Sleeping Bear Dunes site, respectively. These figures show the distribution of the various wells and well clusters at the site. The data from the wells and well clusters provide information on the general spatial distribution of the aromatic acids in relation to depth and distance from the source.

The other set of samples were collected from underground storage tank (UST) sites near Houston, Texas. These samples were provided by Dr. Joseph P. Salanitro of the Shell Development Company Westhollow Research Center in Houston, Texas. All three sites consisted of BTEX (benzene, toluene, ethylbenzene, and xylenes) plumes and were located in low permeability clay settings. The ground water movement at these sites was noted to be very slow.

All the monitoring well samples had been collected from within the plume at each site except for one (MW3).

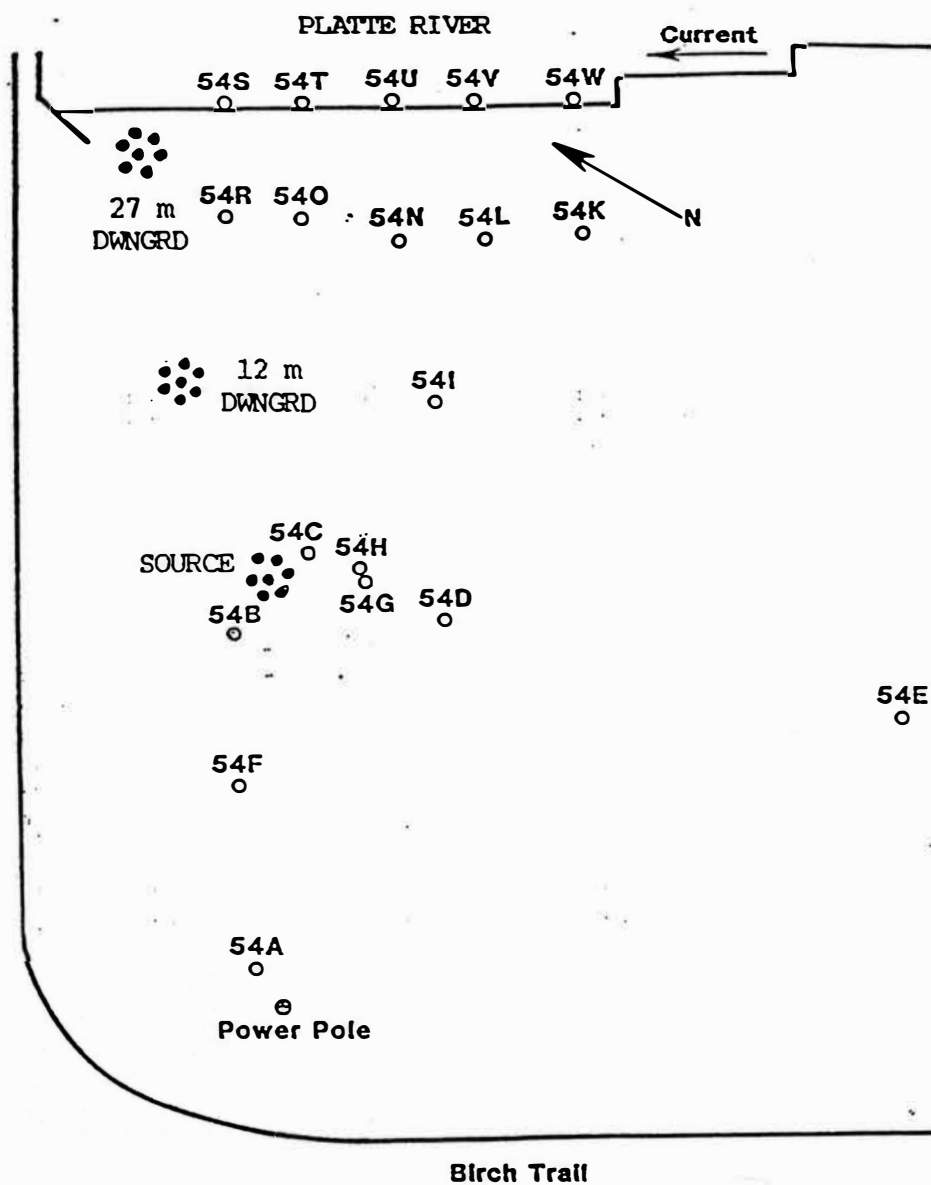


Figure 9. Aerial Layout of the Sleeping Bear Dunes Site.

Source: The Traverse Group, Inc., Traverse City, MI.

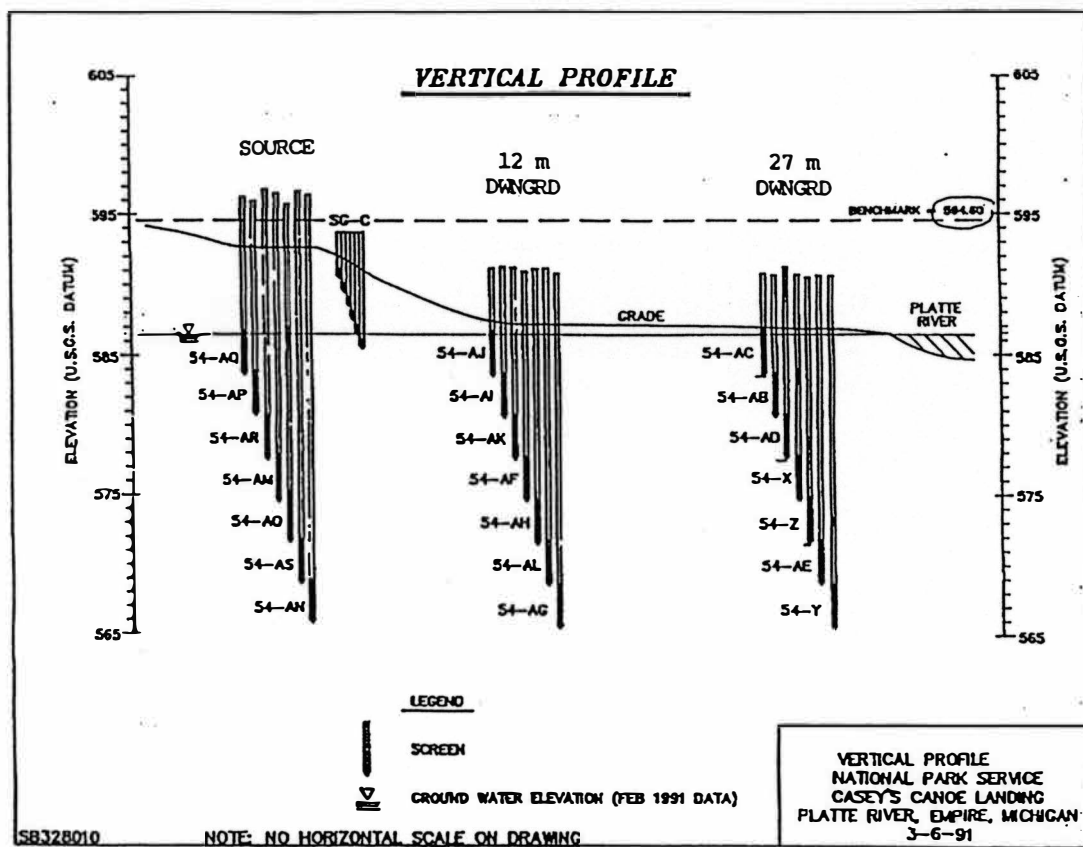


Figure 10. Vertical Profile of the Sleeping Bear Dunes Site.

Source: The Traverse Group, Inc., Traverse City, MI.

CHAPTER IV

DISCUSSION

Underground Storage Tank (UST) Sites

The results for the aromatic acid determinations of the ground water samples from the UST sites near Houston, Texas, are shown in Tables 6 and 7. These results demonstrate that the aromatic acids are transformation products of the fossil-fuel compounds in the subsurface. The concentrations of the aromatic acids varied and reached as high as $1.39 \mu\text{M}$ for salicylic acid. The concentrations for benzoic acid, o-, m-, and p-toluic acids were at similar levels to the plume concentrations of the same acids identified in the study by Cozzarelli, Baedecker, Eganhouse, and Goerlitz (1993). Overall the results help to show the coupling of organic acid formation to presumed anaerobic degradation of toluene and the xylenes.

Dissolved iron(II) concentrations exceeding 1.0 mg/L were found at some of the sample sites, indicating possible iron reducing conditions in the subsurfaces. Figure 11 is a plot of the total aromatic acid concentrations as a function of the dissolved iron(II) concentrations from the monitoring wells. With an increase in dissolved iron(II), possibly indicating more strongly reducing conditions, a general increase in total aromatic acid concentrations was observed. The oxidation of the

Table 6
Characterization of the Underground Storage
Tank (UST) Sites

WELL	BTEX (ppb)	Fe2+ (ppm)	DISS O2 (ppm)	TEMP (oC)	SAMPLING DATE
DP-1	2051	2.6	1.5	20-21	6/92
DP-3	2	0.2	1.7	20-21	6/92
NWF-4	3343	1.25	0.5	19	6/92
NWF-5	3648	1.6	0	19	6/92
HV-13S	31379		2.3	22-23	6/92
HV-13D	1892		0.8	22-23	6/92

Table 7

Aromatic Acid Concentrations From the Underground Storage Tank (UST) Sites

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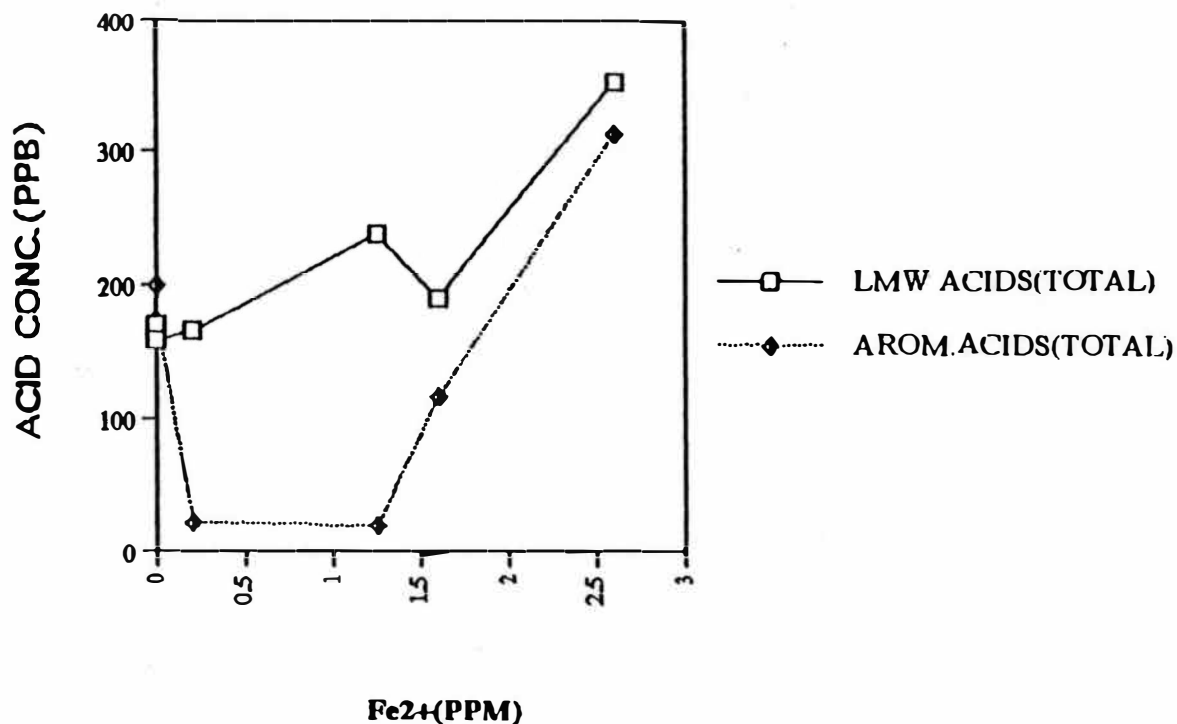


Figure 11. Plot of Total Aromatic Acid Concentrations as a Function of Dissolved Iron(II) Concentrations From UST Sites.

fossil-fuel compounds (BTEX) to form the aromatic acids therefore appeared to be coupled to iron(III) reduction. This hypothesis is consistent with work which demonstrated that the anaerobic oxidation of aromatic compounds can be coupled to iron(III) reduction through microbial activity (Lovley et al., 1989; Lovley and Lonergan, 1990).

Although there were no other inorganic indicators measured from the UST monitoring wells, iron(III) reduction does appear to be an important coupled redox process in the anaerobic degradation of BTEX compounds. With the aromatic hydrocarbons as the sole electron donors and iron(III) as the sole

electron acceptor, the production of aromatic acids as intermediates becomes possible in these environments.

Sleeping Bear Dunes Site

The results for aromatic acid determinations of the ground water samples from Sleeping Bear Dunes State Park in Empire, Michigan, are contained in Tables 8 and 9. This data suggests that the formation of the aromatic acids areas hydrocarbon transformation products under anaerobic subsurface conditions.

The characterization of the contaminated site was done by Kampbell, Cook, and Wilson (personal communication, 1991). Figure 12 illustrates a depth profile of total dissolved BTEX concentrations at the source region, 12 meters downgradient, and 27 meters downgradient of the contaminant source. The BTEX profile curves in this figure show a decrease in concentration with an increase in distance downgradient from the source. The curves also show that the dissolved BTEX was most concentrated at approximately two meters below the water table at all three sampling locations. This may indicate that the dissolution and movement of the lighter than aqueous phase compounds restrict them to a near-surface lateral distribution in the ground water flow.

The distribution of the aromatic acids at the site showed the highest concentrations in the source region and generally decreasing concentrations with increasing distance from the source. Therefore at the time of sampling, the aromatic acid metabolites were found most predominantly in the source region.

Table 8
Characterization of the Sleeping
Bear Dunes Site

WELL	SITE	DEPTH (m-bwts)	TOTAL Fe (ppm)	METHANE (ppb)	SULFATE (ppm)	NITRATE (ppm)	AMMONIA (ppm)	DISS O2 (ppm)	ALKALINITY (ppm CaCO3)	TOC (ppm)	SAMPLING DATE
54AQ	SOURCE	1	13	143	5	<0.3	5.6	0.1	294	170	7/24/92
AP	SOURCE	2	17.5	1198	4	0.4	5.7		430	100	11/17/92
54AR	SOURCE	3	10	2983	3	<0.3	7.6	0.6	314	54	7/24/92
AM	SOURCE	4	7	126	15	<0.3	6.6		278	26	11/17/92
54AO	SOURCE	4.7	2.5	126	15	<0.3	6.6	0.7	142	5	7/24/92
54AJ	12 m dwngrd	1	12	528	10	<0.3	5.4		159	220	7/24/92
AI	12 m dwngrd	2	7	1128	4	<0.3	4.1		427	110	11/17/92
54AK	12 m dwngrd	3	8	217	31	<0.3	5.2		436	30	7/24/92
AF	12 m dwngrd	4	6	304	3	<0.3	1.2		290	15	11/17/92
54AH	12 m dwngrd	4.7	3.5	113	24	<0.3	1.2		219	11	7/24/92
54AC	27 m dwngrd	1	12	1641	7	<0.3	2.4	1	277	110	7/24/92
AB	27 m dwngrd	2	6	2500	4	<0.3	2.8	0.15	369	40	11/17/92
54AD	27 m dwngrd	3	4	119	14	<0.3	1.4	2	219	11	7/24/92
54Z	27 m dwngrd	5	3.5	44	23	0.3	2.2	1.5	187	<5	7/24/92
AE	27 m dwngrd	5.7	0.4	36	12	<0.3	0.5	1.05	10.3	<5	11/17/92

Cozzarelli, Baedecker, Eganhouse, and Goerlitz (1993) conducted a study on the geochemical evolution of organic acids from the degradation of petroleum contaminants from a defined source over a five year period (1986-90). They found that over time (through the time span of their study) the total aromatic acid concentrations increased with increasing distance from the plume.

This study could not account for the effect of time on the concentrations of the aromatic acid metabolites since only one sample event was possible. What

Table 9
Aromatic Acid Concentrations From the
Sleeping Bear Dunes Site

WELL	BENZOIC (μM)	BENZOIC (ppb)	o-TOLUIC (μM)	o-TOLUIC (ppb)	m-TOLUIC (μM)	m-TOLUIC (ppb)	p-TOLUIC (μM)	p-TOLUIC (ppb)	SALICYLIC (μM)	SALICYLIC (ppb)	TOTAL ACIDS (μM)	TOTAL ACIDS (ppb)
S4AQ			2.93	399	0.082	11.2	0.108	14.7	0.754	104	3.88	529
AP			2.46	335	0.437	59.5	0.287	39.1	0.774	107	3.96	541
S4AR	0.178	21.7	1.27	173	1.1	150	0.645	87.8	1.85	255	5.05	688
AM											0.729	
S4AO			0.053	7.22			0.053	7.22	0.623	86	0.729	100
S4AJ									0.215	29.7	0.215	29.7
AI			0.133	18.1	0.784	107	0.232	31.6			1.15	157
S4AK					0.015	2.04	0.008	1.09	0.404	55.8	0.427	58.9
AF												
S4AH												
S4AC			0.094	12.8			0.07	7.53	0.026	3.59	0.19	25.9
AB												
S4AD									0.216	29.8	0.216	29.8
S4Z												
AE			0.315	42.9							0.315	42.9

this study did help to show was that aromatic acid metabolites formed during the anaerobic degradation of the BTEX compounds in the subsurface. Comparison of the profiles of the aromatic acid concentrations to the profiles of inorganic electron acceptor concentrations yields a picture of the coupling of redox reactions in the aquifer. This study also gives a better understanding of the degradation pathways of BTEX compounds, particularly toluene and the xylenes, in various aquifer settings.

Sleeping Bear Total BTEX Profiles

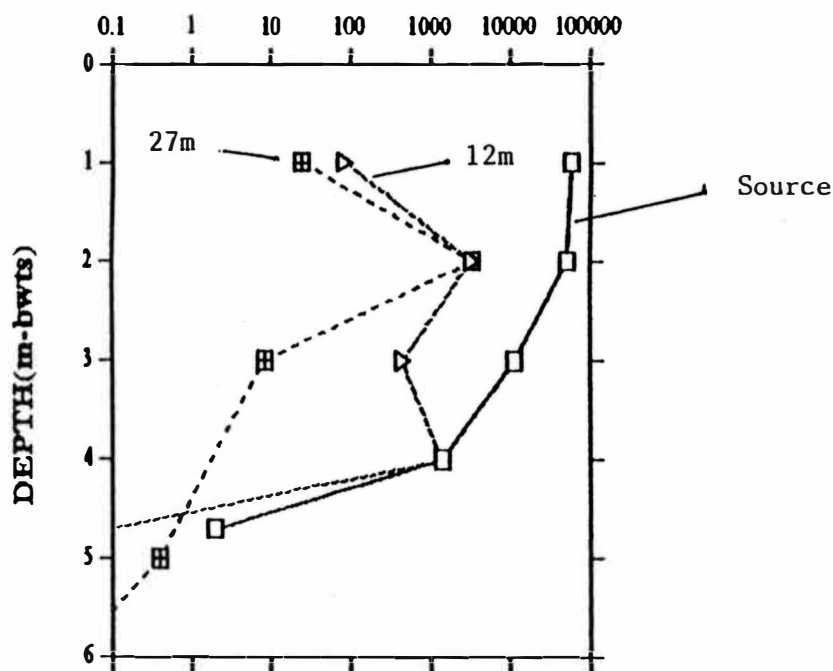


Figure 12. Depth Profile of Total Dissolved BTEX Concentrations (ppb) at Sleeping Bear Dunes.

Figures 13, 14, and 15 illustrate the relationship between the concentrations of various inorganic electron acceptors and the concentrations of the aromatic acids at the three well locations. These figures may help to give a better understanding of whether the degradation processes at this site are of nitrate reduction, sulfate reduction, or methanogenesis.

Figure 13 displays the correlation between sulfate concentrations and aromatic acid concentrations at the three well locations. Microcosm studies have been done to show how sulfate can serve as the terminal electron acceptor in the

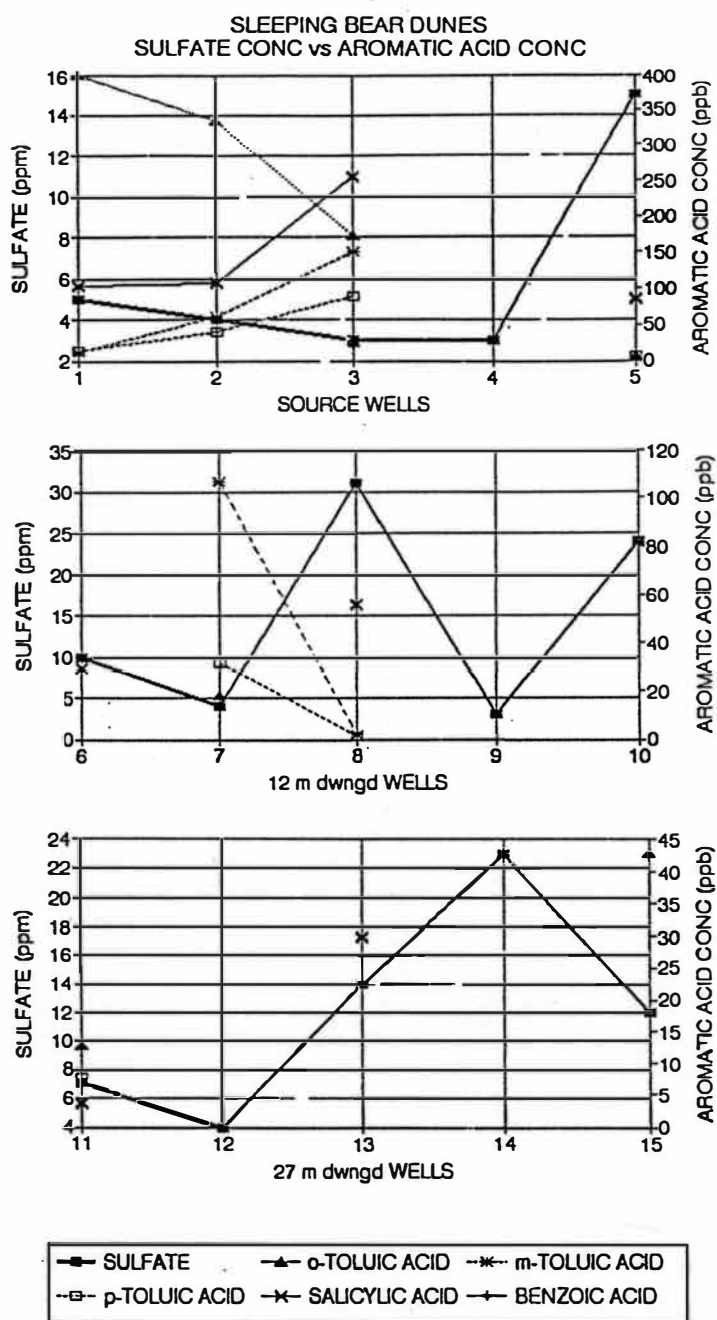


Figure 13. Correlation Between Sulfate Concentrations and Aromatic Acid Concentrations at Sleeping Bear Dunes.

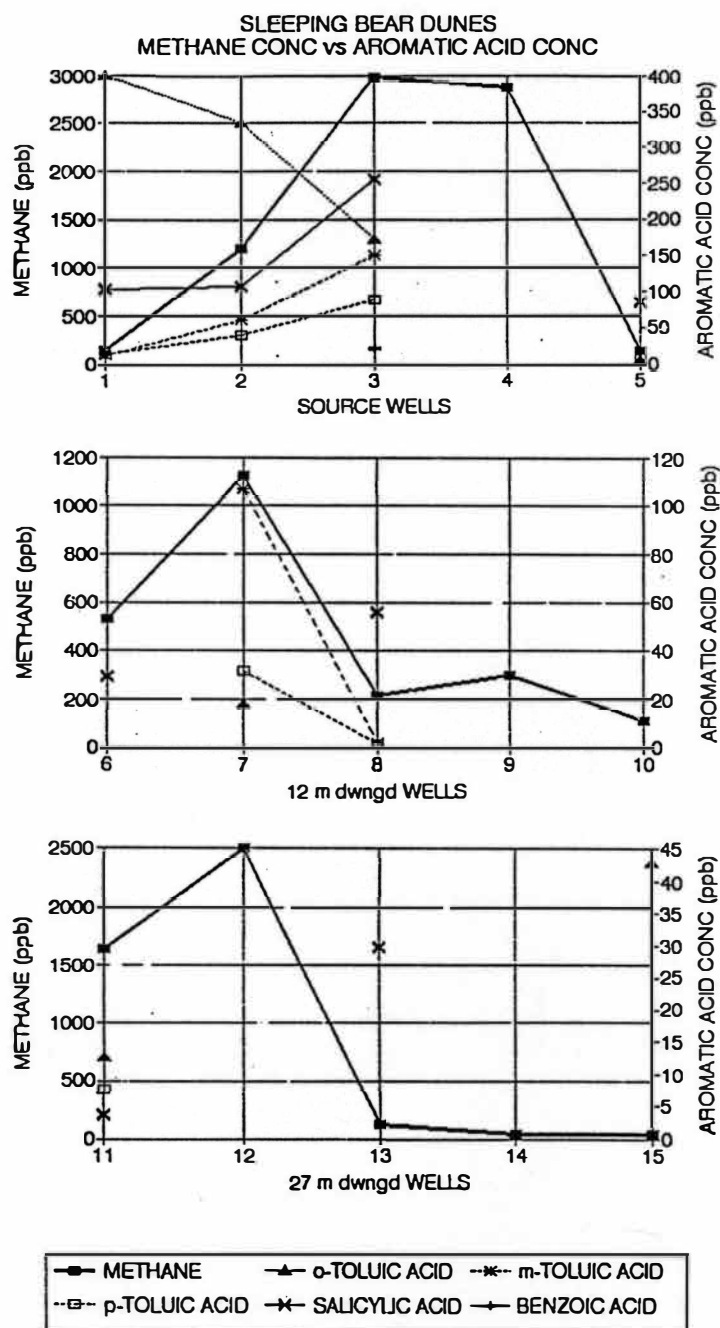


Figure 14. Correlation Between Methane Concentrations and Aromatic Acid Concentrations at Sleeping Bear Dunes.

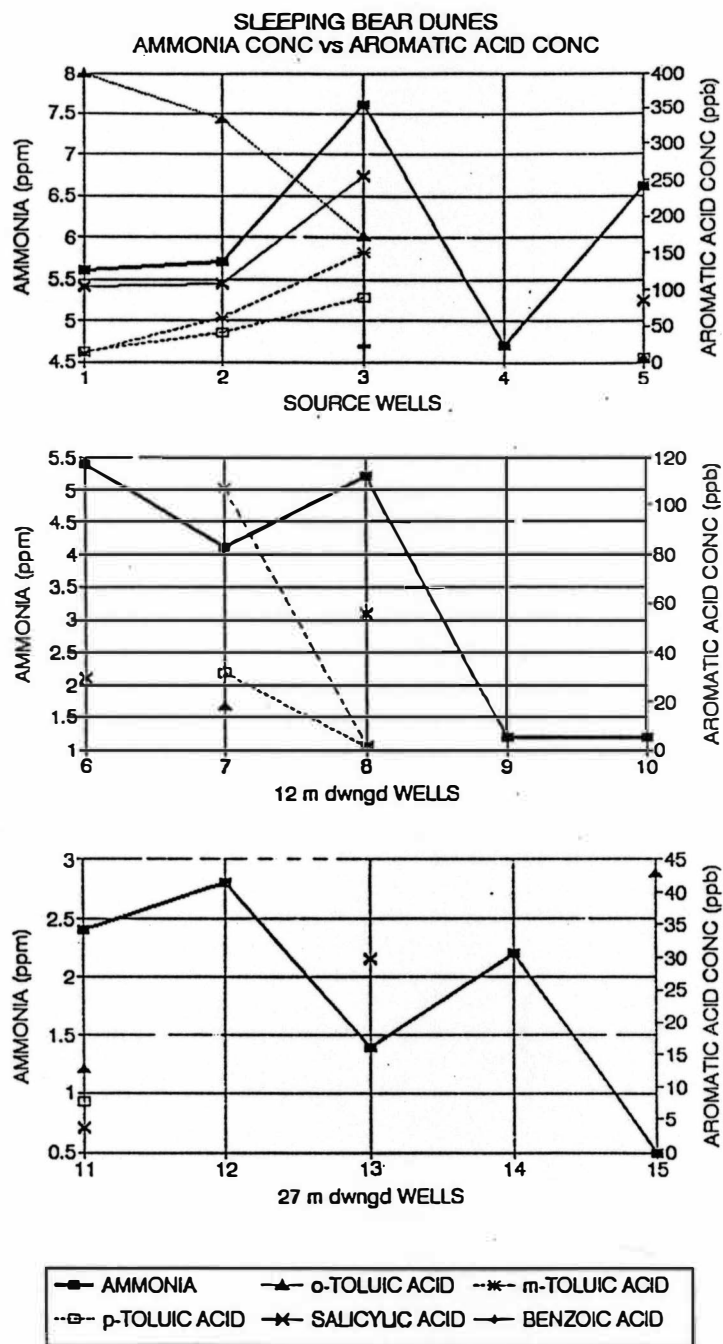


Figure 15. Correlation Between Ammonia Concentrations and Aromatic Acid Concentrations at Sleeping Bear Dunes.

anaerobic degradation of toluene and the xylenes (Edwards, Reinhard, and Grbic-Galic, 1992). In the source region at Sleeping Bear Dunes it can be seen that the sulfate concentrations are low, possibly indicating sulfate reducing conditions within the plume. As distance downgradient from the source increases, the sulfate concentrations appear to generally increase. This increase in sulfate concentration outside the source region suggests that the redox intensity of the aquifer was less reducing.

There may be a reasonable correlation between the changes in aromatic acid concentrations and changes in sulfate concentrations near the source. As the sulfate concentrations decrease within the source region, aromatic acid concentrations generally increase. The exception to this characterization is seen with o-toluic acid. For m-, p-toluic acids, and salicylic acid, there is an increase in acid concentration with a decrease in sulfate concentration. This suggests that as the sulfate is reduced to sulfide, the aromatic hydrocarbons (BTEX) are oxidized to form the aromatic acids.

Figure 14 suggests a correlation between methane concentrations and aromatic acid concentrations at the three well locations. Previous microcosm studies have been performed to show how aromatic hydrocarbons (BTEX) can be degraded anaerobically in methanogenic conditions (Wilson et al., 1986; Grbic-Galic and Vogel, 1987). At the Sleeping Bear Dunes site, methane concentrations within the source region were significantly higher than those at the downgradient well locations. In addition, there appears to be a direct correlation

between changes in methane concentrations and changes in aromatic acid concentrations within the plume. This correlation is not seen at the downgradient locations, possibly indicating that methanogenic conditions were no longer significant.

The study done by Cozzarelli, Baedecker, Eganhouse, and Goerlitz (1993) identified changes in the degradation processes over time. Their study showed that the anaerobic degradation of contaminants shifted from iron(III) reduction to methanogenesis over the five year period that the site was studied. At the Sleeping Bear Dunes site, it is not known if there had been a shift in the degradation processes. However it does appear that both sulfate reduction and methanogenesis are involved in the anaerobic degradation of the BTEX compounds to form the aromatic acids.

A third possible degradation mechanism to be considered at the Sleeping Bear Dunes site may be nitrate reduction. In anaerobic conditions nitrate can serve as an electron acceptor in the degradation of aromatic hydrocarbons (Kuhn et al., 1988; Zeyer et al., 1990). The reduction of nitrate will produce ammonia. Figure 15 illustrates the relationship between ammonia concentrations and aromatic acid concentrations at each of the three well locations. Since the nitrate concentrations were either near or below the detection limit (<0.3 mg/L) for all three well locations, it is plausible that the nitrate had been reduced to ammonia.

Figure 15 shows a plot suggesting a degree of correlation between ammonia and aromatic acid concentrations with depth in the source region. With

an increase in the ammonia concentrations, indicative of more strongly reducing conditions, there is an increase in aromatic acid concentrations. As the distance downgradient from the source region increases, the redox conditions become less reducing and the correlation is no longer seen.

The inorganic indicators which have been presented do appear to be involved in the anaerobic degradation of the fossil-fuel contaminants. Sulfate reduction, methanogenesis, and nitrate reduction are three degradation processes which were correlated with the accumulation of the aromatic acids at the Sleeping Bear Dunes site. Microcosm studies have illustrated that all three degradation processes can be associated with the anaerobic degradation of certain aromatic hydrocarbons. This study has shown that these processes are also active in the in-situ degradation of the BTEX compounds in subsurface environments.

Transformation Pathways

Based on the information which has been presented, possible transformation pathways can be formulated in order to better understand the anaerobic degradation of aromatic contaminants. Figure 16 shows how methyl oxidation of toluene and o-, m-, and p-xylenes can result in the intermediates of benzoic acid and o-, m-, and p-toluic acids respectively. These four intermediate organic acids were all identified in various concentrations in the ground water samples analyzed for this study. These organic acids have also been identified in

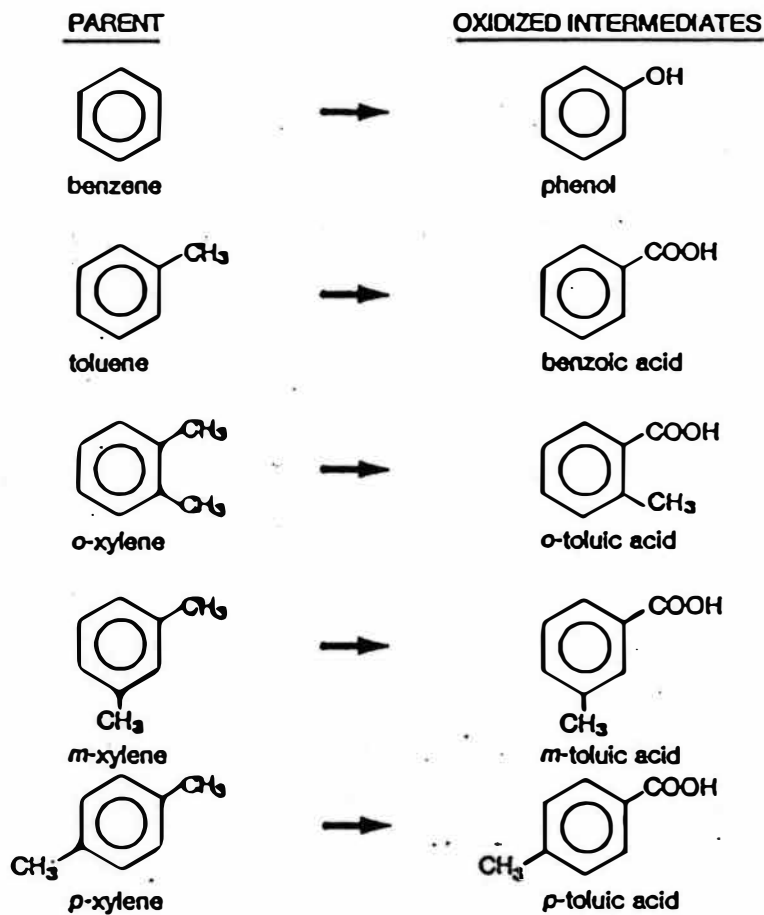


Figure 16. Oxidation of Selected BTEX Compounds.

Source: Cozzarelli, I.M., R.P. Eganhouse and M.J. Baedecker, Transformation of Monoaromatic Hydrocarbons to Organic Acids in Anoxic Ground Water Environment, Environ. Geol. Water Sci., 16, 2, 135-141, 1990.

other subsurface environments with anaerobic conditions (Cozzarelli et al., 1990; Cozzarelli et al., 1993).

A more detailed pathway description can be seen in Figure 17. This pathway diagram also shows how the methyl oxidation of toluene results in the benzoic acid intermediate. In addition this pathway shows how the ring oxidation of toluene will produce cresol intermediates. This is consistent with the work of Vogel and Grbic-Galic (1986) in which they were able to show the incorporation of oxygen from ^{18}O -labeled water into toluene and benzene under anaerobic conditions.

The o-cresol intermediate can be further oxidized to form 2-hydroxybenzoic acid (salicylic acid). The salicylic acid intermediate was found in high concentrations relative to the other aromatic acids which were identified. In the source region at the Sleeping Bear Dunes site the concentration of salicylic acid was as high as $1.85\ \mu\text{M}$ and at the UST sites the concentration was as high as $1.39\ \mu\text{M}$.

Since the benzoic acid intermediate was only found at one well site at Sleeping Bear Dunes, one may conclude that the salicylic acid (2-hydroxybenzoic acid) intermediate forms via o-cresol. In addition, if hydroxybenzoic acid intermediates were forming via benzoic acid, one would expect to see 3-hydroxybenzoic acid as the predominant isomer due to the meta-directing characteristic of the carbonyl group for electrophilic aromatic substitution (Vollhardt, 1987). But 3-hydroxybenzoic acid was never found in the samples

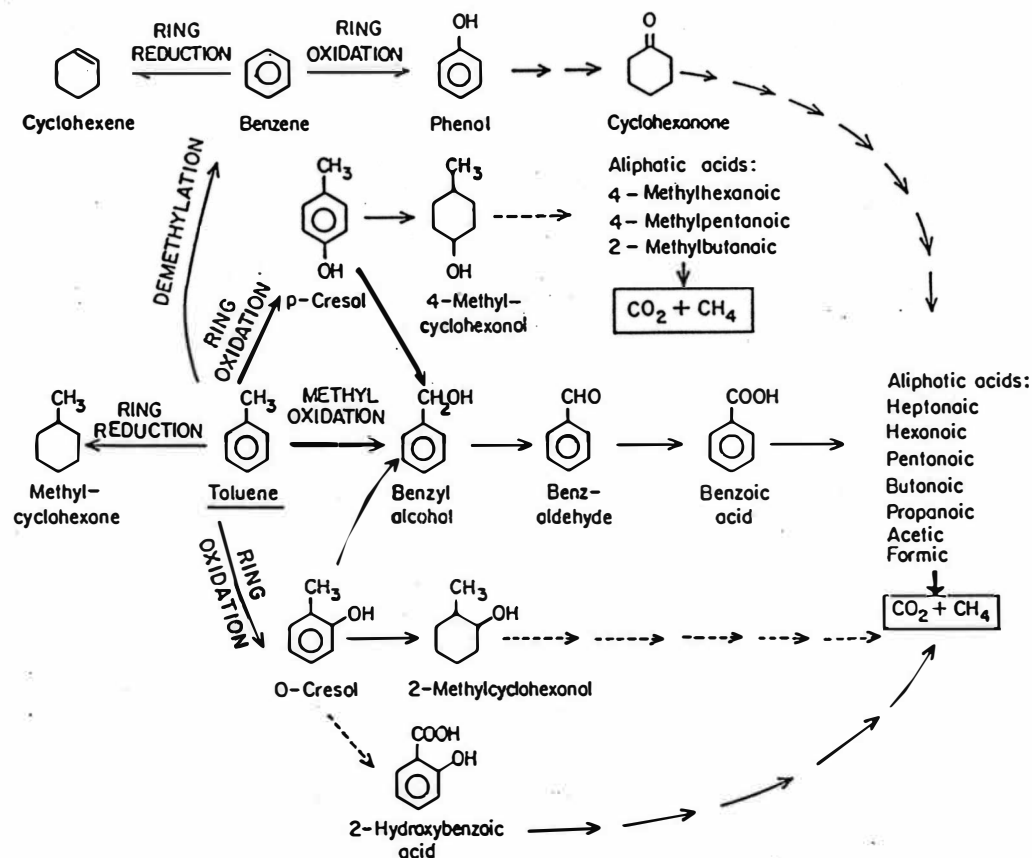


Figure 17. Detailed Degradation Pathways of Benzene and Toluene.

Source: Grbic-Galic, D. and T.M. Vogel, Transformation of Toluene and Benzene by Mixed Methanogenic Cultures, Appl. Environ. Microbiol., 53, 2, 254-260, 1987.

which were analyzed. Further studies could be done on cresol intermediates to determine if o-cresol is a major transformation product of toluene and whether or not there exists a correlation with the formation of the salicylic acid intermediate.

CHAPTER V

CONCLUSIONS

Geochemical investigations of anaerobic subsurface environments can be aided through the sensitive and accurate quantitation of organic acids. The detection of biosynthesized aromatic acids in the subsurface as intermediates allows for a better understanding of the preferred breakdown pathways of BTEX compounds. These acids are also helpful in understanding the microbial involvement in redox reactions occurring in aquifer systems.

The dissolved aromatic acids represented a small fraction of the total organic mass in the contaminated aquifers. However these organic acids provided important information on the localized occurrence and degree of contaminant breakdown under a range of redox conditions. The formation of aromatic acids under reducing conditions was found to be coupled with iron reduction, nitrate reduction, sulfate reduction, and methanogenesis.

Many scientists and researchers have an interest in understanding the fate of contaminant compounds once they enter a subsurface environment. This research project can be viewed as a contribution to further understanding in-situ anaerobic biodegradation processes.

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