THE FATE OF ^{14}C-PHENANTHRENE LABELLED DIESEL FUEL #2 IN SELECTED MANITOBA SOIL

BY

ROBERT D. MAURICE

A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfillment of the Requirements
for the Degree of

MASTER OF SCIENCE

Department of Soil Science
University of Manitoba
Winnipeg, Manitoba

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ABSTRACT

Maurice, Robert Daniel. M.Sc., The University of Manitoba, May, 1998. <u>The Fate of ^{14}C-Phenanthrene Labelled Diesel Fuel #2 In Selected Manitoba Soils.</u> Major Professor; Dr. David L. Burton.

The release of petroleum hydrocarbons into the environment represents a human and environmental hazard. The treatment of the hazardous waste found in soil in the past has been through the excavation and treatment off-site. The realization of the costs and the potential to contaminate the surrounding environment through this method has increased interest *in situ* bioremediation. *In situ* bioremediation allows intrinsic or supplemented microbial populations to degrade hydrocarbons on site with little soil disruption. In order to understand the potential for bioremediation, environmental factors such as landscape, soil type, texture, water content, organic carbon content, and microbial species diversity must be understood in order to optimize this process. To date, little research has been done on the effects of soil type, landscape and moisture status on bioremediation potential. This research deals with the mineralization of phenanthrene in diesel fuel a) in a survey of soils, b) along a landscape, and c) under wetting and drying conditions in soil.

In order to determine the remediation capacity of a range of soils, a survey was undertaken of 10 sites in the province of Manitoba. Along with uncontaminated soil samples, soil having previous contamination of hydrocarbons was also examined. The

mineralization, volatilization and sorbed or residual ^{14}C labeled phenanthrene in diesel was monitored in microcosms. Results indicated naturally occurring microbial species were able to degrade phenanthrene but there was poor mineralization in the surface and subsurface with the majority of the compound remaining adsorbed in the soil. Prior hydrocarbon sensitization did not ensure significant mineralization and factors other than texture, organic carbon, and microbial diversity contributed to total mineralization. Texture, organic carbon and microbial metabolic diversity were poor indicators of the total mineralized phenanthrene, yet organic carbon and microbial metabolic diversity were good indicators of the rate of mineralization.

The effect of landscape on the mineralization of phenanthrene in diesel fuel was assessed using air flow through systems in intact soil columns and static microcosms. The results, after 343 days of monitoring, indicated mineralization and volatilization were minor fates while the majority of the phenanthrene remained in the soil in an adsorbed state. Transport or water soluble phenanthrene or degradation intermediates was a major fate with significant amounts moving 50cm down the soil profile. The same result was seen in the total extractable hydrocarbon experiment. There appeared to be no effect of landscape on the mineralization of phenanthrene and subsurface environments were not significantly different from the surface, however total extractable hydrocarbons did decrease slightly from the knoll to the depression. Static microcosms had 15 times less mineralization than intact soil columns with air flow systems, therefore, aeration may be an important factors influencing phenanthrene mineralization.

The mineralization of glucose and phenanthrene under various moisture conditions was monitored using flow through microcosm systems. The soil was subjected to wet-dry,

continuously dry and continuously wet conditions. Wetting and drying of the soil did not significantly increase nor detrimentally affect the mineralization of glucose or phenanthrene in the soils investigated. Landscape also had no effect on the mineralization of either phenanthrene or glucose. This experiment also calls into question the role of aeration in the degradation of hydrocarbons because up to 50% of phenanthrene was mineralized, significantly greater than the passive systems.

FOREWORD

The following thesis was prepared using the manuscript format outlined in the Guide to Thesis Preparation for Graduate Students in the Department of Soil Science. All of the manuscripts presented in this thesis (Chapters 3, 4 and 5) will be submitted for publication to refereed journals. The manuscripts will also include a co-author, Dr. David L. Burton, who is also the major professor and advisor.

CHAPTER 1

Introduction

The exposure of petroleum hydrocarbons to the environment is a growing concern due to the detrimental effects on human health and the environment (Canadian Environmental Protection Act 1994). The clean up of these spills can be quite costly depending on the risk and urgency of the accident. The traditional recommendation of excavation and treatment off site is quite costly to perform and can damage the ecosystem. Intrinsic bioremediation, utilizing undisturbed soil parameters and microorganisms in degradation, has become a commonly accepted approach in the remediation industry due to the relatively inexpensive reclamation procedure (Widrig and Manning 1995). The decision to remediate a site via this method relies on the understanding of the properties of the soil and the microorganisms contained there. It is, therefore, important to understand the physical, chemical and biological properties of the soil as they influence the potential for enhanced bioremediation.

Phenanthrene was chosen as a model compound in order to understand the properties of hydrocarbons that accumulate in soil after a diesel spill. It is a PAH (polyaromatic hydrocarbon) with 3 aromatic rings. It has a high molecular weight, log K_{ow}, and potential for adsorption in soil. It has also been shown to cause cancer in biological cells (Barfknecht et al. 1981) Determining the potential rate of phenanthrene

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mineralization in diesel fuel is an important component in assessing the risk of long-term environmental impact and allows an evaluation of the suitability of *in situ* bioremediation.

The objectives of this study were to determine the remediation capabilities of soils found across the province of Manitoba and how parameters influence contaminant availability and the microbial environment (texture, organic carbon, structure, moisture potential etc.) influence this rate. To do this, a survey of the phenanthrene mineralization capabilities across a range of soils was undertaken. The survey will help to determine the capacity of microbial populations to degrade hydrocarbon constituents and determine the relationships between mineralization and physical and biological properties of the soil. The results obtained in this study will help in assessing the fate of hydrocarbons and risk to the environment. Soils varying in texture, organic carbon and climate were selected from across the province of Manitoba. The phenanthrene mineralization potential was determined for each site. A second study determined the degradation potential across a landscape simulated by a severe spill of diesel fuel (100,000kg diesel/ha to a depth of 0-15cm). This experiment determined the capacity of microorganisms to degrade diesel at very high concentrations. The information will be useful in risk assessment to determine areas in the landscape supporting degradation. This study also looked at the effect of structure comparing intact soil columns to microcosms. The effect wet-dry cycles on the mineralization of glucose and phenanthrene in diesel fuel was also determined. This study focused on the properties of water as a regulator of microbial activity. The soils were subjected to continuously wet, continuously dry and wet-dry cycles to determine the effect moisture had on mineralization.

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CHAPTER 2

Literature Review

2.1 Introduction

Hydrocarbon contamination of soil can occur via many pathways. Accidental release by tankers, storage tanks and transportation of toxic petroleum products can result in adverse affects on the environment. When contamination occurs, a remediation strategy must be implemented in order to satisfy both the reduction in risk to human health and the environment and cost of clean up. Remediation technologies can include intensive, costly techniques such as excavation, soil washing, soil flushing, thermal desorption or more extensive *in situ* remediation which may be less costly. The potential to use *in situ* remediation relies on many factors such as pH, temperature, oxygen availability, water potentials, inorganic nutrients and presence of toxins along with the presence of microbial population capable of degradation (Skladany and Metting 1993; Atlas and Cerniglia 1995). Understanding the effects wetting and drying, landscape, organic matter, texture, and microbial populations will have on degradation will help in understanding the factors limiting the process of *in situ* bioremediation. The potential for the remediation of diesel contaminated sites with different physical and biological influences is the focus of this study.

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2.2 Diesel Fuel Degradation

2.2.1 Composition of Diesel

Diesel fuel is made up of hundreds of different compounds including aliphatic, polycyclic and aromatic compounds (Widrig and Manning 1995). The composition of diesel fuel varies due to the differences in source, fractionation cracking and formulation (Gillespie et al. 1989). Table 2.1 and Table 2.2 indicate the relative distribution of hydrocarbons seen in diesel fuel #2, with the first table indicating the number of carbons in each fraction, while the second table compares individual compounds found in different diesel sources. C_{10} to C_{19} paraffins, cycloparaffins and aromatic compounds dominate diesel fuel #2 (Table 2.1). Also the source and preparation of diesel is a factor in determining the types and amounts of compounds involved (Table 2.2). Some hydrocarbon compounds such as phenanthrene can be identified in some diesel isolates while being undetectable in others.

To simplify the description of diesel fuel #2 it is easier to group compounds into n-alkanes, monoaromatics, and polynuclear aromatics (McGill et al. 1981; Atlas and Bartha 1993). N-Alkanes can be branched or cyclic and may make up the greatest component in diesel. Monoaromatics, such as benzene and toluene, and polyaromatics (phenanthrene and naphthalene) can also make up a significant component of diesel varying from 10-30% (Block et al. 1991).

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Table 2.1 Composition of diesel fuel #2 (Clewell 1981).

Component	Concentration (% Volume)	Component	Concentration (% Volume)
C_{10} paraffins	0.9	C_{15} paraffins	7.4
C_{10} cycloparaffins	0.6	C_{15} cycloparaffins	5.5
C_{10} aromatics	0.4	C_{15} aromatics	3.2
C_{11} paraffins	2.3	C_{16} paraffins	5.8
C_{11} cycloparaffins	1.7	C_{16} cycloparaffins	4.4
C_{11} aromatics	1.0	C_{16} aromatics	2.5
C_{12} paraffins	3.8	C_{17} paraffins	5.5
C_{12} cycloparaffins	2.8	C_{17} cycloparaffins	4.1
C_{12} aromatics	1.6	C_{17} aromatics	2.4
C_{13} paraffins	6.4	C_{18} paraffins	4.3
C_{13} cycloparaffins	4.8	C_{18} cycloparaffins	3.2
C_{13} aromatics	2.8	C_{18} aromatics	1.8
C_{14} paraffins	8.8	C_{19} paraffins	0.7
C_{14} cycloparaffins	6.6	C_{19} cycloparaffins	0.6
C_{14} aromatics	3.8	C_{19} aromatics	0.3

Table 2.2 Diesel fuel composition (mg/g) from PTS (petroleum and shale oil and tar sands) sources (Millner et al. 1992).

Sample Compound	4801 GeoKinetics Suntech Add.	9101 Philips lot C345	DF-2-1 Ft. Carson DIO	PTS Coprocessed 9523-1990 DF
C_8	5.8	-	1.0	-
C_9	5.1	3.6	4.3	9.0
C_{10}	9.3	10.1	7.7	9.9
C_{11}	17.3	17.1	13.4	10.1
3-Me-C_{11}	1.2	1.8	1.4	1.6
Napthalene	1.7	1.6	1.9	5.7
C_{12}	22.3	17.7	13.9	9.6
2-Me-C_{12}	2.5	2.7	2.2	2.1
2-Me-Nap	1.9	8.4	9.6	12.7
C_{13}	24.2	20.4	16.7	8.1
1-Me-Nap	1.1	4.6	4.7	5.8
3-Me-C_{13}	1.3	2.0	1.5	0.9
Biphenyl	-	-	-	-
C_{14}	21.4	20.8	19.1	7.6
1,3-DiMe-Nap	1.0	8.6	9.4	8.0
1,5-DiMe-Nap	-	2.7	2.8	2.4
1,4-DiMe-Nap	1.8	1.8	1.6	1.2
2-Me-C_{14}	11.3	5.0	3.8	0.9
C_{15}	20.6	26.2	24.0	7.0
Fluorene	0.5	1.4	0.9	0.7
C_{16}	19.2	24.8	21.9	6.0
C_{17}	15.8	23.6	19.7	10.5
Pristane	9.7	7.4	4.7	1.9
Phenanthrene	-	3.0	1.9	1.6
C_{18}	12.2	17.0	16.0	5.2
Phytane	7.1	5.5	4.9	2.3
C_{19}	8.8	9.2	11.7	4.5
C_{20}	5.8	3.7	8.4	4.3
C_{21}	5.1	1.6	7.0	4.7
2-Me-Phenan	-	1.6	1.8	2.1
C_{22}	2.5	-	3.8	3.4
C_{23}	2.0	-	2.4	2.8
C_{24}	-	-	-	1.9
C_{25}	-	-	-	2.0
Total Compounds Identified	239	255	245	156

2.2.2 Diesel Fuel Metabolism

ZoBell (1946) characterized the metabolism of hydrocarbons by microorganisms. His work lead researchers to believe that not only could organisms be used for industrial purposes but also for *in situ* bioremediation (Rainwater et al. 1993). The idea for *in situ* bioremediation did not gain popularity until the mid 1980's when oil spills were examined for natural attenuation near the coast of Alaska (Atlas and Cerniglia 1995), in particular, the Exxon Valdez spill where *in situ* remediation was quite successful. Petroleum spills were monitored along shorelines and over time appeared to decrease in concentration. From these observations there was a rapid growth in the field of microbial mediated remediation and now represents a billion or even trillion dollar industry (Rainwater et al. 1993; Widrig and Manning 1995).

2.2.3 N-Alkane Degradation

In soil, n-alkanes are the most rapidly degraded compounds contained in diesel fuel and are degraded usually aerobically where oxygen atoms are incorporated into the structure of the chemical (McGill et al. 1981). Increasing chain length and branching increases the time required for degradation.

The enzymes catalyzing these processes are called monooxygenase and dioxygenase. In the case of monooxygenase (Figure 2.1), one atom of oxygen is incorporated into the alkane forming a primary alcohol and water. Dioxygenase (Figure 2.2) incorporates two oxygen atoms into the structure initially forming a hydroperoxide. This molecule is then reduced to form an alcohol and water (Atlas and Bartha 1993). In either case the terminal methyl group is attacked thereby forming an alcohol functional group. The primary alcohol is then converted to aldehydes and fatty acids where β-

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oxidation can occur (Figure 2.3). In β-oxidation a fatty acid is converted to an acyl coenzymeA and from here can form acetyl CoA which may now enter the TCA cycle (McGill et al. 1981). The β-oxidation sequence does not require the presence of oxygen, and therefore can occur under anaerobic conditions (Figure 2.4) allowing the degradation without the presence of oxygen.

$$R\text{-}CH_2\text{-}CH_3 + O_2 + NADPH_2 \rightarrow R\text{-}CH_2\text{-}CH_2\text{-}OH + NADP + H_2O$$
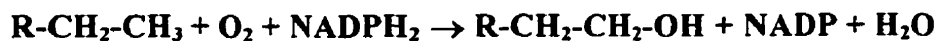
Figure 2.1 Monooxygenase mediated conversion of an alkane molecule forming a primary alcohol (Atlas and Bartha 1993).

$$R\text{-}CH_2\text{-}CH_3 + O_2 \rightarrow R\text{-}CH_2\text{-}CH_2\text{-}COOH$$
$$R\text{-}CH_2\text{-}CH_2\text{-}OOH + NADPH_2 \rightarrow R\text{-}CH_2\text{-}CH_2\text{-}OH + NADP + H_2O$$
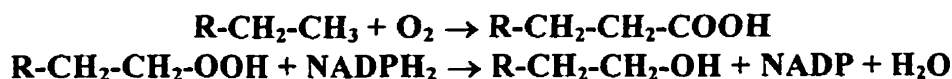
Figure 2.2 Dioxygenase mediated conversion of an alkane molecule ultimately forming a primary alcohol (Atlas and Bartha 1993).

$$R\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}COOH \xrightarrow[+CoA]{} R\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}COCoA \xrightarrow[-2H^-]{} R\text{-}CH_2\text{-}CH=CH\text{-}COCoA$$
$$R\text{-}CH_2\text{-}CH=CH\text{-}COCoA \xrightarrow[+H_2O]{} R\text{-}CH_2\text{-}CHOH\text{-}CH_2\text{-}COCoA \xrightarrow[-2H^-]{} R\text{-}CH_2\text{-}CO\text{-}CH_2\text{-}COCoA$$
$$R\text{-}CH_2\text{-}CO\text{-}CH_2\text{-}COCoA \xrightarrow[+CoA]{} R\text{-}CH_2\text{-}COCoA + CH_3\text{-}COCoA$$
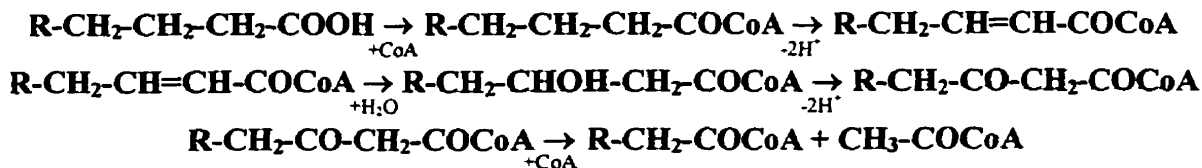
Figure 2.3 β-oxidation sequence of a primary alcohol to acetyl CoA units (Atlas and Bartha 1993).

$$R\text{-}CH_2\text{-}CH_3 \xrightarrow[-2H^-]{} R\text{-}CH=CH_2 \xrightarrow[+H_2O]{} R\text{-}CHOH\text{-}CH_3$$
$$R\text{-}CHOH\text{-}CH_3 \xrightarrow[-2H^-]{} R\text{-}CH_2\text{-}CHO \xrightarrow[-2H^-, +H_2O]{} R\text{-}CH_2\text{-}COOH$$
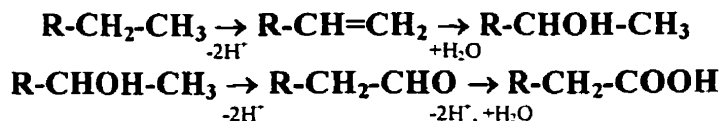
Figure 2.4 Proposed anaerobic degradation of hydrocarbons through the β-oxidation sequence pathway (Atlas and Bartha 1993).

2.2.4 Degradation of Monoaromatics

Monoaromatic degradation (e.g. Benzene) relies on initial oxygenation of the aromatic ring by either monooxygenase or dioxygenase (Figure 2.5). Bacteria have been shown to use dioxygenase to begin the degradation thereby forming cis dihydrodiol which

are oxidized to catechol. Eukaryotic cells (mammals and fungi) tend to use monooxygenase. The incorporation of one oxygen atom forms an areneoxide which is then hydrated to form trans-dihydrodiol and then is oxidized to form a catechol (McGill et al. 1981). Once catechol is formed the aromatic ring can be opened by either ortho or meta cleavage. In ortho cleavage catechol forms cis,cis-muconic acid which is further oxidized to β-ketoadipic acid which can form TCA cycle intermediates. Meta cleavage results in the formation of cis,cis-moconic semialdehyde which may now form pyruvic acid, formic acid or acetaldehyde (Ribbons and Eaton 1982; Atlas and Bartha 1993). The pathway of oxygenation is important as fungi and mammals form trans-diols which can have carcinogenic effects while cis diols, formed by bacteria, do not (Figure 2.6).

2.2.5 Polyaromatics Degradation

In the case of polyaromatic degradation, in this case phenanthrene, the reaction is much more complicated due to the fused ring structure. The initial oxygenation occurs using an oxygenase enzyme forming a dihydrodiol. The dihydrodiol is further oxidized to naphthalene and from here, through salicyclic acid to catechol where it follows the same degradation in monoaromatics (Figure 2.7). After entering the TCA cycle compounds are mineralized to carbon dioxide and water.

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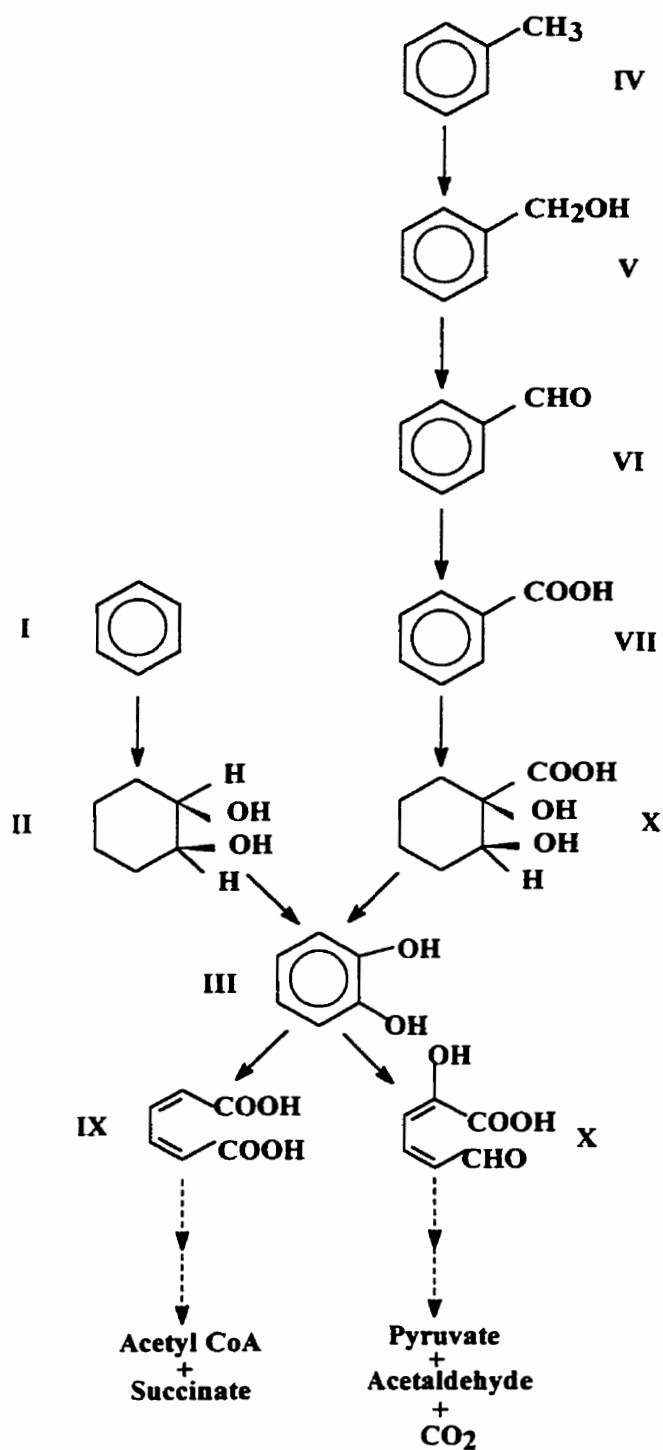
Figure 2.5 Metabolism of monoaromatics benzene (I) and toluene (IV) to catechol (III), then through meta (X) or ortho (IX) clevage to TCA cycle intermediates (Ribbons and Eaton 1982).

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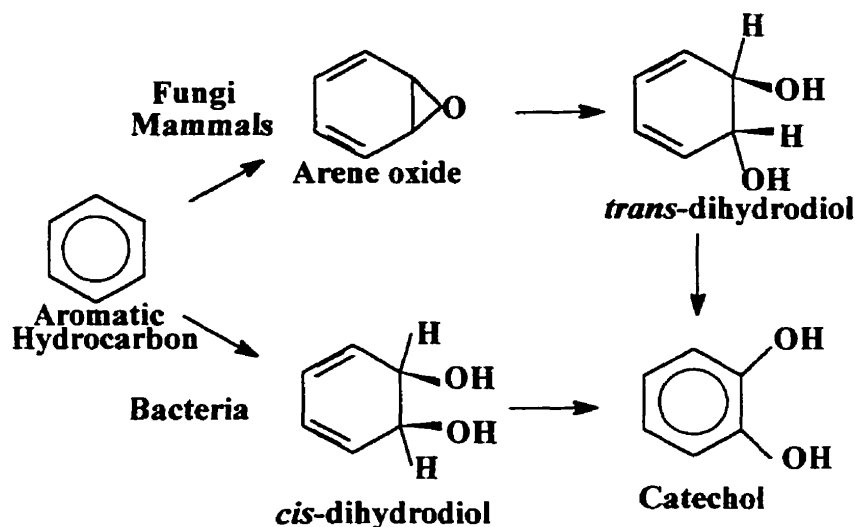
Figure 2.6 Oxidation of aromatic hydrocarbons by eukaryotic and prokaryotic organisms (Atlas and Bartha 1993).

2.2.6 Cometabolism

A second factor to consider in diesel fuel degradation is the cometabolism of compounds. In cometabolism, transformation of an organic molecule occurs without the organism utilizing the compound biosynthetically or as a source of energy (Stringfellow and Aitken 1995). There are two possible types of cometabolism. The first will occur only in the presence of a second organic substrate used for carbon and or energy. The compound being cometabolized is broken down due to its similar structural characteristics. The degradation enzymes are only induced when the second substrate is present resulting in the fortuitous break down of the compound of interest. The second process occurs in the absence of a structurally similar co-substrate. The degradative enzymes are constitutively produced or activated by the cometabolized compound. The compound is broken down to intermediates but no further. This may be due to suppression by pathway intermediates of the later enzymes in the pathway capable of further degradation and thereby preventing carbon and energy production. Alternatively, the degradative reaction may be catalyzed by

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enzymes capable of reaction with many substrates. These enzymes attack any closely related molecules producing end products that may or may not be utilized by the microorganisms. An example of this would be the production of lignin peroxidase. This enzyme initially oxidizes both PAHs and lignin to form products which may then be mineralized (Tatarko and Bumpus 1993). Diesel fuel contains a wide range of compounds that have the potential to be mineralized yielding energy to the organism, as well as those which may only be used co-metabolically. Examples of compounds that may be used metabolically or cometabolically include toluene, benzene, phenanthrene and cyclohexane (Tatarko and Bumpus 1993). Enzymes usually responsible for cometabolism have varying substrates that can be reacted upon (Brodkorb and Legge 1992). These enzymes are sensitive to the concentration of the compound because at one concentration cometabolism may occur and at another metabolism may dominate the system.
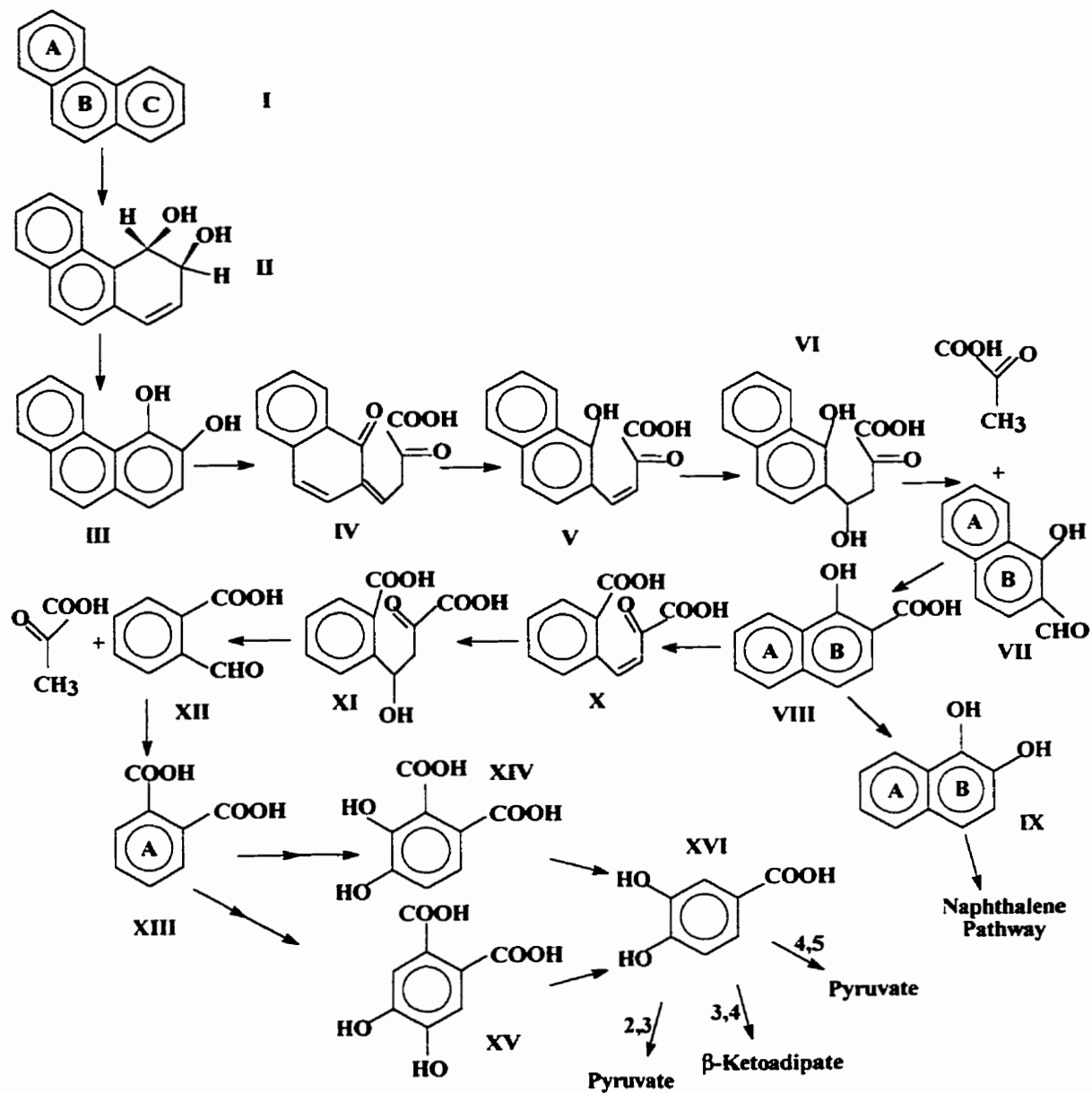
Figure 2.7 Degradation of phenanthrene to TCA cycle intermediates
(Ribbon and Eaton 1982).

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2.2.7 Microbial Populations

Many organisms have been shown to metabolize specific diesel compounds, but in some instances they may not be present in a given soil. A number of heterotrophic bacteria along with fungi all can have the ability to degrade diesel (Table 2.3). Kästner et al. (1994) have shown not all soils contain organisms capable of PAH degradation. In addition to having organisms capable of biodegrading these compounds, the genes encoding for these pathways must also be induced and expressed before degradation can occur. The organisms in question must also be competitive enough to survive in the soil environment (Brodkorb and Legge 1992).

Table 2.3 Heterotrophic bacteria and fungi capable of hydrocarbon degradation.

Organisms Capable of Hydrocarbon (Phenanthrene) Degradation	Reference
Pseudomonas stutzeri	Stringfellow and Aitken (1994)
Pseudomonas aeruginosa	Providenti et al. (1995)
Pseudomonas chrysosporium	Broadkorb and Legge (1992)
Pseudomonas sp.	Devare and Alexander (1995)
Aeromonas sp.	Kiyohara and Nagao (1978)
Alcaligenes sp.	Møller and Ingvorsen (1993)
Arthrobacter polychromogenes	Keuth and Rehm (1991)
Acidovorax delafieldii and Spingomonas paucimobilis	Shuttleworth and Cerniglia (1996)
Mycobacterium, and Rhodococcus	Kästner et al. (1994) and Bouldrin et al. (1993)
Canidia, Cryptococcus, Rhodotorula, Torulopis, and Trichosporon	MacGillivray and Shiaris (1993)

The metabolic diversity of microbial populations can be characterized by using a Biolog system to measure substrate intensity and substrate richness (Zak et al. 1994). The results characterize the metabolic diversity of the microflora inhabiting the soil matrix.

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The microbial populations were examined for their ability to use a diverse array of substrates indicating the enzymatic biodiversity of each soil tested.

2.3 Fate of Phenanthrene

The accumulation of phenanthrene and other polycyclic aromatic hydrocarbons in the environment cause a health risk due to carcinogenic and mutagenic properties (Keuth and Rehm 1991; Scribner 1973). Phenanthrene also has a high molecular weight (172.2 g/mol), log K_{ow} (4.53 at 26°C), and log K_{oc} (4.36) (Piatt et al. 1996; Kenaga and Goring 1980). Because of these chemical properties it will adsorb readily to organic matter and resist degradation. It is also a CCME priority pollutant when concentrations exceed 0.1, 5, and 50 ppm in agricultural, residential/parkland or commercial/industrial soil systems respectfully (Canadian Council of Ministers of the Environment 1991). This compound can be found in diesel fuel which is used around the world in automobiles, heavy equipment, hydroelectric power stations etc.. Phenanthrene can also found in crude oil, creosote, and gasoline. The popular use of diesel fuel sometimes leads to accidental release of this fluid to the environment. This pollutant is also toxic to microorganisms plants and animals found in and around diesel spill sites (Oesch et al. 1981; Barfknecht et al. 1981; Black et al. 1983).

2.3.1 Biological Transformation

Biological degradation of phenanthrene and diesel has been extensively documented throughout the literature (Stringfellow and Aitken 1995; Møller and Ingvorsen 1993; Kästner et al. 1994; MacGillivray and Shiaris 1993). Varying degrees of

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in situ mineralization of phenanthrene occurs due to the complexity of the molecule (Brodkorb and Legge 1992). Table 2.4 indicates some examples of phenanthrene degradation in soil. The degradation half-life of phenanthrene has also been characterized by many researchers (Table 2.5).

Table 2.4 Phenanthrene mineralization in the literature.

Reference	% Phenanthrene Mineralized	Rate of Mineralization (%/day)	Concentration of Phenanthrene	Previous Contamination of Hydrocarbons
Manilal and Alexander (1991)	50*	4.5[t]	10 mg/kg (Soil)	No
Manilal and Alexander (1991)	50*	12.5[t]	10 mg/L (Inorganic Salts Solution)	--
Møller and Ingvorsen. (1993)	12	0.3[t]	1 mg/kg (Soil)	No
Møller and Ingvorsen. (1993)	96*	10.7[t]	1 mg/kg (Soil)	No
Keuth and Rehm (1991)	47*	44.2[t]	150 mg/L (Mineral Salts Medium)	--
Brodkorb and Legge (1992)	19.5	0.9[t]	~15 mg/kg (Soil Slurry)	No
Brodkorb and Legge (1992)	37.7*	1.8[t]	~15 mg/kg (Soil Slurry)	No
Berry (1995)	6.2	0.01	1.41 mg/kg in 10,000 ppm Diesel (Soil)	No
Berry (1995)	7.2	0.075	0.708 mg/kg in 5000 ppm Diesel (Soil)	No

*Organisms were isolated from previously contaminated soil and added to the medium
[t] Calculated mineralization rate (% mineralized / length of experiment)

Table 2.5 Degradation half-life of phenanthrene in the environment (Howard et al. 1991).

Degradation	Half-Lives (days)
Soil	16 - 200
Air	0.0838 - 0.838
Surface Water	0.125 - 1.04
Ground Water	32 - 402
Aqueous Aerobic Biodegradation	16 - 200
Aqueous Anaerobic Biodegradation	2.7 - 799
Photolysis (Air)	0.125 - 1.04

The biodegradation of phenanthrene in diesel fuel is dependent on many factors in the soil. One such factor is hypothesized to be wetting and drying. Degradation of this molecule appears to be fairly slow in soil due to the high molecular weight and high K_{ow} properties (Boldrin et al. 1993). Wetting and drying may cause increased bioavailability, degradation, and activity by the soil microflora, thereby supporting enhanced mineralization of phenanthrene. The physical, chemical, and biological attenuation of the soil during varying moisture potentials may become an important factor in remediation of contaminated sites.

2.3.1.1 Effect of Moisture on Phenanthrene Degradation The first effect drying has on the soil environment is population control. As the moisture potential drops, bacteria are limited first before fungi, therefore lyse and supplement the environment with substrate (Sommers et al. 1981). If a white rot fungi such as *Phanerochaete chrysosporium* (Brodkorb and Legge 1992) survives the moisture stress, enhanced decomposition of phenanthrene may occur due to its polyfunctioning enzyme system. Many other bacteria including *Pseudomonas* have also shown resistance to low water potentials allowing them to flourish under these conditions (Stringfellow and Aitken 1995). The decrease in biomass when moisture becomes unavailable relieves the competition between organisms

and may in fact promote the degradation of phenanthrene. On the other hand if microbial populations capable of degrading phenanthrene are adapted to a continuous moist climate, then biodegradation will not be favored and phenanthrene will accumulate in the environment. Along with population selection comes cell death of organisms unable to survive the moisture stress. The cellular components released from the biomass provides substrate and energy to the system enhancing growth, secondary metabolism, and mineralization and cometabolism of hydrocarbons by surviving populations (Hartel and Alexander 1987).

Wetting and drying cycles have been well established in the literature (Lebedjantzev 1924; Birch 1958; Stevenson 1956; Shelton et al. 1995). Many of the researchers concluded varying moisture potentials increased the degradation of substrate in soil. Van Gestel et al. (1993), Sorensen (1974), and Bottner (1985) concluded wetting and drying increased the decomposition of added plant substrate compared to continuously moist or continuously dry. Amato et al. (1983) concluded continuously wet soil incubated with plant pods had 65.2% of the ^{14}C remaining in the soil while wetting and drying (3 weeks wet-1week dry) had only 48.1%. Taylor and Parkinson (1988) found similar results during leaf decay attributing some of the degradation to hydrolysis attack of cellulose during the drying treatment. They found the weight of aspen leaves decreased more during alternating wet-dry conditions (4.77g; wet 4 days, dried quickly and rewetted) compared to continuously moist controls (4.89g) after 4 months. Researchers including Van Veen et al. (1985) and Haider and Martin (1981) found wetting and drying had the same as or less degradation of glucose-ammonium sulfate (63%[wet 4 days-dry 10 days], 65%[wet] - 101 day experiment) and coniferyl alcohols (59%[dried and wetted at 6, 12,

18 months], 60%[wet] - 2 year experiment) respectfully. It appears the effects of moisture potentials may be soil specific and dependent on the natural conditions from which the microbial populations have evolved.

Aggregate breakdown is a physical process involved in release of non accessible organic matter, nutrients and possibly phenanthrene. Wetting and drying disrupts aggregates promoting this process. The net result is enhanced microbial activity and fertility of the soil due to the availability of substrate and nutrients (Van Gestel et al. 1991). Along with aggregates, clay lattices may also be subject to physical disruption releasing some substrates to enrich the environment (Birch 1958).

Finally, hydrolysis reactions occurring during dry periods may in fact enhance biodegradation. Jager and Bruins (1975) indicated when soil is heated, disruption of organic matter and other compounds occurs. The organic matter is now accessible to organisms allowing a flush of activity. This microbial activity may now mineralize or cometabolize phenanthrene present in the matrix.

The drying of soil can be one factor in determining the bioavailability of phenanthrene in the environment. Shelton et al. (1995) found the extraction efficiency of atrazine decrease up to 22% after drying and rewetting. This can affect bioavailability if the compounds are no longer accessible to the biomass. In this example wet-dry periods may in fact hinder the mineralization process and decrease overall degradation.

The literature has indicated both enhanced and decreased mineralization of compounds in soil when subjected to wetting and drying. As stated earlier, Sorensen (1974) incubated ^{14}C labeled plant material for up to two years prior to subjecting the soil to wet-dry intervals. After 284 days they concluded increased degradation of the organic

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material created from plant tissue under wet-dry conditions (30.1%) compared to continuously wet conditions (13.9%). Widrig and Manning (1995) monitored the biodegradation of diesel fuel in soil columns. They subjected the soils to a continuous supply (~50% degradation) and a periodic supply (wet-dry, ~70% degradation) of water and nutrients and found enhanced degradation under wet-dry conditions. Shelton et al. (1995) and Shelton and Parkin (1991) concluded increased adsorption of carbofuran and atrazine occurred during drying conditions. This corresponds to less bioavailability which in turn affects degradation. Though this trend may be important, the two pesticides have different physical, chemical and biological characteristics compared to plant material, which in turn have different properties compared to hydrocarbons. The effect wetting and drying may have on the degradation of phenanthrene may be compound. soil and microflora specific.

2.3.1.2 Effect of Nutrients on Phenanthrene Degradation The availability of nutrients such as nitrogen (amino acid synthesis), phosphorus (ATP), and sulfur (protein synthesis) may limit microbial proliferation in the environment. Other nutrients necessary for contaminant destruction include sodium, calcium, iron, and many other elements. If these factors are not present in the environment, degradation of phenanthrene and other hydrocarbons will not occur. Fertilizers are added to the soil to introduce nutrients that may be absent from the medium (Widrig and Manning 1995). In the case of hydrocarbon contamination, many researchers have used and demonstrated the need for the addition of nutrients (Phelps et al. 1994; Prado-Jatar et al. 1993). Most sub-soil systems are nutrient poor, therefore may decrease the rate of biodegradation. Manilal and Alexander (1991) have studied the properties that affect phenanthrene mineralization. They found that the

addition of phosphate increased the rate of mineralization (~49% degraded in 8 days compared to 12 days in unamended soil) while nitrate actually decreased net mineralization (~42% in 12 days compared to ~49% in unamended soil), therefore nutrients play a large role in the degradation of hydrocarbons in soil.

2.3.2 Adsorption

The retention of phenanthrene in a soil matrix is another property of the fate in the environment. A chemical can be no longer available for degradation, volatilization or transport once sorbed to organic and inorganic components in the soil. Texture affects the bioavailability of hydrocarbons in the environment. Adsorption of phenanthrene to soil particles may inhibit degradation thereby decreasing bioavailability. Heavy textured soils have greater surface areas and high CEC's compared to coarse soils which may increase adsorption and decrease bioavailability of some compounds. In the case of non polar organics such as phenanthrene, sorption due to surface area and charge will play a minor role in bioavailability (Tsomides et al. 1995). When phenanthrene is degraded to pathway intermediates, its polarity changes therefore sorption to negative clay surfaces may occur.

The high hydrophobic nature of phenanthrene makes it a candidate for sorption, thereby decreasing bioavailability. Once sorbed, the compound is usually inaccessible to microbial attack until desorption occurs (Edwards et al. 1994). Compounds such as polysaccharides, polypeptides, phenols, amino acids, aminosugars, fatty acids and carbohydrates exist in organic matter and can serve as substrates or building blocks in biosynthesis. Organic matter association has been characterized in the literature by two mechanisms. The first deals with the partitioning or diffusion of the molecule into the solid phase of organic matter. In other words the compound is distributed in the entire organic

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matter matrix. The second theory deals with compounds adsorbing to the outer surfaces of organic matter. The molecule is physically or chemically bound to the surface of the organic solid (Alexander 1994). Whatever the case, phenanthrene may be lost from the available system. Manilal and Alexander (1991) varied the amounts of organic matter in a soil and found the same soil with high organic matter (36.7%) had less degradation (42% in 20 days) compared to the soil with lower amounts (5.9% OM had 46% mineralization in 20 days). The data reflect the role sorption to organic matter plays in influencing bioavailability to microorganisms. Table 2.6 indicates some organic carbon adsorption coefficients related to compounds found in diesel.

Table 2.6 Organic carbon adsorption coefficients for some components of diesel (Kenaga and Goring 1980).

Compound	K_{ow}	K_{oc}
Benzene	135	83
Napthalene	2040	1300
Phenanthrene	32,900	23,000
Anthracene	22,000	26,000
Pyrene	800,000	650,000

Adsorption (sorption to the surface) and absorption (sorption within mass) properties are highly dependent on the soil matrix, charge and size of the chemical involved. Soils containing clay contribute to a large surface area compared to sands, which allows for enhanced sorption. Clay minerals can be negatively charged having Cation Exchange Capacities (CEC) where positively charged molecules may be associated on the exchange. The associated negative charge on the clay particles can vary from clay to clay. Soils containing 2:1 expanding clays (montmorillonite) will also have greater surface areas and potential for adsorption compared to nonexpanding 2:1 and 1:1 clays (Alexander, 1994).

When considering neutral organics and negatively charged pesticides the sorption is usually associated with the organic matter. Organic matter contains polyfunctioning groups which makes these molecules very reactive in sorption. The neutral, positive, and negative charged chemicals can thus interact with the multiple functioning groups in organic matter and form bonds retaining the chemicals in the soil. The solubility and hydrophobic nature of hydrocarbons affects the sorption kinetics and dynamics because a hydrocarbon "forced" out of solution will have a greater tendency to sorb than one in solution (Chiou 1989).

Desorption is considered to be another important parameter in determining the fate of phenanthrene in the environment. The kinetics of desorption are usually slower than the sorption process which indicates a slow release mechanism or hysteresis (Green and Karickhoff 1990). Carmichael et al. (1997) determined the sorption of phenanthrene in a soil to be 100 μg L^{-1} h^{-1} while the desorption was 13.8 μg L^{-1} h^{-1}. With the desorptive process occurring, there will be a potential for mineralization and loss of the hydrocarbon from the soil system via movement and transport. Important sorptive characteristics of a chemical can be measured via the determination of sorptive constants such as K_p and K_{oc}. These constants will change depending on charge, K_{ow}, clay content, organic carbon etc. of the molecules and soil matrix. K_p is the soil partitioning coefficient with units of L kg^{-1} and is defined as the ratio of the concentration of sorbed chemical (C_s) to the concentration in solution (C_w). The K_{oc} is known as the organic carbon partitioning coefficient and is used because of the importance of organic carbon in neutral organics sorption (Green and Karickhoff 1990; Karickhoff 1981).

$$K_p = C_s/C_w \qquad K_{oc} = K_p / f_{oc} \qquad K_{oc} = 0.41*K_{ow}$$

<div align="right">**Equation 2.1**</div>

f_{oc} = fraction of organic carbon in soil
K_{ow} = octanol-water partitioning coefficient

With the above equations it is evident that the greater the organic carbon content and hydrophobicity of the molecule, the greater the sorption will be. In the case of phenanthrene the adsorption may be the greatest fate in the majority of the soils in the environment.

2.3.3 Humification

The humification and decreased bioavailability of organic molecules can result due to irreversible chemical bond formation. Humification is different from sorption because the association is usually irreversible due to the change in structure and nature of the compound. The interactions between organic matter and hydrocarbons tend to be stable with covalent bonds resulting in the formation of humus. The humus structure is very complex and cannot be defined due to great variability. The composition of humus includes carbohydrates, amino acids, proteins, aliphatic fatty acids, alkanes, lignins and aromatics (Paul and Clark 1989). The formation of this soil constituent is microbial mediated resulting in the partial decomposition of organic residues added to the soil (plant material, microbial biomass etc.). The aromatic structure of humus is similar to phenanthrene and other hydrocarbons found in diesel therefore these compounds may be a candidate for humification (Bollag and Loll 1983). The non polar constituents react with each other creating chemical bonds. These can be either electrostatic bonding, hydrophobic bonding or charge-transfer complexing (Bollag and Loll 1983). Researchers have also found that the degradation of aromatics to phenols can also lead to humification.

Phenols can polymerize via oxidative coupling to form a repeated structure which in turn may bind to organic matter (Bollag and Loll 1983) creating humus.

2.3.4 Volatilization

The loss of contaminant to the atmosphere in a soil system is usually low due to the high adsorption rate of hydrocarbons to the soil matrix and low vapour pressure of compounds (Table 2.7) (Carmichael et al. 1997).

Table 2.7 Vapour pressures of some significant components of diesel fuel. (Mackay and Shiu 1981)

Compound	Vapour Pressure (kPa)
n-Hexane	20.2
Benzene	12.7
Toluene	3.8
Napthalene	1×10^{-2}
Phenanthrene	2.67×10^{-5}
Anthracene	1.44×10^{-6}
Octadecane	7.44×10^{-6}
Pyrene	8.86×10^{-7}
3,4-Benzopyrene	6.67×10^{-13}

During an initial spill there may be significant loss of contaminant as the diesel pools on top of the soil. Over time, the contaminant usually enters the soil system where the volatilization potential decreases (Norris et al. 1994). In the end volatilization can contribute to a small fraction of the fate of diesel fuel in the environment.

2.4 Current State of Literature

We know from the literature:

- Phenanthrene can be mineralized, volatilized, transported, and sorbed in soil.
- Phenanthrene and diesel fuel can be degraded in the environment.
- Phenanthrene may have a large potential for sorption.
- Soils are diverse.
- Surface environments are much different from the subsurface.
- Moisture potentials affect microbial populations and activity.
- Phenanthrene can be used to represent the fraction of PAH's found in diesel with similar properties.

But we don't know:

- Do all soils have the same potential for phenanthrene mineralization?
- What properties can we use to predict phenanthrene mineralization in soil?
    - Physical, chemical and biological...
- Will previous exposure of hydrocarbons affect mineralization?
- Will landscape position influence the degradation of phenanthrene?
- Is there a difference between the mineralization of phenanthrene in microcosms and intact soil columns?
- What effect, if any, will soil wetting and drying have on phenanthrene mineralization?

CHAPTER 3

Survey of the Remediation Capacity of Phenanthrene in Diesel Fuel Contaminated Soil

3.1 Abstract

In order to interpret the relationship between the physical, chemical, and biological properties of soils and the rate of biodegradation of phenanthrene, a component of diesel fuel, a survey across a range of Manitoba soils was undertaken. The organic matter content, texture and climate of the soils varied dramatically. Samples from the surface (0-10 cm) and subsurface (90-100 cm) were collected to compare the rates of degradation. Mineralization rates were determined in microcosms incubated with field moist soil from each site. Radiolabeled phenanthrene was added as a component of diesel fuel in order to assess the mineralization potential of that compound. The fate of phenanthrene along with the metabolic diversity of the microbial populations was determined for each soil type. Phenanthrene mineralization in freshly sampled soil without prior hydrocarbon exposure occurred indicating the potential for *in situ* mineralization. The majority of the sites had mineralization less than 5% of the total added phenanthrene after 259 days. When considering the sites with previous hydrocarbon exposure, only one of the two had significant mineralization (>45%). This indicates prior exposure did not ensure rapid mineralization, other factors must limit the mineralization process. Surface soils did not

consistently have greater mineralization than the subsurface, though, when mineralization was above 5%, the surface soils had greater mineralization rate. The major fate of phenanthrene in soil was sorption and decreased bioavailability. Texture, organic carbon, and microbial metabolic diversity did not have consistent influences on the total mineralization of phenanthrene in uncontaminated soils. Organic carbon and microbial metabolic diversity did have an effect on the phenanthrene mineralization rate.

3.2 Introduction

Diesel fuel spills are of concern to many industrial agencies due to toxic effects on the environment. When they do occur two remedial options may be used, either excavation followed by treatment or treatment *in situ* (Prado-Jatar et al. 1993; Atlas and Cerniglia 1995). The relative costs and degree of site disturbance of these two approaches vary dramatically. The decision as to the most appropriate method depends upon an understanding of the potential threat to surrounding environments. McGill (1977) and Atlas and Cerniglia (1995) have reported that if certain environmental properties are present, then the *in situ* remediation of contaminated land can occur without a threat to its surroundings. The potential for transport of the contaminant off site or into more sensitive components of the ecosystem may limit the ability to treat the contaminant *in situ*. Soil texture, organic matter content, and the presence of hydrocarbon degraders affect the degradation and transport of contaminants in the environment (Rainwater et al. 1993; Phelps et al. 1994). Texture, through its impact on pore size distribution, can affect oxygen flow to the microorganisms, contaminant adsorption, water availability, and

presence of nutrients (Hillel 1982: Karickhoff and Morris 1985). Organic matter also affects adsorption of hydrocarbons, presence of nutrients, water availability and aggregate formation (Smith et al. 1993). The presence of hydrocarbon degraders and the relative bioavailability of diesel fuel can also affect the potential for mineralization (Edwards et al. 1994). The ability to predict the fate of diesel fuel, based on fundamental soil properties, would allow estimation of potential risk of impact prior to site investigation.

3.3 Objective of Study

The objective of this study was to determine the presence of phenanthrene degrading organisms and to examine the mineralization of this compound contained in diesel fuel, in a range of soils varying in texture, organic material, and climatic locations in Manitoba.

3.4 Materials and Methods

3.4.1 Soils

Soils selected from across the province and their characteristics are listed in Tables 3.1, 3.2, and 3.3. The organic carbon and particle size analysis was performed by Manitoba Soil Survey (Winnipeg, MB) according to the method of Haluschak (1986). The soils selected for the study represent a wide range of organic matter, texture and climatic conditions (Figure 3.1). Sites # 8 and #10 had prior hydrocarbon exposure in the last 5 years as a result of diesel fuel and crude oil spills respectfully. Site #8 was the site a

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of a long term, leaking, below ground diesel storage tank located near Bakers Narrows Manitoba. Site #10 was located south of Somerset Manitoba and was the site of a crude oil pipeline rupture in 1994. Sampling of the soil was conducted in late July (1996) using Dutch Augers. Soil was collected at intervals of 0-10 cm and 90-100 cm. Replicates were completed in quadruplicate and stored in air tight plastic bags in the dark at 4°C until the beginning of the experiment.

3.4.2 Microcosms

Microcosms were purchased from Richards Packaging (Winnipeg, MB) as 500 mL glass jars with sealed metal lids. To the internal base of each microcosm a 3 cm length of polyvinylchloride (PVC) was attached using silicone to hold a 7 mL scintillation vial for $^{14}CO_2$ trapping. A polyurethane foam plug (PUF) was added to trap any volatile phenanthrene and a 20 mL scintillation vial containing 10 mL water (pH 3) was included to humidify the air. The lid of the microcosm also contained a sampling port (Rubber Suba Seal #9) to allow the sampling of gas. At various times a sample of the air inside the microcosm was analyzed by GC to determine whether the vessel was aerobic. The field moist soil equivalent to 40 g oven dry mass was added to 50 mL glass beakers then brought to field capacity. The beaker was inserted into a microcosm and incubated at 20 ± 2°C for one week to allow equilibration of the microbial biomass.

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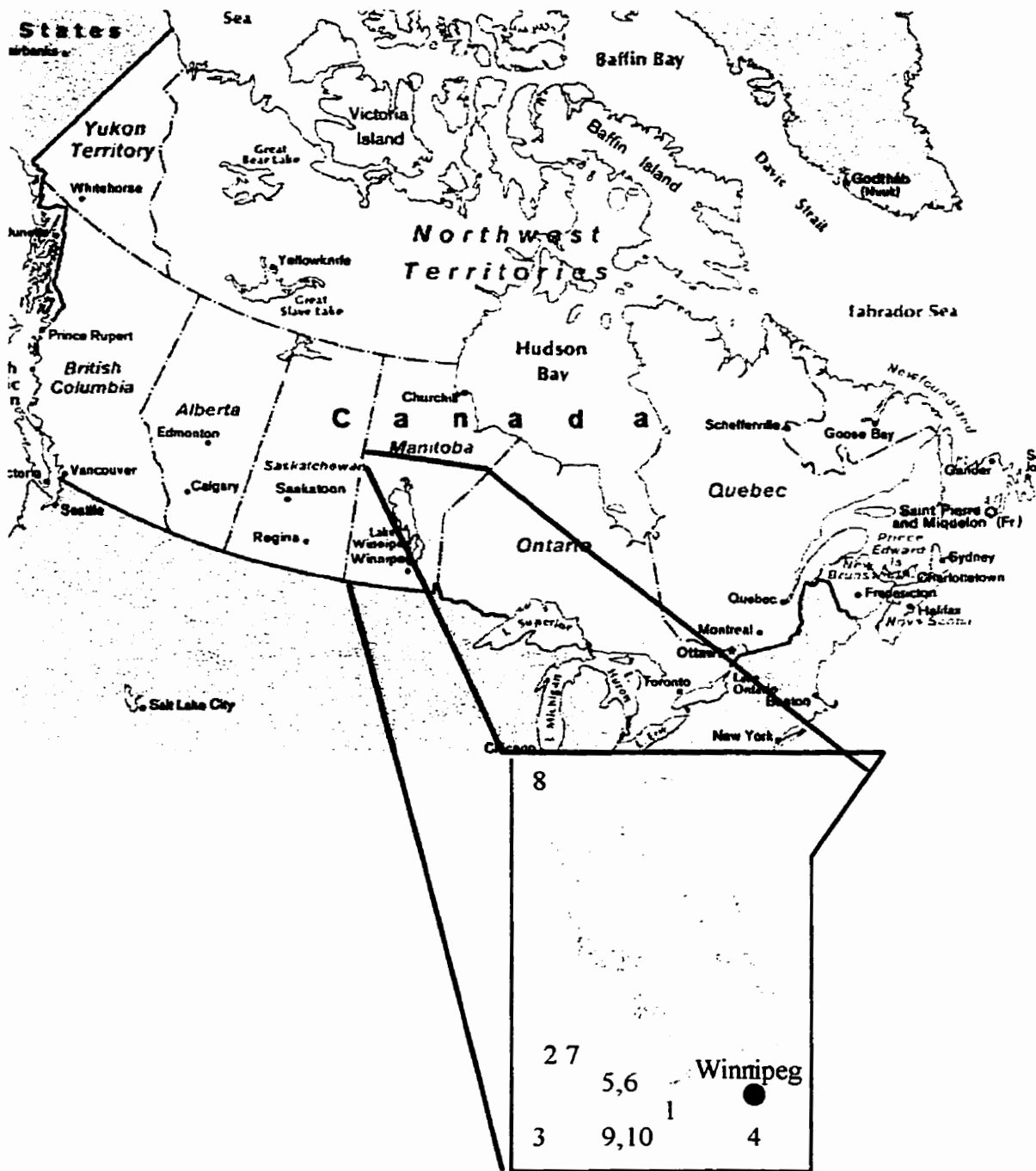
Figure 3.1 Soil selected for the mineralization survey across the province of Manitoba
(refer to Site numbers in Table 3.1).

Table 3.1 Site classifications for remediation study.

Site	Classification	Site Location	Site Location
Site #1	Calcareous Black	South facing catena sampled at mid-slope	5 km East of St. Claude Manitoba (SE 13-8-8W)
Site #2 *	Orthic Dark Grey	South facing catena sampled at mid-slope	10 km North of Rossburn Manitoba (NW 14-19-23W)
Site #3	Orthic Black	South facing catena sampled at mid-slope	3 km West of Melita Manitoba (SE 22-4-29W)
Site #4	Gleyed Rego Black	Sampled on moderate incline slope	5 km South of Glenlea Manitoba (SW 18-8-3E)
Site #5 ^	Orthic Black	South facing catena sampled at mid-slope	10 km North of Brandon Manitoba (NE 31-12-18W)
Site #6	Humic Luvic Gleysol	Sampled in a depression area	10 km North of Brandon Manitoba (NE 31-12-18W)
Site #7	Orthic Grey Luvisol	South facing catena sampled at mid-slope	10 km North of Oakburn Manitoba (SE 4-20-23W)
Site #8	Gravel Fill	Diesel storage tank leak	Bakers Narrows Manitoba (5-66-28W)
Site #9	Gleyed Rego Black	Control for Site #10	5 km South of Somerset Manitoba (SW 32-4-9W)
Site #10	Gleyed Rego Black	Crude oil spill in 1994 sampled in a depression area	5 km South of Somerset Manitoba (SW 32-4-9W)

* Site used in Chapter 5

^ Site used in Chapter 4

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Table 3.2 Particle size analysis of sites selected for remediation survey.

Site	% Sand	% Silt	% Clay	Class	% Organic Carbon	Field Capacity (%)
Site #1 0-10 cm	87	4	9	Sand	2.1 ± 0.3	25.3 ± 2.1
90-100 cm	91	2.5	6.5	Sand	0.1 ± 0.1	11.3 ± 1.5
Site #2 0-10 cm	43.5	29	27.5	Clay Loam	2.5 ± 0.2	26.8 ± 4.1
90-100 cm	72	14.5	13.5	Sandy Loam	0.1 ± 0.1	18.6 ± 8.6
Site #3 0-10 cm	45	28.5	26.5	Loam	3.2 ± 0.1	28.5 ± 2.3
90-100 cm	39	36.3	24.7	Loam	0.2 ± 0.01	24.8 ± 1.7
Site #4 0-10 cm	4	19	77	Heavy Clay	3.7 ± 0.1	42.2 ± 1.7
90-100 cm	1	16	83	Heavy Clay	0.9 ± 0.1	38.3 ± 7.7
Site #5 0-10 cm	28.5	33.5	38	Clay Loam	5.7 ± 0.1	34.4 ± 2.1
90-100 cm	32.5	34.5	33	Clay Loam	0.6 ± 0.3	21.9 ± 1.4
Site #6 0-10 cm	31.5	35.5	33	Clay Loam	4.7 ± 0.3	30.7 ± 6.4
90-100 cm	34.5	30.5	35	Clay Loam	0.4 ± 0.2	29.4 ± 1.3
Site #7 0-10 cm	34.5	33.5	32	Clay Loam	1.1 ± 0.01	27.5 ± 3.6
90-100 cm	33	35.5	31.5	Clay Loam	0.3 ± 0.02	22.8 ± 3.7
Site #8 0-1 m *	91	8	1	Sand	0.3 ± 0.1	4.4 ± 2.1
1-2 m *	95	4.5	0.5	Sand	0.3 ± 0.02	27.2 ± 2.2
Site #9 0-10 cm	13	44	43	Silty Clay	4.9 ± 1.5	34.0 ± 2.1
90-100 cm	47.5	32.5	20	Loam	0.3 ± 0.02	24.7 ± 4.8
Site #10 0-10 cm *	16.3	44	39.7	Silty Clay	9.3 ± 0.3	42.5 ± 3.2
90-100 cm *	32.5	42	25.5	Loam	0.7 ± 0.3	29.8 ± 3.6

* Prior sensitization of hydrocarbons on site

Table 3.3 Particle size analysis of sites with sandy soil fractions.

Site	Total Sand	Very Coarse Sand	Coarse Sand	Medium Sand	Fine Sand	Very Fine Sand
Site #1 0-10 cm	87	0	0	3.3	66.3	17.3
Site #1 90-100 cm	91	0	0	2.5	68.5	20
Site #2 90-100 cm	72	26	18	13	9.5	5.5
Site #8 0-1 m	91	34.3	16	13	16	11.7
Site #8 1-2 m	95	0	0	11	66.5	17.5

3.4.3 ^{14}C Phenanthrene and Diesel Fuel

^{14}C phenanthrene was purchased from the Sigma Chemical Co. (St. Louis, MO) as phenanthrene-9-^{14}C (8.3 nCi/mmol, Radio-chemical purity >98%) (Figure 3.2). Stock solutions were first made up in hexane then transferred to diesel fuel #2 for addition to soil. At the beginning of each experiment 5000 ppm (5000 μg/g soil) of diesel-^{14}C phenanthrene mixture was added containing 0.05 μCi of ^{14}C phenanthrene. Stock diesel fuel added contained about 0.7059μg unlabelled and 0.0586μg of labeled phenanthrene per gram of soil.
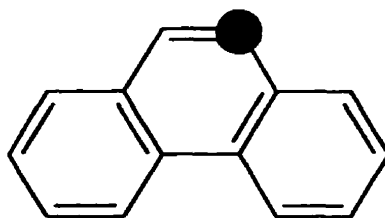
Figure 3.2 ^{14}C Phenanthrene added to soil microcosms.

3.4.4 Volatilization

When the experiment was completed, the polyurethane foam plugs were removed from the microcosms and placed in 100ml French Square Bottles. To these bottles 20ml methanol was added, making sure 5ml was in excess, for total extraction of the phenanthrene. Bottles were then sealed and shaken on a lateral shaker for 2 minutes. The

foam plugs were then removed and placed in a 50ml syringe to extract all of the methanol. The procedure was repeated to ensure the total extraction of phenanthrene. From the extract, a 1 mL subsample was collected and counted by the scintillation counter.

3.4.5 $^{14}CO_2$ Traps

0.5 mL (+/-)-α-phenylethylamine and 0.5 mL methanol were added to a 7 mL scintillation vial in order to determine the amounts of $^{14}CO_2$ evolved from the soil (the methanol prevents crystallization). During the experiment, traps were changed at weekly intervals until there was no longer any significant radioactivity recovered (<0.1%). At the end of the experiment 5 g of soil was removed and combined with 20 mL 1M HCl in a 50 mL beaker. The beaker was then placed in a separate microcosm to determine ^{14}C associated with soil carbonates and dissolved in soil water. A 1 mL phenylethylamine-methanol CO_2 trap was included to trap any residual ^{14}C remaining in the soil . The trap was then counted in the scintillation counter and added on to the overall mineralization recoveries.

The liberation of ^{14}C as CO_2 would probably occur at the mid point of the degradation pathway (Refer to Figure 2.7 in Chapter 2). The degradation begins at one of the two "outside" aromatic rings and proceeds to the 9th carbon in the molecule at either the 12th step or at the completion on the degradation pathway (Ribbon and Eaton 1982). Assuming organisms follow this pathway, the placement of the ^{14}C at this position ensures there must be sufficient degradation to at least a phenyl group or to complete degradation.

3.4.6 Liquid Scintillation Counting

Each 1 mL CO_2 trap removed from the microcosm was combined with 5 mL Ecolite (+) Liquid Scintillation Fluid (ICN Biochemicals Inc. Aurora, OH). The cocktail

was then allowed to equilibrate in the dark for 24 hours before counting to prevent any erroneous readings from ion interaction with the scintillation fluid. A Beckman LS 7500 scintillation counter was used with a quench curve correction to give final results of disintegrations per minute (DPM). Final DPMs were corrected for background and blanks then related to the original radioactivity added to each microcosm to give the percent mineralization of phenanthrene in diesel.

3.4.7 Kinetic Analysis of $^{14}CO_2$ Evolution

A first order rate model was used to determine the rate of mineralization of phenanthrene (Knaebel et al. 1994). The calculation describes the reaction over time as a single first order component where P is the percent phenanthrene mineralized at time t. A is the percent of compound evolved as CO_2 at t = ∞, and k is the rate constant for $^{14}CO_2$ evolution (day^{-1}).

$$P = A[1-e^{(-kt)}]$$

Equation 3.1

The constants were estimated using a non-linear least squares procedure (Figure 3.3). Parameters were estimated for each replicate and an analysis of variance was performed on the resulting data set. Once the rate constant was determined a mineralization half life can be calculated using the following equation:

$$t_{1/2} = \ln2 / k$$

Equation 3.2

Quadruplicate reps were modeled, then an average of the curve fitting constants were determined including a standard deviation.

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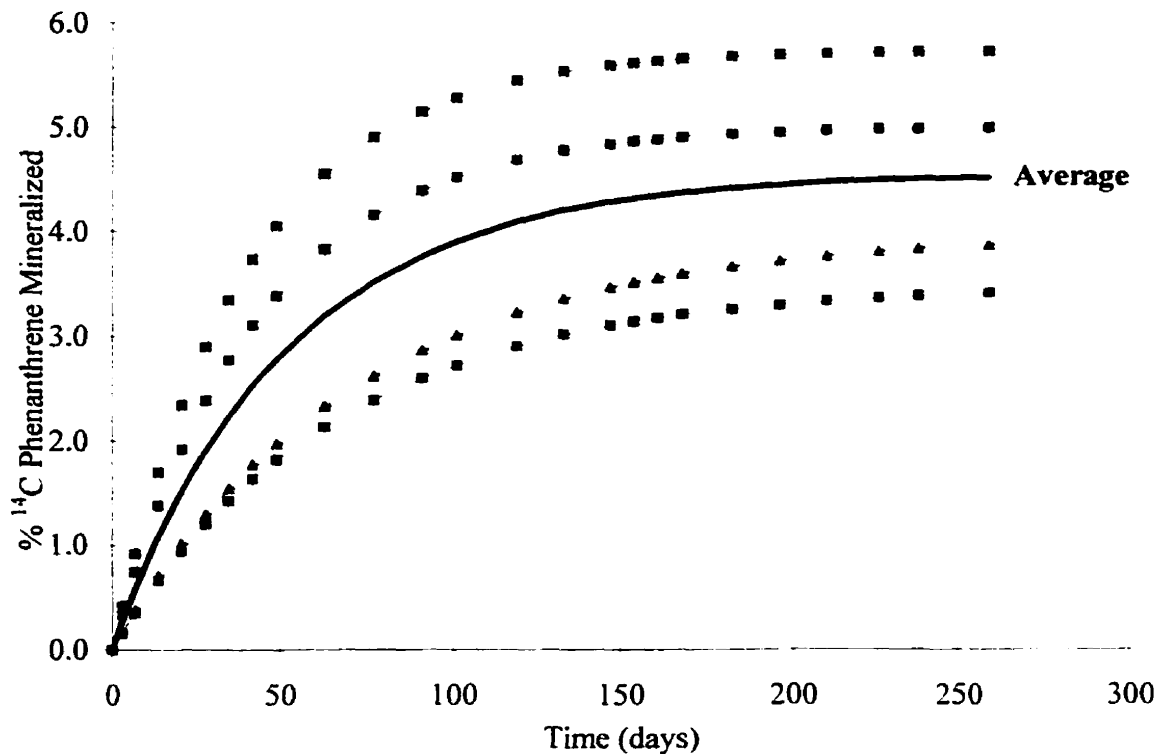
Figure 3.3 Example model data for the mineralization of ^{14}C phenanthrene over time. Quadruplicate reps were each modeled then an average and standard deviation were calculated.

3.4.8 Sequential extraction of ^{14}C from soil

Residual ^{14}C was extracted to determine the properties of the remaining fraction after 259 days of incubation. A step-wise extraction using water, methanol, and methylene chloride followed by total digestion of the organic carbon was used to remove any radioactivity in the soil. The water extractable ^{14}C would indicate the possible free phenanthrene and degradation products in soil. These fractions would then be considered in the mobile phase. The methanol extract would contain the weakly associated phenanthrene and degradation products sorbed to soil. This fraction would be considered no longer bioavailable and have some potential for desorption. The soxhlet extractable fraction would contain the highly sorbed and stable ^{14}C. Wet digestible ^{14}C would be

considered residual and may be in the humified fraction in the soil. At the end of the experiment, 5g of oven dry equivalent soil was removed from the microcosms and placed in 50 mL acid washed Teflon tubes. Following an adsorption method proposed by Knaebel et al. (1994), to this tube 25 mL of 10 mM NaN_3 (5:1 ratio of soil to liquid) was added along with 5 glass beads to aid in agitation. The tubes were then vortexed for 10 seconds and placed on a lateral shaker for 24 hours. The next day the tubes were centrifuged at 12,000 xg for 15 minutes then the supernatant was removed and a 1 mL subsample was analyzed in a scintillation counter. The remaining pellet was then combined with 25 mL of methanol, and subjected to the same procedure as the above. After pouring off the methanol, the pellet was allowed to air dry to aid in the transfer of the soil into cellulose extraction thimbles (EPA Method 3540A). The thimble containing soil was placed in a soxhlet extraction apparatus and extracted for 24 hours with 100 mL methylene chloride and 3 boiling chips. After the 24 hour extraction, the solvent was removed from the extraction vessel and a 1 mL subsample was counted in a liquid scintillation counter. Again the soil was allowed to air dry to aid in transfer of 0.8 g of soil into a wet digestion tube (Voroney et al. 1991). To this tube 6 mL of digestion solution (189.4 g CrO_3 in 250 mL 14.7M H_3PO_4 and 500 mL 18M H_2SO_4) was added to the soil along with a glass rod stand to hold 2 mL of 2M NaOH in a 6 mL glass scintillation vial (NaOH traps $^{14}CO_2$ liberated from oxidation of organic carbon). The tube was then sealed with a #49 Rubber Suba Seal and placed in a digestion block and heated at 145°C for 1 hour. The $^{14}CO_2$ was then trapped for 24 hours after which a 0.3 mL subsample was removed and combined with scintillation fluid and analyzed for radioactivity. The above

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procedure was also run on a control soil (in triplicate) using a spiked sample of ^{14}C phenanthrene to determine the efficiency of extraction.

In order to mathematically calculate the adsorption coefficient (K_p), the method uses the octanol-water partitioning coefficient (K_{ow}) of phenanthrene, the organic carbon partitioning coefficient (K_{oc}), and the fraction of organic carbon in the soils (f_{oc}). K_p has units of L kg^{-1} and is defined as the ratio of the concentration of sorbed chemical (C_s) to the concentration in solution (C_w). (Green and Karickhoff 1990; Karickhoff 1981).

$$K_p = C_s/C_w$$
$$= K_{oc} * f_{oc}$$
$$K_{oc} = 0.41 * K_{ow}$$

Equation 3.3

$K_{ow} = 32900$ (Kenaga and Goring 1980) $K_{oc} = 0.41*32900 = 13489$

3.4.9 Biolog Test for Metabolic Diversity

Gram Negative Biolog plates containing 95 sole carbon sources were purchased from Biolog Inc. (Hayward, CA). Each plate has 96 wells, 1 control with no carbon source and 95 wells having different carbon sources. Along with the carbon source a dried mixture of peptone, nutrients, salts, and redox tetrazolium dye are included to ensure growth of organisms and provide an indicator for metabolism. Wells, after inoculation, were colorless until metabolic activity reduced the tetrazolium dye to a purple color indicating a positive test (Zak et al. 1994). Physiological saline (0.85%, 8.5g NaCl in 1000 mL distilled water) and water agar (0.2%, 2g purified agar in 1000 mL distilled water) were prepared and dispensed into 100 mL milk dilution bottles. Bottle 1 contained 90 mL of 0.2% water agar solution and 10-5 mm glass beads while Bottle 2 contained 99 mL of 0.85% saline. All bottles were then autoclaved. Once the cycle was complete, water agar

bottles were cooled in a water bath and shaken on a lateral shaker to prevent solidification of the agar. 10 g of oven dry equivalent soil, stored at 4°C since sampling (quadruplicate reps of surface and subsurface soils - 160 total), was added to Bottle 1 and shaken on a lateral shaker for 30 minutes. A 1 mL subsample of this solution was then transferred to Bottle 2 (10^{-3} dilution) and shaken. Final dilutions were then poured into sterile plastic reagent reservoirs (Eppendorf, Inc.) while inside a sterile hood (LABCONCO® Purifier™ Clean Bench). Soil solutions were dispensed into the 96 well plates in 100μL volumes using a multichannel pipette (Eppendorf 8-channel Repeater™ Pipette) and incubated at 25°C. At intervals of 25, 48, 72, and 96 hours plates were scored for a change in the tetrazolium dye color and assigned a ranking value (Table 3.4). Values ranged from 1 to 4 with 1 signifying a color change after 24 hours and 4 indicating a color change after 96 hours. Data was summarized with respect to intensity and substrate richness. Average intensity, the average time it takes to get a positive result, indicates the activity and density of the population. Substrate richness represents the percentage of substrates metabolized over the 96 hour test and is an indicator of the functional diversity or metabolic capability of the microbial community.

Table 3.4 Arbitrary values assigned for color change in biolog plates

Time (hours)	Intensity Code
25	4
48	3
72	2
96	1
No Reaction	null value

3.4.10 Drying of Soil and Further Addition of ^{14}C Phenanthrene

The objective of this treatment was to determine the effect of drying and grinding of the soil on further phenanthrene mineralization. The addition of another spike of a phenanthrene-diesel fuel mixture was to determine if the microbial populations could again mineralize phenanthrene. At the completion of the sequential extraction and initial mineralization experiments (259 days of incubation), the remaining soil (~30 g oven dry equivalent) was divided into two beakers. One set was placed in microcosms and incubated at 20°C while the other set was dried in an oven for 2 days at 60°C, ground, then inserted into individual microcosms. Each microcosm also contained a 5 mL vial containing 1 mL $^{14}CO_2$ trapping solution (1:1 methanol to phenylethylamine) and a vial containing 10 mL water (pH 3) to humidify the air. After about 24 hours all soils were rewetted to field capacity and incubated at 20 $\pm$ 2°C to determine if there was further degradation of the added phenanthrene. After about 35 days (295 days total), 5000 ppm (5000 µg/g soil) of diesel-^{14}C Phenanthrene mixture was added with approximately 0.05 µCi of activity to each microcosm. The production of $^{14}CO_2$ was monitored to determine further mineralization of the fresh spike of phenanthrene (total incubation of soil was 343 days).

3.4.11 Statistical Analysis

Single factor ANOVAs and Duncan's New Multiple Range Tests were conducted using SuperANOVA (Abacus Concepts Inc., Berkley, CA). Treatments included site, depth and site versus depth properties at the 5% significance level. First order curve fitting of the mineralization data was performed using JMPIN (SAS Institute Inc., Cary, NC).

3.5 Results And Discussion

3.5.1 Phenanthrene Fate in Non Hydrocarbon Exposure Sites

The mineralization of radiolabeled phenanthrene in diesel fuel was monitored in soils sampled across the province of Manitoba. The previously uncontaminated soils represented a range of physical (texture, organic carbon), chemical, and biological (metabolic diversity) properties allowing the determination of the mineralization capacity. The soil microcosms allowed the simultaneous study of mineralization, volatilization and adsorption throughout a 259 day experiment.

3.5.1.1 Phenanthrene Volatilization Based on the low vapour pressure (0.113 Pa at 25°C) and high log K_{ow} (4.53 at 26°C) (Piatt et al. 1996), the volatility of phenanthrene in soil was considered to be a minor fate. The high K_{ow} correlates to a high partitioning rate into the organic carbon of the soil where it is absorbed thereby preventing other fates such as volatilization (Piatt et al. 1996). Limited transfer of phenanthrene was observed in the traps, consistent with a low vapour pressure of this compound (Table 3.5 and Figure 3.4). In Figure 3.4, volatilization appears to be higher in the subsurface compared to the surface. This may indicate increased volatilization in the parent material due to decreased organic matter or some other unique factor. There was not a significant correlation between soil texture and volatilization. Organic carbon, on the other hand, shows a weak relationship to volatilization (Figure 3.5). This occurrence may be due to the fact that organic matter is usually the major factor controlling adsorption (Carmichael et al. 1997). When organic matter increases in the surface so does the potential for adsorption of organic compounds to the soil matrix (Green and Karickhoff 1990).

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3.5.1.2 Phenanthrene Mineralization The results indicated there were microbial populations present mineralizing phenanthrene but the production of $^{14}CO_2$ did not vary greatly between the freshly sampled soils (Table 3.5. Figure 3.4). The greatest mineralization resulted in Site #4 (Heavy Clay) at the 0-10 cm depth. The least amount of mineralization was found in the surface of the Orthic Dark Grey soil (Site #2). The interaction between total mineralization in the surface and subsurface was not consistent (Table 3.6). There was only a slight treatment effect between sites with one site (Site #4) being statistically different. This result is interesting because it indicates all soils had a similar potential for total mineralization in the environment. There appeared to be no significant differences in mineralization of phenanthrene between the surface and subsurface sites but there was an interaction between site and depth indicating not all sites behaved the same way. In the examples where mineralization was higher than 5%, the surface had greater mineralization than the subsurface. In most cases the rate of mineralization was less that 5% of the added phenanthrene in diesel fuel. Where large amounts of mineralization was observed, the results were often inconsistent having standard deviations as large as the mean (Site #4 and #9). The mineralization rates in these soils were higher than the soils with less than 5% mineralized phenanthrene. This indicates Site #4 and Site #9 microorganisms were able to access the phenanthrene before it became no longer bioavailable through sorption. The mineralization rates in the surface were significantly different than the subsurface (Table 3.6). This indicates there will be greater active microbial mineralization of the phenanthrene before it becomes less bioavailable due to organic matter. The mineralization rates also indicate there will be little mineralization differences between sites in the province. The inconsistency of elevated mineralization in

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freshly sampled soil indicates that the occurrence of populations capable of enhanced phenanthrene mineralization may be relatively sporadic. It is unknown whether the nature of these soils to degrade phenanthrene is due to microbial populations or due to conditions in the soil conducive to mineralization (bioavailability, nutrients etc.). The mineralization half lives observed in this work are comparable to those reported for the aerobic mineralization of 16-200 days reported by Howard et al. (1991). Shiaris (1989) also demonstrated slow degradation (<5%) of the phenanthrene molecule in the absence of diesel fuel in 5 soils analyzed. The half life calculation in the present study is for the mineralized fraction. It appears mineralization is relatively quick but not much is degraded. The low amounts of degradation could be due to degradation intermediate accumulation or bioavailability.

Mineralization occurred in the soils examined, therefore indicating a potential for mineralization in the environment. The results indicate if a spill occurs at anyone of these sites there will be a consortium of microorganisms capable of remediation. The findings support *in situ* bioremediation because organisms are present, but cause some concern with the amounts of labeled phenanthrene mineralized (<16%). It appears bioavailability may be limiting further mineralization of phenanthrene. The organisms initially mineralized the phenanthrene rapidly but the mineralization soon decreased over time (Appendix). This process indicates the phenanthrene is being sorbed and becomes no longer bioavailable. It may then be prudent to look at ways of enhancing the already present microorganisms in the environment by decreasing sorption.

Clay content is an important characteristic of the degradative environment providing extensive surfaces for the stabilization and/or reaction of organic compounds

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(Devare and Alexander 1995). The surface of the Gleyed Rego Black (Site #4) had the greatest mineralization and had the greatest clay content. The sub-surface of this site had a relatively low rate of mineralization despite having a clay content greater than 80%. Thus. clay content alone cannot account for differences in mineralization. Fuller et al. (1995) also concluded texture alone had no significant relationship with the mineralization of toluene and trichloroethylene in vadose sediments. There appeared to be no relationship between mineralization and clay content for a) all sites and b) subsurface sites (Figure 3.6 and 3.8) There was a correlation between mineralization and clay content in the surface. but this relationship may be based on one or two points (Figure 3.7). It was then believed organic carbon may play a role in mineralization. When looking at the effect organic carbon had on mineralization (Figure 3.9. 3.10. and 3.11). we see that it had very little influence on mineralization in a) all soils. b) surface soils and c) subsurface soils investigated. Manilal and Alexander (1991) found that peat soils high in organic matter (37%) had less mineralization (~42% degraded in 20 days) than soils with less (5.9% organic matter. 46% degraded in 20 days). a much different finding from this study. It is important to note this study used organic soils which do have different properties than mineral soils. The results indicate that another factor or a combination of factors (type and richness of organic matter. sorption. presence of microbial populations etc.) influence the total mineralization of phenanthrene. When considering the relationship between mineralization rate and texture and organic carbon (Figure 3.12 and 3.13). clay content (r^2 = 0.1) had no effect on the rates but organic carbon did have a significant relationship (r^2 = 0.65). It can be concluded organic carbon can be a good indicator of the activity of the microbial populations to mineralize phenanthrene in soil.

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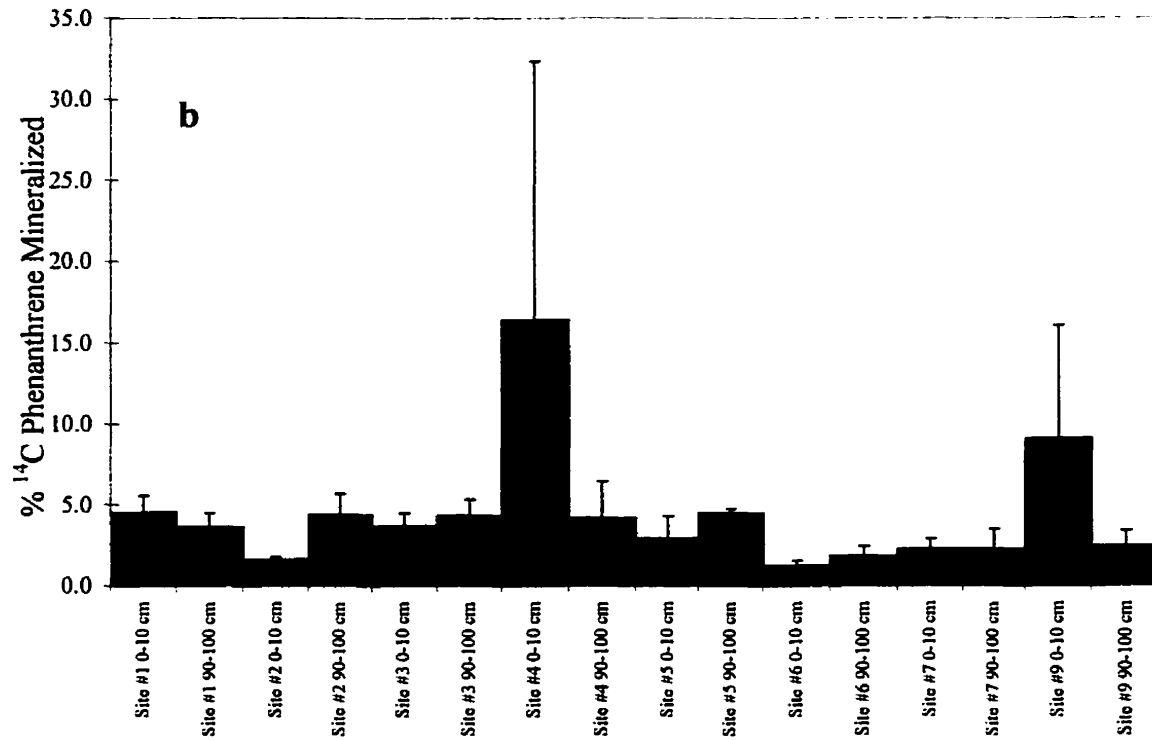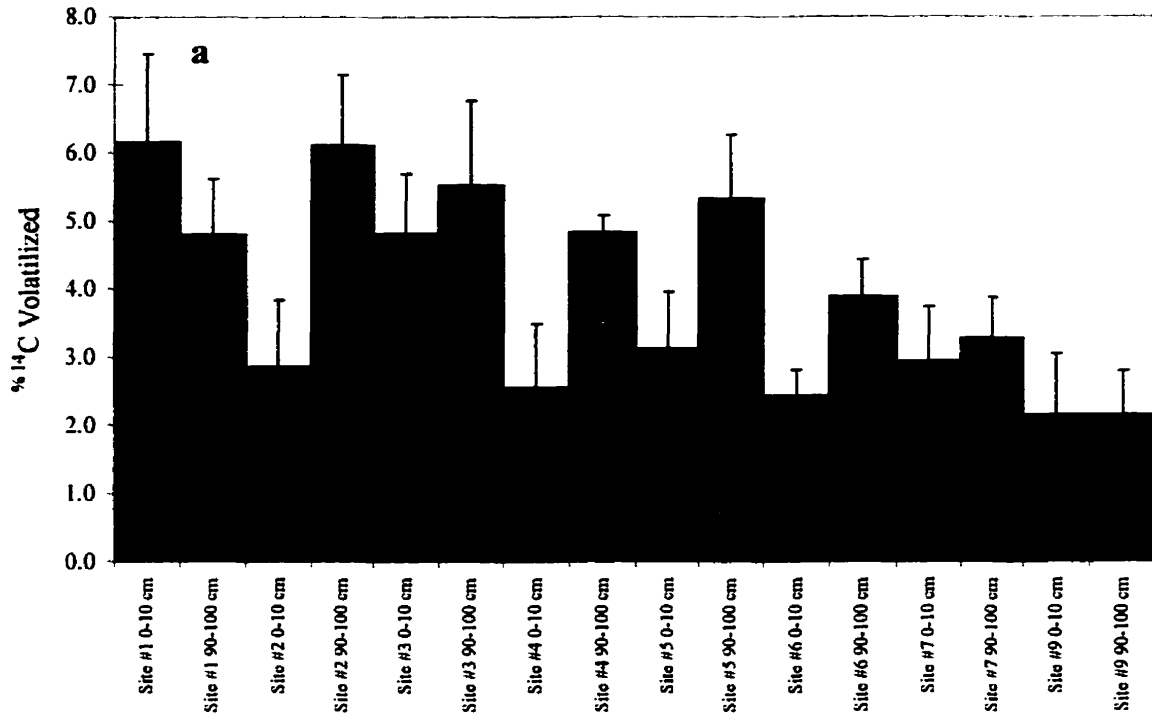
Figure 3.4 Total percent a) volatilization and b) mineralization of ^{14}C phenanthrene in the surface and subsurface of 8 sites investigated without prior hydrocarbon exposure. The experiment lasted 259 days.

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$R^2 = 0.1064$

$R^2 = 0.2813$
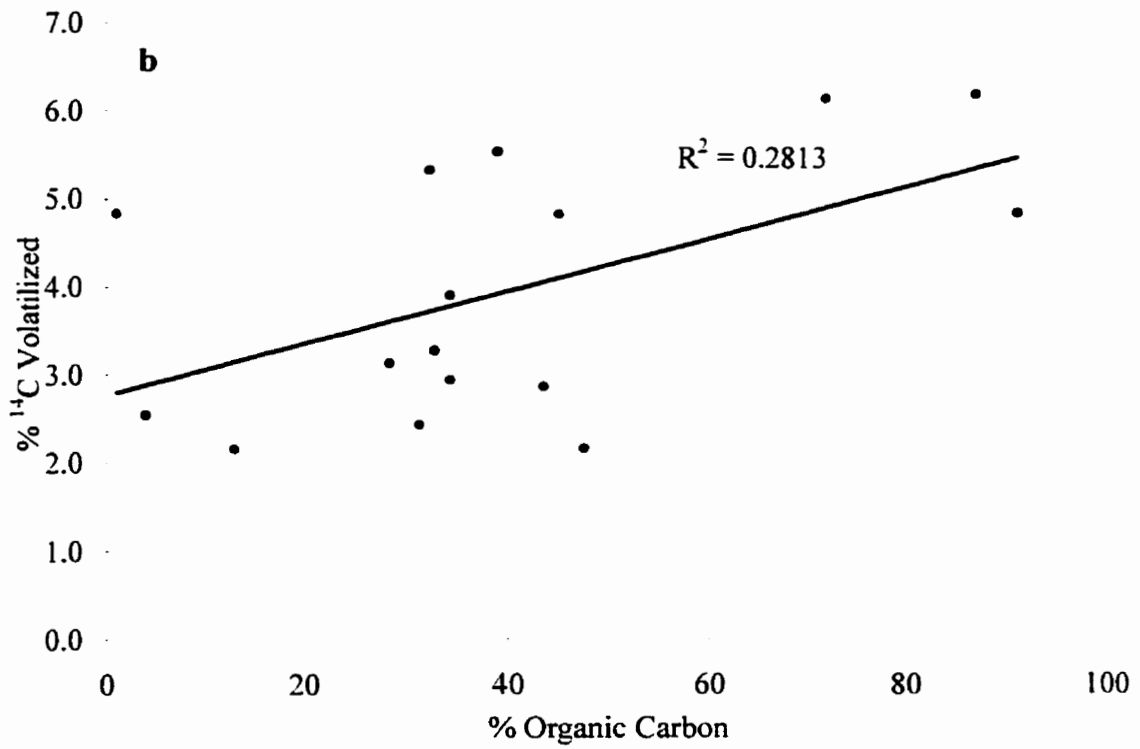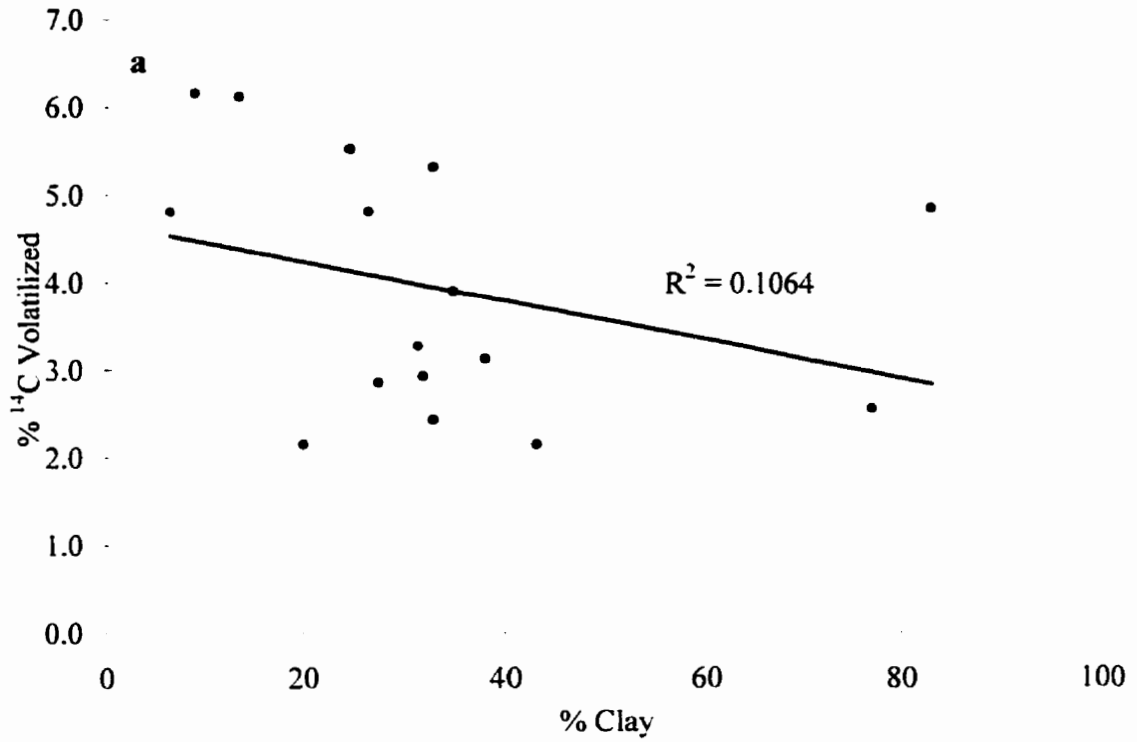
Figure 3.5 Correlation between volatilization and the physical properties clay content (a) and organic carbon (b).

Table 3.5 [14]C Phenanthrene volatilization and mineralization over the course of the microcosm experiment.

Site	% [14]C Volatilized	% [14]C Phenanthrene Mineralized (A)[t]	Rate Constant (k) (day^{-1})[a]	Mineralization Half Life (days)[b]
Site #1 0-10 cm	6.2 ± 1.3	4.5 ± 1.0	0.019 ± 0.006	36
90-100 cm	4.8 ± 0.8	3.6 ± 0.9	0.006 ± 0.001	126
Site #2 0-10 cm	2.9 ± 1.0	1.6 ± 0.2	0.021 ± 0.011	33
90-100 cm	6.1 ± 1.0	4.4 ± 1.3	0.005 ± 0.001	132
Site #3 0-10 cm	4.8 ± 0.9	3.7 ± 0.8	0.014 ± 0.002	49
90-100 cm	5.5 ± 1.2	4.3 ± 1.0	0.007 ± 0.001	99
Site #4 0-10 cm	2.5 ± 0.9	16.4 ± 16.0	0.032 ± 0.012	22
90-100 cm	4.8 ± 0.2	4.2 ± 2.3	0.007 ± 0.002	107
Site #5 0-10 cm	3.1 ± 0.8	2.9 ± 1.4	0.029 ± 0.031	24
90-100 cm	5.3 ± 0.9	4.5 ± 0.2	0.005 ± 0.001	139
Site #6 0-10 cm	2.4 ± 0.4	1.2 ± 0.3	0.015 ± 0.005	45
90-100 cm	3.9 ± 0.5	1.8 ± 0.6	0.007 ± 0.004	96
Site #7 0-10 cm	2.9 ± 0.8	2.3 ± 0.6	0.022 ± 0.012	32
90-100 cm	3.3 ± 0.6	2.3 ± 1.2	0.008 ± 0.003	89
Site #9 0-10 cm	2.2 ± 0.9	9.1 ± 7.0	0.045 ± 0.014	15
90-100 cm	2.2 ± 0.6	2.4 ± 1.0	0.005 ± 0.003	132

[t] Total Mineralization after 259 day experiment.

[a] Rate of Mineralization (% per day).

[b] Time for half of the Total Mineralized Phenanthrene to be liberated as CO_2.

Table 3.6 Mean values for each site and sampling depth for ^{14}C Phenanthrene volatilization and mineralization over the course of the microcosm experiment.

	% ^{14}C Volatilized	% ^{14}C Phenanthrene Mineralized	Rate Constant (k) (day^{-1})
Site Comparisons[t]			
Site #1	5.5 e	4.1 a	0.012 a
Site #2	4.5 cd	3.0 a	0.013 a
Site #3	5.1 de	4.0 a	0.01 a
Site #4	3.7 bc	10.3 b	0.019 a
Site #5	4.2 c	3.7 a	0.017 a
Site #6	3.2 ab	1.5 a	0.011 a
Site #7	3.1 ab	2.3 a	0.015 a
Site #9	2.6 a	5.7 a	0.025 a
Depth Comparison[x]			
0-10 cm	3.4 a	5.2 a	0.025
90 -100 cm	4.6 b	3.4 a	0.006
ANOVA			
Site	***	*	ns
Depth	***	ns	***
Site x Depth	***	*	*

[t]Average of 8 replicates.
[x]Average of 32 replicates.
Means followed by a common letter are not significantly different at the 5% level.
***-highly significant, **-significant, *-low significance, ns-not significant (α = 0.05)

Figure 3.6 Correlation between mineralization and clay content in all sites.
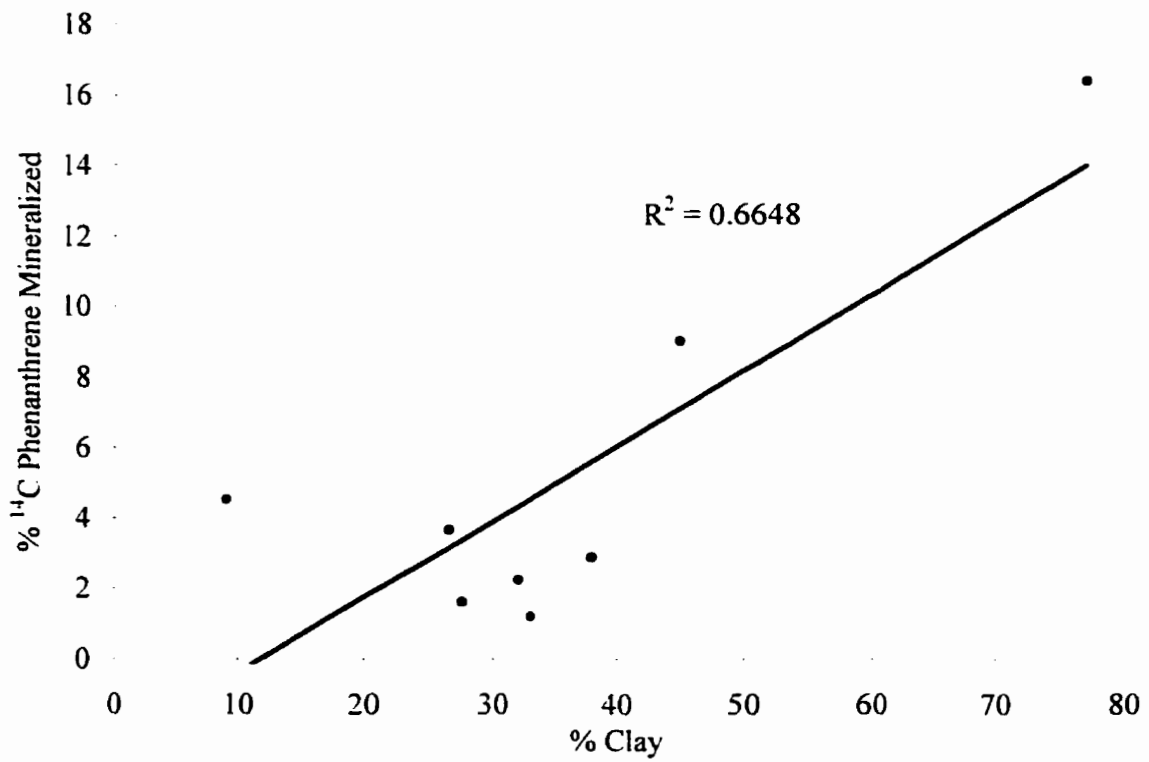
Figure 3.7 Correlation between mineralization and clay content in the surface sites.

51
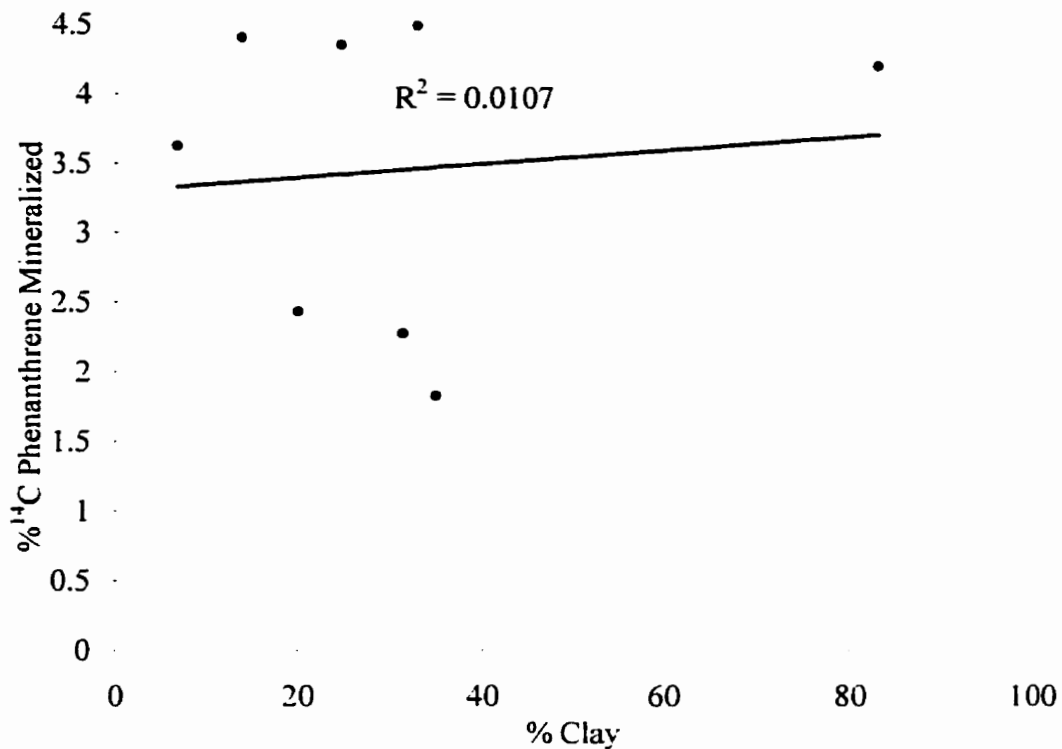
Figure 3.8 Correlation between mineralization and clay content in subsurface soils.
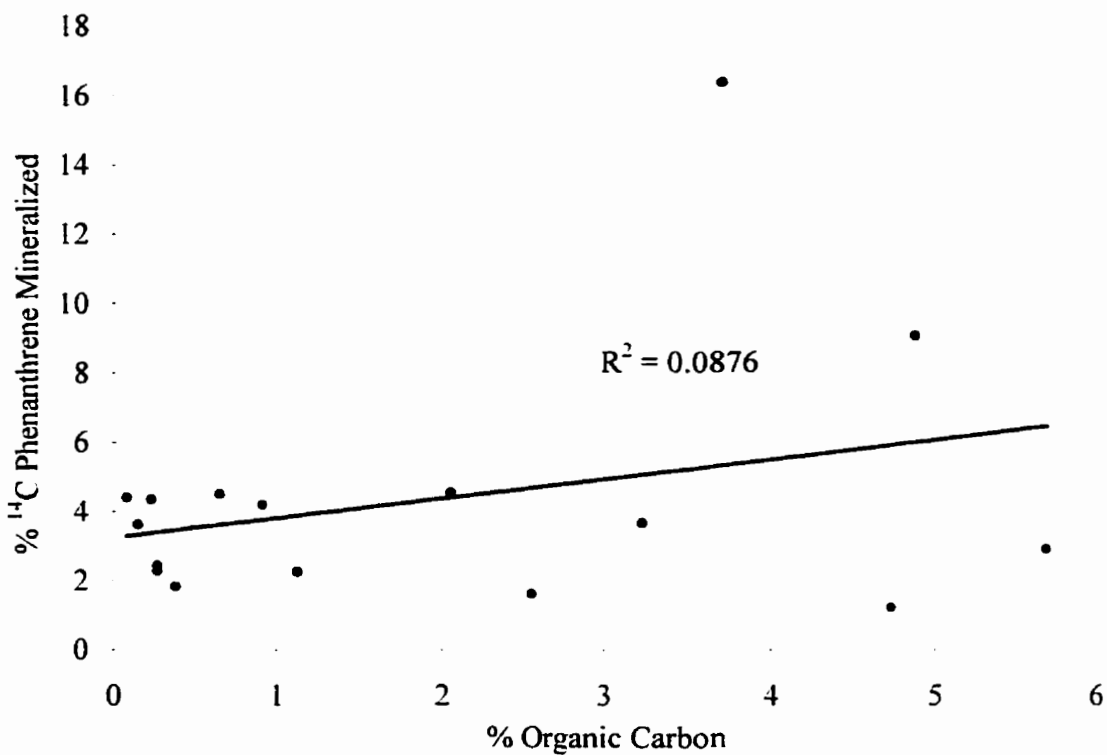
Figure 3.9 Correlation between mineralization and organic carbon in all sites.

52
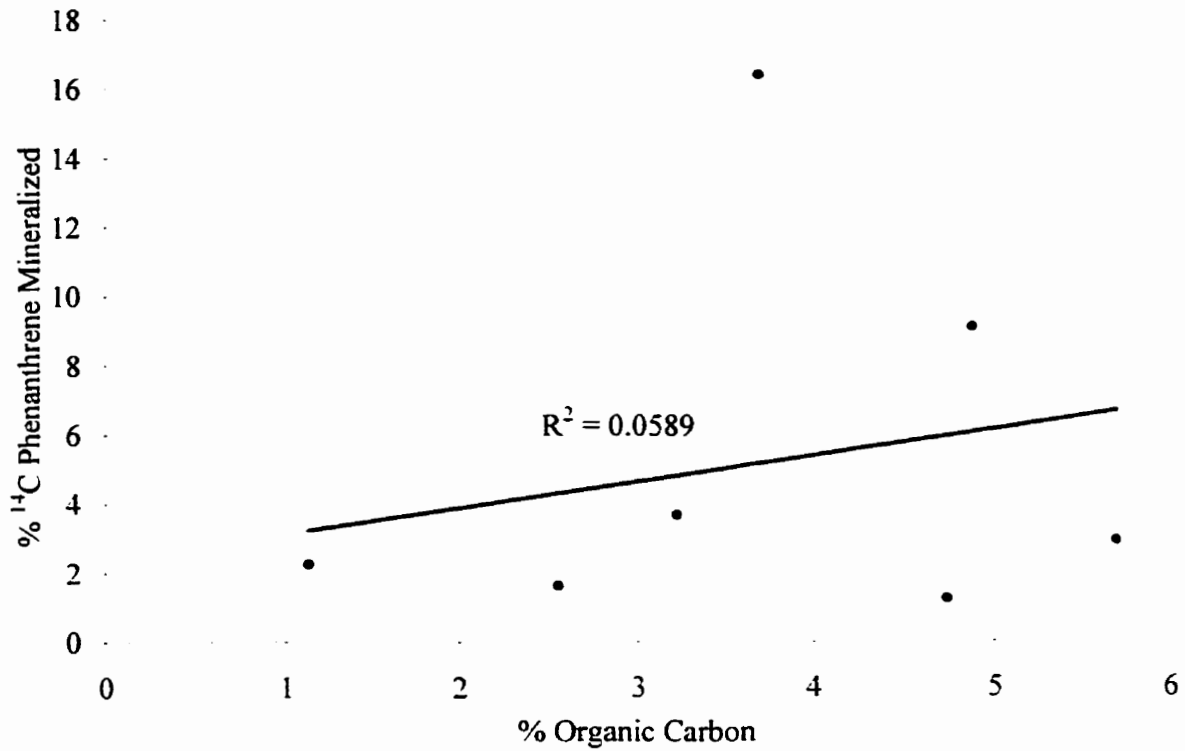
Figure 3.10 Correlation between mineralization and organic carbon in surface sites.
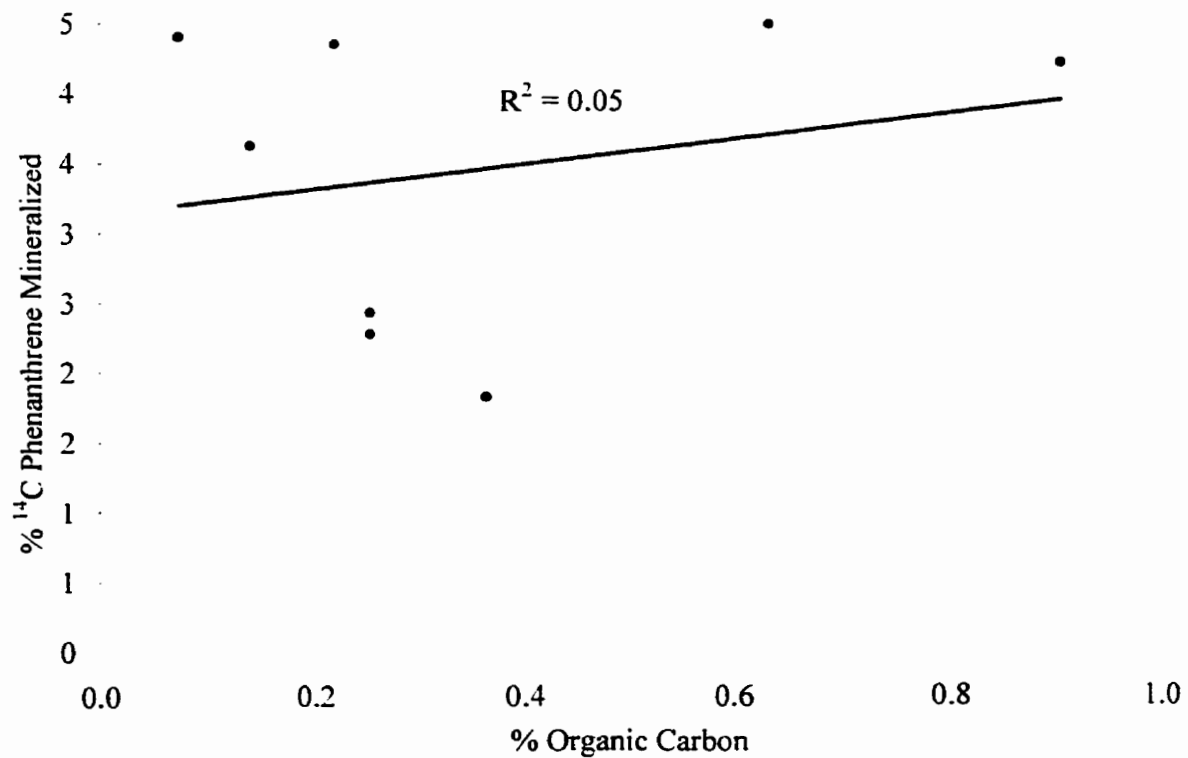
Figure 3.11 Correlation between mineralization and organic carbon in subsurface soils.

53
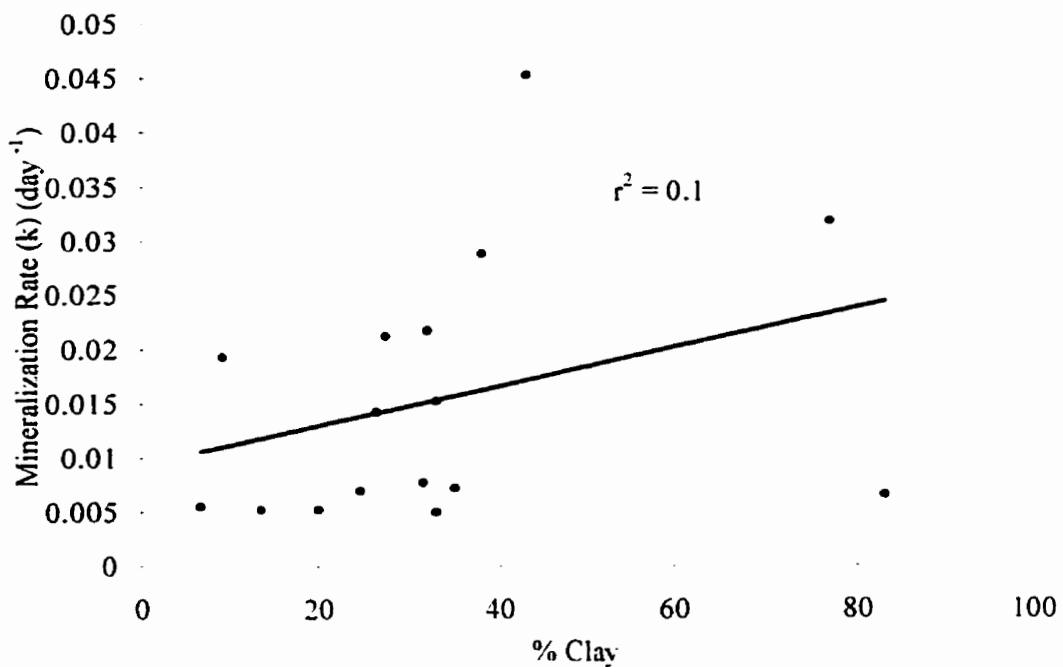
Figure 3.12 Correlation between mineralization rate and clay content in all soils.
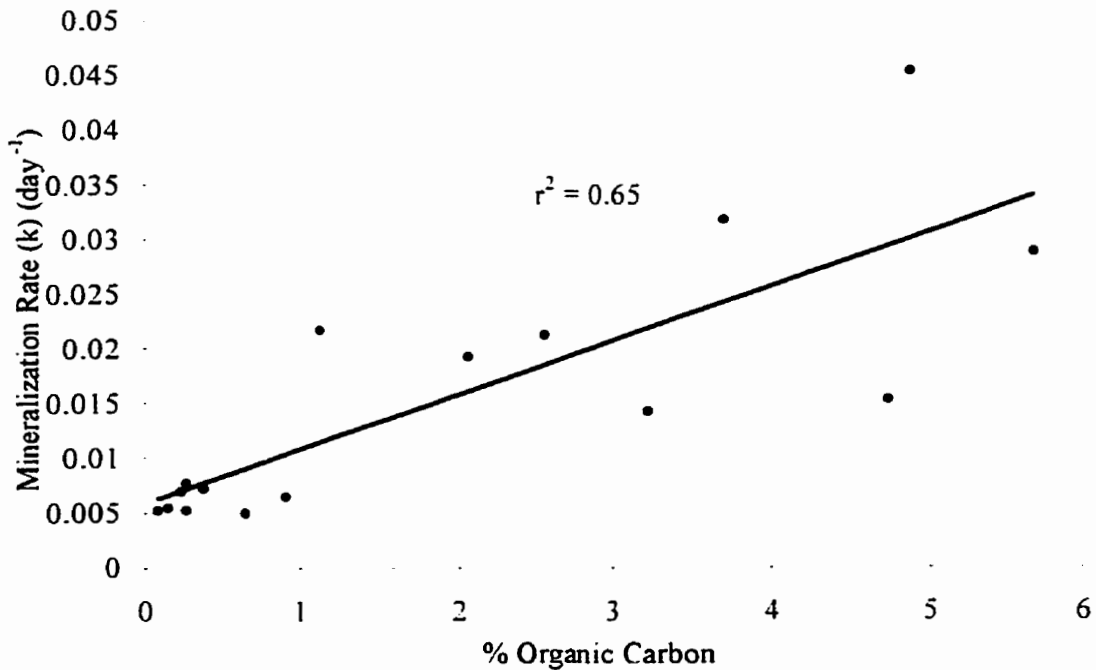
Figure 3.13 Correlation between mineralization rate and organic carbon in all soils.

3.5.1.3 Sequential Extraction of ^{14}C from Soil The results of the extraction of the remaining ^{14}C in the soil after the experiment are listed in Table 3.7. Most sites, if not all, had greater than 30% of the radioactivity recovered in a methanol wash. Site #1 (Sand) and Site #5 (Clay Loam) in the surface had greater than 60% of the total added radioactivity in a methanol extractable phase, much greater than any other phase. Site #6 (Silty Clay), on the other hand, had 35.3% methanol extracted but only 43% totally recovered. The results indicate phenanthrene or its degradative products were adsorbed to the soil particles thereby decreasing the bioavailability of the compounds. The methanol wash may in fact be extracting degradation intermediates that are more polar than the phenanthrene molecule. If this is the case, then there may have been significant degradation of phenanthrene while mineralization appeared to be low. The methanol wash may be removing only phenanthrene itself because in the control extraction (5000 ppm diesel and radiolabeled phenanthrene spiked in dry soil) up to 57% of the ^{14}C was removed at this stage. It is therefore impossible to determine whether or not the ^{14}C phenanthrene or degradation intermediates. The methanol extract indicates a weak association to soil which may allow the use of surfactants to increase bioavailability. Dohse and Lion (1994), Tsomides et al. (1995), and Providenti et al. (1995) have all indicated the enhanced bioavailability of phenanthrene in the presence of microbial or human origin surfactants. The results in this study support the idea of adding compounds to reduce the adsorption and increase the bioavailability of hydrocarbons in soil during *in situ* bioremediation.

In 6 of 8 sites studied, the water extractable phase had more ^{14}C extracted in the subsurface compared to the surface. Sites with less extractable ^{14}C in the subsurface included Site #4 (Heavy Clay) and Site #7 (Clay Loam). This indicates there was less

adsorption in the subsurface therefore leading to a potential for greater transport in these environments. When considering the methanol extraction, the surface soils had greater ^{14}C extracted than the subsurface in 6 of the 8 sites investigated. The exceptions in this example included Sites 3 (Loam) and 4 (Heavy Clay). The results indicate there was greater adsorption of phenanthrene and its mineralization intermediates in organic carbon rich environments. The soxhlet extraction yielded similar results with all sites having less ^{14}C extracted in the subsurface compared to the surface with some overlaps of the standard deviations. Finally, upon wet digestion, all sites except Site #7 (Clay Loam) had more ^{14}C extracted in the surface compared to the subsurface indicating the greater adsorption in surface soils. The implications of adsorption on *in situ* bioremediation are enormous. Surface soils will have an immense potential for adsorption thereby reducing the bioavailability of hydrocarbons. Manilal and Alexander (1991) monitored the mineralization of 10 mg/kg of labeled (9-^{14}C) and non labeled phenanthrene in soil microcosms. They varied the amounts of organic matter in a soil and found the soil with high organic matter (36.7%) had less mineralization (42% in 20 days) compared to the soil with lower amounts (5.9% OM had 46% mineralization in 20 days). There was not as much of a pronounced difference in mineralization of phenanthrene in surface soils but there was the indication of the role of organic carbon in bioavailability.

It appears adsorption of the phenanthrene in the soils studied was the major fate. The total recovered ^{14}C ranged from 43% in Site #6 to 110% in Site #5. Broadkorb and Legge (1992) found 55.3% of the total added phenanthrene remaining in the soil after a 21 day incubation. Adsorption coefficients calculated using the organic carbon from each soil indicate the majority of added phenanthrene will be readily adsorbed (Table 3.9),

consistent with results in the literature. Site #5 (0-10cm) had the highest calculated adsorption coefficient and the highest recoverable ^{14}C in the soil. Site # 1 (90-100cm) had one of the lowest calculated adsorption coefficients and one of the lowest recoverable ^{14}C. The results indicate organic carbon influences the adsorption and bioavailability of phenanthrene in soil. The ^{14}C extracted in the methanol phase indicates the compound, or its degradative intermediates, was loosely bound to soil and may be desorbed in time. The rate of desorption controls the potential for further mineralization of this compound or its degradative intermediates.

The majority of the ^{14}C in the control (5000 ppm diesel and radiolabeled phenanthrene spiked in dry soil) was found in the methanol phase, consistent with the experimental data presented. It should be noted that the efficiency of extraction for the controls was high with 91% of the added ^{14}C being recovered (Table 3.7).

Table 3.7 Sequential extraction of ^{14}C from soil at the end of the soil microcosm experiment.

Sample	% ^{14}C Water Extracted	% ^{14}C Methanol Extracted	% ^{14}C Soxhlet Extracted	% ^{14}C Wet Digested	%Total ^{14}C Extracted
Site #1 0-10 cm	1.8 ± 0.7	60.1 ± 12.1	8.5 ± 0.9	6.3 ± 4.2	76.6 ± 11.8
Site #1 90-100 cm	2.7 ± 0.4	49.4 ± 5.9	3.5 ± 0.6	1.7 ± 1.1	57.3 ± 5.1
Site #2 0-10 cm	1.1 ± 0.5	51.3 ± 6.2	12.0 ± 2.2	9.6 ± 2.1	74.0 ± 7.6
Site #2 90-100 cm	2.2 ± 0.2	47.1 ± 6.8	3.0 ± 2.2	1.9 ± 0.7	54.2 ± 5.9
Site #3 0-10 cm	1.3 ± 0.8	45.9 ± 3.5	7.5 ± 4.4	10.7 ± 2.2	65.4 ± 7.2
Site #3 90-100 cm	1.9 ± 0.3	48.8 ± 3.9	3.4 ± 1.6	1.5 ± 0.6	55.6 ± 4.6
Site #4 0-10 cm	1.4 ± 0.9	42.4 ± 13.6	8.1 ± 10.6	1.9 ± 14.3	53.8 ± 15.7
Site #4 90-100 cm	0.8 ± 0.4	50.5 ± 5.1	5.4 ± 2.6	1.5 ± 0.6	58.2 ± 5.5
Site #5 0-10 cm	0.6 ± 0.4	64.5 ± 10.8	29.6 ± 11.2	15.3 ± 8.7	110.0 ± 8.1
Site #5 90-100 cm	1.5 ± 0.3	43.1 ± 3.0	1.4 ± 1.8	3.9 ± 3.2	50.0 ± 1.8
Site #6 0-10 cm	0.6 ± 0.4	56.3 ± 6.7	10.7 ± 3.0	22.5 ± 7.1	90.1 ± 15.5
Site #6 90-100 cm	2.3 ± 0.7	35.3 ± 6.1	4.3 ± 2.2	1.0 ± 0.2	43.0 ± 7.7
Site #7 0-10 cm	1.4 ± 0.1	42.4 ± 8.1	8.1 ± 5.5	1.9 ± 1.1	53.8 ± 13.0
Site #7 90-100 cm	1.1 ± 0.7	39.6 ± 4.7	6.1 ± 1.1	2.5 ± 1.6	49.4 ± 3.5
Site #9 0-10 cm	0.9 ± 0.4	43.1 ± 10.4	6.1 ± 0.5	15.7 ± 3.8	65.8 ± 9.1
Site #9 90-100 cm	1.4 ± 0.4	42.1 ± 11.3	4.0 ± 2.7	3.0 ± 0.6	50.5 ± 12.5
Controls	8.4 ± 3.2	56.9 ± 4.2	8.7 ± 0.3	16.9 ± 2.8	90.9 ± 8.2

Table 3.8 Duncan New Multiple Range Test for comparing the sites for sequential extraction of ^{14}C from soil at the end of the soil microcosm survey experiment.

	% ^{14}C Water Extracted	% ^{14}C Methanol Extracted	% ^{14}C Soxhlet Extracted	% ^{14}C Wet Digested	%Total ^{14}C Extracted
Site Comparisons[t]					
Site #1	2.2 d	54.8 c	6.0 a	4.0 a	67.0 b
Site #2	1.7 c	49.2 abc	7.5 a	5.7 ab	64.1 b
Site #3	1.6 c	47.3 abc	5.4 a	6.1 ab	60.5 ab
Site #4	0.9 a	42.6 a	9.5 a	2.2 a	51.6 a
Site #5	1.1 ab	53.8 bc	15.5 b	9.6 bc	80.0 c
Site #6	1.5 bc	45.8 ab	7.5 a	11.8 c	66.6 b
Site #7	1.3 abc	41.0 a	7.1 a	2.2 a	51.6 a
Site #9	1.1 abc	42.6 a	5.1 a	9.3 bc	58.1 ab
Depth Comparison[x]					
0-10 cm	1.1 a	49.8 b	12.0 b	11.8 b	74.7 b
90 -100 cm	1.7 b	44.5 a	3.9 a	2.1 a	52.3 a
ANOVA					
Site	***	**	***	**	***
Depth	***	**	***	***	***
Site x Depth	**	***	***	**	***

[t]Average of 8 replicates.

[x]Average of 32 replicates.

Means followed by a common letter are not significantly different at the 5% level.

***-highly significant, **-significant, *-low significance, ns-not significant ($\alpha = 0.05$)

Table 3.9 Calculated organic carbon adsorption coefficients for each soil investigated without prior hydrocarbon exposure.

Sample	Fraction of Organic Carbon (f_{oc})	K_p $(K_{oc}*f_{oc})^t$ (L kg^{-1})
Site #1 0-10 cm	0.021	283.3
Site #1 90-100 cm	0.001	13.5
Site #2 0-10 cm	0.025	337.2
Site #2 90-100 cm	0.001	13.5
Site #3 0-10 cm	0.032	431.6
Site #3 90-100 cm	0.002	27.0
Site #4 0-10 cm	0.037	499.1
Site #4 90-100 cm	0.009	121.4
Site #5 0-10 cm	0.057	768.9
Site #5 90-100 cm	0.006	80.9
Site #6 0-10 cm	0.047	634.0
Site #6 90-100 cm	0.004	54.0
Site #7 0-10 cm	0.011	148.4
Site #7 90-100 cm	0.003	40.5
Site #9 0-10 cm	0.049	661.0
Site #9 90-100 cm	0.003	40.5
Piatt et al. (1996)[B]	0.02	2.7
Carmichael et al. (1997)[B]	0.029	14125.4
Carmichael et al. (1997)[B]	0.0026	15848.9

[B] Experimentally observed values.

When comparing the amounts of ^{14}C in each extractable phase to the clay content of the soil, the results showed no correlation for any factors (Table 3.10). The comparison of percent clay to water extractable ^{14}C has a very weak correlation but is not significant. The results prevent the use of texture in an initial risk assessment determining the adsorption potential of phenanthrene in the environment. There was, however, a relationship between ^{14}C contained in water, methanol, and soxhlet extracts and soil organic C. A significant relationship existed between the soil organic C and the ^{14}C released on wet digestion and total extracted radioactivity. This is consistent with the partitioning of phenanthrene or degradation products into soil. In soils with greater amounts of organic carbon, more phenanthrene will sorb and be released during oxidative

digestion. The relationship between phenanthrene adsorption and organic carbon can be used in assessing the bioavailability and initial risk of movement of compounds off site.

Table 3.10 Correlation between each sequential extractable phase and clay content or organic carbon in sites investigated.

Soil Property of Sites	% ^{14}C Water Extracted	% ^{14}C Methanol Extracted	% ^{14}C Soxhlet Extracted	% ^{14}C Wet Digested	%Total ^{14}C Extracted
% Clay	0.25*	0.00005	0.013	0.0008	0.002
% Organic Carbon	0.47	0.30	0.51	0.71	0.64

* r^2 values obtained in regression

3.5.1.4 Metabolic Diversity of Soil The determination of the metabolic diversity of the microbial populations using biolog plates helps to understand the substrate diversity of the organisms. The results indicate the majority of the color change (degradation) occurred between 48 and 72 hours after inoculation of the soil extracts (Table 3.11). Surface soils were more active in degrading substrates than the subsurface. This can be explained because the majority of the microbial activity will be found at the surface due to the presence of organic matter and other nutrients (Smith et al. 1993). When comparing the metabolic diversity of the sites, the results indicate there was a significant effect of the site, depth and site by depth treatments. Surface soils had greater metabolic diversity than the subsurface because there are reduced amounts of substrate in the subsurface environments for organisms to utilize. Site #2 and #3 had the greatest metabolic diversity yet had less than 5% phenanthrene ^{14}C mineralized. Site #4 had significantly less metabolic diversity yet had greater mineralization (~16%). This indicates metabolic diversity is a poor indicator of total phenanthrene mineralization in soil. The mineralization rate had different results. It was correlated to the metabolic diversity of the soil. This indicates there is a direct relationship between the diversity of microbial populations and their activity in the

mineralization of phenanthrene (Figure 3.14). Biolog plates may be used in the determination of the potential for a soil to mineralize phenanthrene.

Table 3.11 Average intensity and substrate richness for the 8 sites investigated without prior hydrocarbon exposure. The properties of the microorganisms was determined using Biolog plates.

	Average Intensity (Time of Color Change)	Substrate Richness (% Substrates Utilized)
<u>Site Comparisons[t]</u>		
Site #1	2.4 b	50.1 ab
Site #2	2.1 a	65.2 c
Site #3	2.2 a	65.2 c
Site #4	2.5 b	56.9 bc
Site #5	2.6 b	40.8 a
Site #6	2.5 b	54.6 bc
Site #7	2.1 a	64.5 c
Site #9	2.2 a	63.0 c
<u>Depth Comparison[x]</u>		
0-10 cm	2.4 b	71.4 b
90 -100 cm	2.3 a	43.7 a
<u>ANOVA</u>		
Site	***	***
Depth	*	***
Site x Depth	ns	***
<u>Correlation</u>		
% Total Mineralized (r^2)	0.07	0.0002
Mineralization Rate (k) (r^2)	0.07	0.6

[t]Average of 8 replicates.
[x]Average of 32 replicates.
Means followed by a common letter are not significantly different at the 5% level.
***-highly significant, **-significant, *-low significance, ns-not significant ($\alpha = 0.05$)
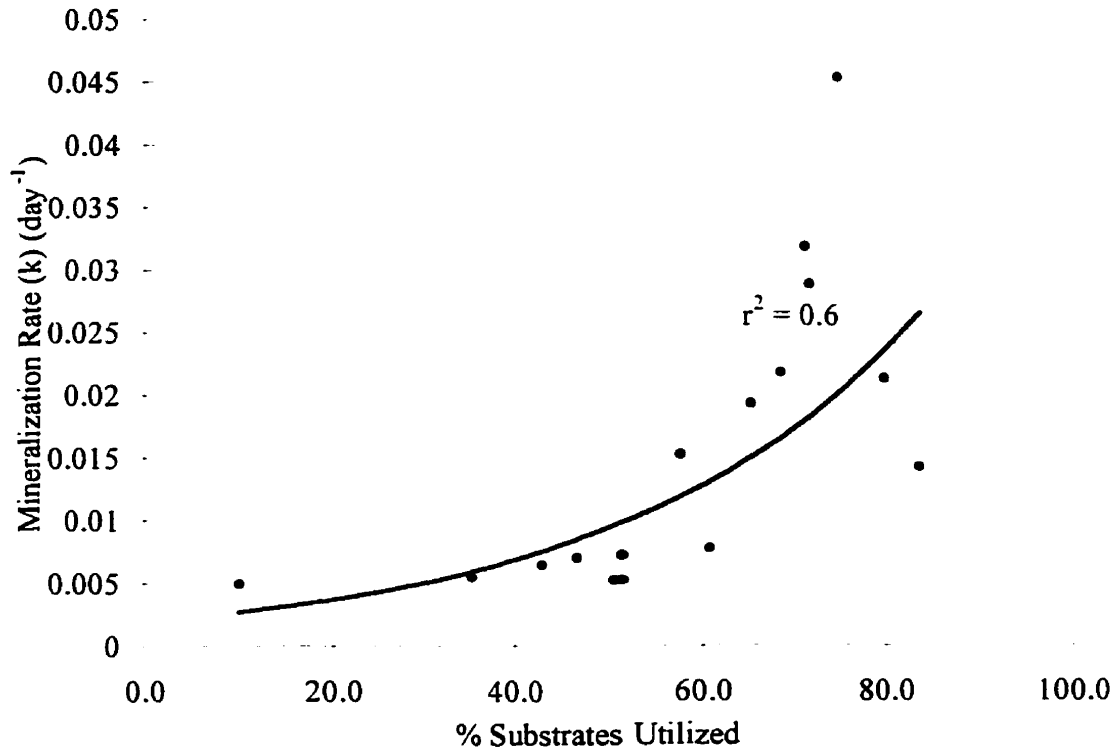
Figure 3.14 Correlation between the mineralization rate and the diversity of substrates utilized by the microorganisms in soil.

3.5.1.5 Fate of Phenanthrene and Mass Balance The mass balance of radioactivity added to the soil is listed in Table 3.12. The summing of mineralization, volatilization, and residual ^{14}C results in recoveries ranging from 44 to 116% of the added ^{14}C. The data indicates the majority of the ^{14}C phenanthrene introduced into virgin soil *in situ* (i.e. no hydrocarbon exposure) is adsorbed (Table 3.12). Bioavailability is a factor due to the inability to extract ^{14}C from the soil using water. The majority of the radioactivity was found in the methanol extractable phase which is probably not accessible by the microorganisms at this time. If this phase was available due to surfactants, mineralization of phenanthrene and its degradative products should have been greater in the soil analyzed. Volatilization and mineralization of the ^{14}C label were minor factors involved in the fate of phenanthrene in unexposed soil. Values ranged from 0 to 16% of the total ^{14}C added,

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while soil extractions made up the remaining portion. $^{14}CO_2$ traps were shown to be effective in the second part of this experiment because a large amount of ^{14}C was trapped in a short period of time (Site #10).

Table 3.12 **Mass balance of ^{14}C recovered from the addition of phenanthrene. Values were take at the end of the experiment (259 days).**

Site	% ^{14}C Volatilized	% ^{14}C Phenanthrene Mineralized	% ^{14}C Extracted	Total ^{14}C Recovered
Site #1 0-10 cm	6.2 ± 1.3	4.5 ± 1.0	76.6 ± 11.8	87.3 ± 13.2
90-100 cm	4.8 ± 0.8	3.6 ± 0.9	57.3 ± 5.1	65.7 ± 4.1
Site #2 0-10 cm	2.9 ± 1.0	1.6 ± 0.2	74.0 ± 7.6	78.4 ± 7.9
90-100 cm	6.1 ± 1.0	4.4 ± 1.3	54.2 ± 5.9	64.7 ± 5.4
Site #3 0-10 cm	4.8 ± 0.9	3.7 ± 0.8	65.4 ± 7.2	73.9 ± 5.8
90-100 cm	5.5 ± 1.2	4.3 ± 1.0	55.6 ± 4.6	65.4 ± 3.4
Site #4 0-10 cm	2.5 ± 0.9	16.4 ± 16.0	53.8 ± 15.7	67.2 ± 23.0
90-100 cm	4.8 ± 0.2	4.2 ± 2.3	58.2 ± 5.5	72.7 ± 6.2
Site #5 0-10 cm	3.1 ± 0.8	2.9 ± 1.4	110.0 ± 8.1	116.0 ± 9.9
90-100 cm	5.3 ± 0.9	4.5 ± 0.2	50.0 ± 1.8	59.8 ± 2.4
Site #6 0-10 cm	2.4 ± 0.4	1.2 ± 0.3	90.1 ± 15.5	93.8 ± 15.3
90-100 cm	3.9 ± 0.5	1.8 ± 0.6	43.0 ± 7.7	48.7 ± 8.2
Site #7 0-10 cm	2.9 ± 0.8	2.3 ± 0.6	53.8 ± 13.0	59.0 ± 12.9
90-100 cm	3.3 ± 0.6	2.3 ± 1.2	49.4 ± 3.5	54.9 ± 2.9
Site #9 0-10 cm	2.2 ± 0.9	9.1 ± 7.0	65.8 ± 9.1	77.0 ± 8.1
90-100 cm	2.2 ± 0.6	2.4 ± 1.0	50.5 ± 12.5	55.1 ± 12.9

The partitioning of phenanthrene and its degradation products into the various fates in the environment are indicated in Figure 3.15. The data was derived by averaging the surface (0-10cm) and subsurface (90-100cm) results. Phenanthrene and degradation products, in uncontaminated soils, will adsorb readily to the soil matrix with a small portion of this compound available for transport, mineralization and volatilization. The possible transportable fraction (water soluble extracted ^{14}C) and volatilized compounds after 259 days are not significant fates in either environments, but are higher in the subsurface. Adsorption (methanol and soxhlet extractable ^{14}C) and residual (wet digested

[14]C) are higher in the surface indicating organic carbon may play a large role in the fate of phenanthrene in the environment. It is important to note the data used in the Figure 3.15 is calculated by averaging of all sites in the surface and subsurface then dividing by the total recovered ^{14}C and not the total added to the microcosms.

A

Volatilized 4% Mineralized 6% Transportable 1%

Residual 13%

Sorbed 76%

B

Volatilized 8% Mineralized 6% Transportable 3%

Residual 4%

Sorbed 79%
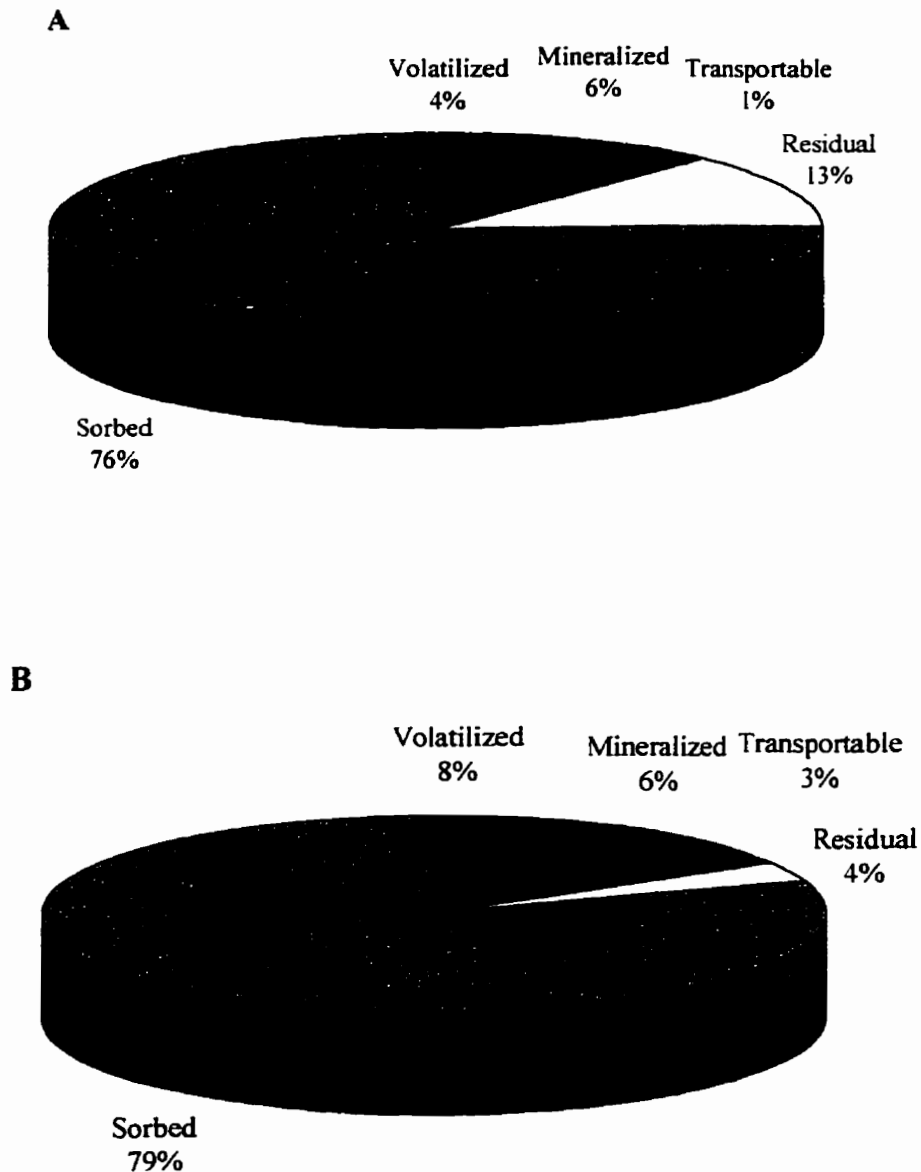
Figure 3.15 Fate of radiolabeled phenanthrene in uncontaminated soil microcosms in the surface (A) and subsurface (B) environments.

3.5.2 Phenanthrene Fate in Soil With Prior Hydrocarbon Exposure

The mineralization of radiolabeled phenanthrene in diesel fuel was monitored in soils previously contaminated with diesel fuel and crude oil. The results in this section will be compared to the previous to determine the effect prior exposure may have on phenanthrene mineralization. The use of soil microcosms allowed the simultaneous study of mineralization, volatilization and adsorption throughout a 259 day experiment.

3.5.2.1 Phenanthrene Volatilization The volatilization of phenanthrene in hydrocarbon sensitized sites and one control site is listed in Table 3.13. The data indicates a low potential for loss of this compound in this system after 259 days. There appeared to be no difference between surface and subsurface soils in either the sites examined, but when comparing Site #10 to the corresponding control of that site there was a slight difference. Site #10 had an extremely low recovery of phenanthrene in the PUF yet the rate of mineralization was high. This indicates that after the compound was introduced to the system, there was complete mineralization of the available phenanthrene preventing any volatilization. Site #9 had a lower rate of mineralization yet a slightly higher amount of phenanthrene volatilized. Results in this section show volatilization will be a minor fate in the disappearance of phenanthrene in soils when there is rapid mineralization.

Table 3.13 ^{14}C Phenanthrene volatilization and mineralization over the course of the microcosm experiment.

Site	% ^{14}C Volatilized	% ^{14}C Phenanthrene Mineralized (A)	Rate Constant (k) (day^{-1})	Mineralization Half Life (days)
Site #8 0-1 m	2.7 ± 0.4	2.0 ± 0.7	0.008 ± 0.002	89
1-2 m	2.7 ± 0.7	4.8 ± 4.4	0.033 ± 0.035	21
Site #9 0-10 cm^P	2.2 ± 0.9	9.1 ± 7.0	0.045 ± 0.014	15
90-100 cm^P	2.2 ± 0.6	2.4 ± 1.0	0.005 ± 0.003	132
Site #10 0-10 cm	0.04 ± 0.05	56.2 ± 12.5	0.357 ± 0.114	2
90-100 cm	1.2 ± 0.7	44.2 ± 8.9	0.091 ± 0.017	8

^{P}Site #9 (no previous hydrocarbon exposure) was a control for Site #10 (previous hydrocarbon exposure)

3.5.2.2 Phenanthrene Mineralization Variable rates of phenanthrene mineralization were observed in soils previously exposed to hydrocarbons (Table 3.13). Site #8 appeared to have low mineralization (surface and subsurface) after 259 days despite being previously contaminated with diesel fuel. This material was a gravel fill containing very little organic matter. The environment would be almost free of nutrients and growth factors making it very harsh for microbial population to grow and survive. Site #10 also had prior hydrocarbon exposure (crude oil) while its control (Site #9 sampled in an adjacent field) had no exposure. The mineralization in this case was rapid, 50% of the added ^{14}C being recovered as CO_2 within the first 2-14 days. The uncontaminated soil at the site had significantly less mineralization. This site (Site #10) was an agricultural soil containing organic matter and high nutrient content, factors conducive to mineralization if the phenanthrene is bioavailable. The mineralization rate indicates it contains microorganisms capable of rapid mineralization of the phenanthrene before the subsequent loss of the bioavailable fraction to adsorption. In the subsurface, where lower amounts of organic matter were present, the crude oil acted as a substrate for microbial growth. Site #10 had

a silty clay texture in the surface and a loam in the subsurface (Table 3.2). These textures allow for an environment more conducive to microbial growth and activity compared to a coarse sand due to the retention of nutrients and organic matter (Donahue et al. 1983). The control for Site #10 (Site #9) had significantly less mineralization yet had similar soil properties. Prior sensitization of hydrocarbons in the contaminated soil (Site #10) decreased the half life and acclimation period of phenanthrene mineralization in diesel fuel relative to the uncontaminated control (Site #9). The same trend was also seen by Kästner et al. (1994) in the enumeration of phenanthrene degrading organisms. They found greater than 10^5 zone forming units on minimal medium plus phenanthrene growth plates in a soil with residual PAH's and none with uncontaminated soil. Sites in the environment with repeated exposure to hydrocarbons (oil fields, storage facilities etc.) may in fact be able to remediate a site without outside intervention promoting *in situ* biodegradation. Previous exposure did not always result in enhanced mineralization, Site #8 resulted in less phenanthrene mineralization (<5%) despite previous exposure to diesel fuel. Other factors besides prior exposure must be controlling the ability to degrade phenanthrene in diesel fuel.

3.5.2.3 Sequential Extraction of ^{14}C from Soil

The extraction of the remaining ^{14}C from the soil at the end of the experiment (259 days) was used to indicate the extent of adsorption. Site #8 and Site #9 followed the trend identified for unexposed soil with the highest extractable ^{14}C in the methanol fraction (Table 3.14). This apparent adsorption seriously affects the bioavailability of phenanthrene in the environment reducing the potential for mineralization *in situ*. These soils also had low mineralization and higher volatilization than Site #10. The surface of Site #10 contained the majority of the

radioactivity in the wet digestible phase while the subsurface had equal proportions in the methanol and wet digestible phases. Though a proportion was found in the methanol phase in the subsurface, the amount was low (16.1%). A reason for having low recoveries (between 33 and 35%) in Site #10 would be the fact that up to 50% of the phenanthrene was mineralized in the first 14 days preventing adsorption of the compound. Wet digestible recoveries were high while methanol extracts were low possibly due to degradative intermediate production which may in turn adsorb or become humified more readily than phenanthrene itself (Ribbon and Eaton 1982). With more intermediates produced, there may have been a greater chance for specific adsorption to occur making it impossible for a methanol wash to remove. Table 3.16 lists the calculated adsorption coefficients for phenanthrene. In this example it can be concluded the majority of the fate of phenanthrene will be adsorbed to the soil fraction if microbial populations are unable to access and degrade the compound. Site #10 had the greatest amount of organic carbon, therefore will have the highest potential for adsorption and humification. In the experiment it appears the microbial populations were able to degrade the phenanthrene before or even during the adsorption process.

The majority of the ^{14}C in the control sequential extraction was found in the methanol phase, consistent with the experimental data presented. It should be noted that the efficiency of extraction was high with 91% of the added ^{14}C being recovered (Table 3.14).

Table 3.14   Sequential extraction of ^{14}C from soils with and without prior hydrocarbon exposure.

Sample	% ^{14}C Water Extracted	% ^{14}C Methanol Extracted	% ^{14}C Soxhlet Extracted	% ^{14}C Wet Digested	%Total ^{14}C Extracted
Site #8 0-1 m	2.0 ± 0.3	49.3 ± 7.6	3.8 ± 2.0	2.0 ± 0.9	57.1 ± 7.3
Site #8 1-2 m	20.0 ± 9.9	28.5 ± 10.5	3.0 ± 2.0	2.4 ± 0.7	53.9 ± 8.9
Site #9 0-10 cm^P	0.9 ± 0.4	43.1 ± 10.4	6.1 ± 0.5	15.7 ± 3.8	65.8 ± 9.1
Site #9 90-100 cm^P	1.4 ± 0.4	42.1 ± 11.3	4.0 ± 2.7	3.0 ± 0.6	50.5 ± 12.5
Site #10 0-10 cm	1.4 ± 0.3	4.8 ± 1.5	3.6 ± 1.7	23.1 ± 5.0	33.0 ± 4.8
Site #10 90-100 cm	1.8 ± 0.6	16.1 ± 7.6	2.9 ± 1.6	13.4 ± 7.8	34.2 ± 12.5
Soil Control	8.4 ± 3.2	56.9 ± 4.2	8.7 ± 0.3	16.9 ± 2.8	90.9 ± 8.2

^{P}Site #9 (no previous hydrocarbon exposure) was a control for Site #10 (previous hydrocarbon exposure)

Table 3.15 Duncan New Multiple Range Test for comparing all sites for sequential extraction of ^{14}C from soils with prior hydrocarbon exposure.

Sample	% ^{14}C Water Extracted	% ^{14}C Methanol Extracted	% ^{14}C Soxhlet Extracted	% ^{14}C Wet Digested	%Total ^{14}C Extracted
Site Comparison^t					
Site #8	11.0 b	38.9 b	3.4 a	2.2 a	55.5 b
Site #9^P	1.1 a	10.5 a	2.3 a	9.3 b	58.1 b
Site #10	1.6 a	42.6 b	5.1 a	18.2 c	33.6 a
Depth Comparison^x					
0-10 cm	1.4 a	32.4 a	4.5 a	13.6 b	52.0 a
90-100 cm	7.8 b	28.9 a	3.3 a	6.2 a	46.2 a
ANOVA					
Site	***	***	ns	***	***
Depth	**	ns	ns	***	ns
Site x Depth	***	**	ns	*	ns

^{P}Site #9 (no previous hydrocarbon exposure) was a control for Site #10 (previous hydrocarbon exposure))

^{t}Average of 8 replicates.

^{x}Average of 12 replicates.

Means followed by a common letter are not significantly different at the 5% level.

***-highly significant, **-significant, *-low significance, ns-not significant ($\alpha = 0.05$)

Table 3.16 Calculated organic carbon adsorption coefficients for each soil investigated with prior hydrocarbon exposure (Green and Karickhoff 1990).

Sample	Fraction of Organic Carbon (f_{oc})	K_p $(K_{oc}*f_{oc})$ [f] (L kg^{-1})
Site #8 0-1 m	0.003	40.5
Site #8 1-2 m	0.003	40.5
Site #9 0-10 cm[A]	0.049	661.0
Site #9 90-100 cm[A]	0.003	40.5
Site #10 0-10 cm	0.093	1254.5
Site #10 90-100 cm	0.007	94.4
Piatt et al. (1996)[B]	0.02	2.7
Carmichael et al. (1997)[B]	0.029	14125.4
Carmichael et al. (1997)[B]	0.0026	15848.9

[A]Site #9 (no previous hydrocarbon exposure) was a control for Site #10 (previous hydrocarbon exposure))

[B] Experimentally observed values.

3.5.2.4 Metabolic Diversity of Soil The results indicate the majority of the color change occurred between 48 and 72 hours after inoculation of the soil extracts (Table 3.17). This is a significant result because it indicates there were equal proportions of organisms inoculated in each well, giving rise to a color change at about the same time. Subsurface soils were as active as surface soils in generating a positive test probably due to the presence of hydrocarbons (substrate) in the subsurface. When comparing the metabolic diversity, the results indicate there was a significant effect of the site, depth and site plus depth treatments. Site #9 was the control for Site #10 and had the most substrates utilized. Having less substrate richness may be due to the selection of organisms who can survive and grow in a somewhat toxic hydrocarbon environment (i.e. selection by repressing other organisms). These organisms have a readily utilizable substrate present and "lose" the ability to degrade other compounds. Site #8 had no control therefore cannot support this hypothesis. Surface soils were significantly different and had more metabolic diversity than the subsurface. There would be reduced amounts of substrate in

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the subsurface environments (Donahue et al. 1983) for organisms to utilize (i.e. organic carbon, nutrients growth factors etc.), thereby limiting their metabolic activity.

Table 3.17   Average intensity and metabolic diversity for the 3 sites investigated with and without prior hydrocarbon exposure.

Sample	Average Intensity (Time of Color Change)	Metabolic Diversity (% Substrates Utilized)
Site Comparison[t]		
Site #8	2.8 c	59.4 b
Site #9[p]	2.2 a	63.0 b
Site #10	2.3 b	43.9 a
Depth Comparison[x]		
0-10 cm	2.4 a	62.0 b
90-100 cm	2.4 a	48.9 a
ANOVA		
Site	***	***
Depth	ns	***
Site x Depth	***	***

[p]Site #9 (no previous hydrocarbon exposure) was a control for Site #10 (previous hydrocarbon exposure)

[t]Average of 8 replicates.

[x]Average of 12 replicates.

Means followed by a common letter are not significantly different at the 5% level using Duncan New Multiple Range Test.

***-highly significant, **-significant, *-low significance, ns-not significant ($\alpha = 0.05$)

3.5.2.5 Fate of Phenanthrene and Mass Balance The major fate of phenanthrene in soils with low amounts of mineralization (<10%) (Sites 8 and 9) was adsorption and reduced bioavailability after 259 days (Table 3.18).   Though there were microbial populations capable of mineralization, it appears adsorption reduced the bioavailability of phenanthrene *in situ*.   The major proportion of phenanthrene was located in the methanol extractable fraction unavailable to microbial mineralization.   If this fraction was accessible (surfactants, wetting and drying) greater than 10% of the phenanthrene might have been mineralized.   In Site #10, a crude oil spill site, the major fate was mineralization (>44%) with some adsorption (<34%).   The enhanced mineralization in soils with prior exposure

allows the implementation of intrinsic *in situ* biodegradation thereby reducing the costs of clean up. Site #9, the control for Site #10, had mineralization up to 9%, but the standard deviation was as high as the mean signifying this property was a sporadic occurrence. The loss of $^{14}CO_2$ was not a factor because a high rate of mineralization occurred in a short interval yet trapping appeared to proceeded normally in Site #10.

Table 3.18 Mass balance of ^{14}C recovered from the addition of phenanthrene. Values were take at the end of the experiment (259 days).

Site	% ^{14}C Volatilized	% ^{14}C Phenanthrene Mineralized	% ^{14}C Extracted	Total ^{14}C Recovered
Site #8 0-1 m	2.7 ± 0.4	2.0 ± 0.7	57.1 ± 7.3	61.8 ± 6.9
1-2 m	2.7 ± 0.7	4.8 ± 4.4	53.9 ± 8.9	61.4 ± 10.9
Site #9 0-10 cm^P	2.2 ± 0.9	9.1 ± 7.0	65.8 ± 9.1	77.0 ± 8.1
90-100 cm^P	2.2 ± 0.6	2.4 ± 1.0	50.5 ± 12.5	55.1 ± 12.9
Site #10 0-10 cm	0.04 ± 0.05	56.2 ± 12.5	33.0 ± 4.8	89.2 ± 13.4
90-100 cm	1.2 ± 0.7	44.2 ± 8.9	34.2 ± 12.5	79.6 ± 12.6

^{P}Site #9 (no previous hydrocarbon exposure) was a control for Site #10 (previous hydrocarbon exposure)

The fate of phenanthrene in the environment is seen in Figure 3.16. Phenanthrene and its degradative products will adsorb readily to the soil matrix and make up the greatest fraction. Mineralization can also be a significant fate with up to 40% of the phenanthrene mineralized before availability became a factor. Site #10 had active microorganisms capable of significant mineralization of phenanthrene before it was adsorbed and no longer available. The water soluble portion of this compound (transportable) was not a significant fate in the surface of these sites but the subsurface had up to 15% of this compound available for transport. Volatilization was not a significant fate in the microcosm experiment but was slightly higher in the subsurface. Residual (humified) ^{14}C was also a significant fate with up to 17% of the phenanthrene in this state. Adsorption

and residual ^{14}C are higher in the surface while volatilization and water soluble phenanthrene are lower indicating organic carbon plays a large role in the fate of phenanthrene in the environment. Organic carbon not only supplies nutrients and substrate to microorganisms but also decreases the bioavailability (sorption, humification) of hydrocarbons in soil (Edwards et al. 1994). It is important to note the data used in the Figure 3.16 is calculated by averaging Sites #8 and #10 in the surface and subsurface then dividing by the total recovered ^{14}C and not the total added to the microcosms.

A

Volatilized
2%

Sorbed
40%

Mineralized
39%

Residual
17%

Transportable
2%

B

Volatilized
3%

Sorbed
35%

Mineralized
36%

Residual
11%

Transportable
15%
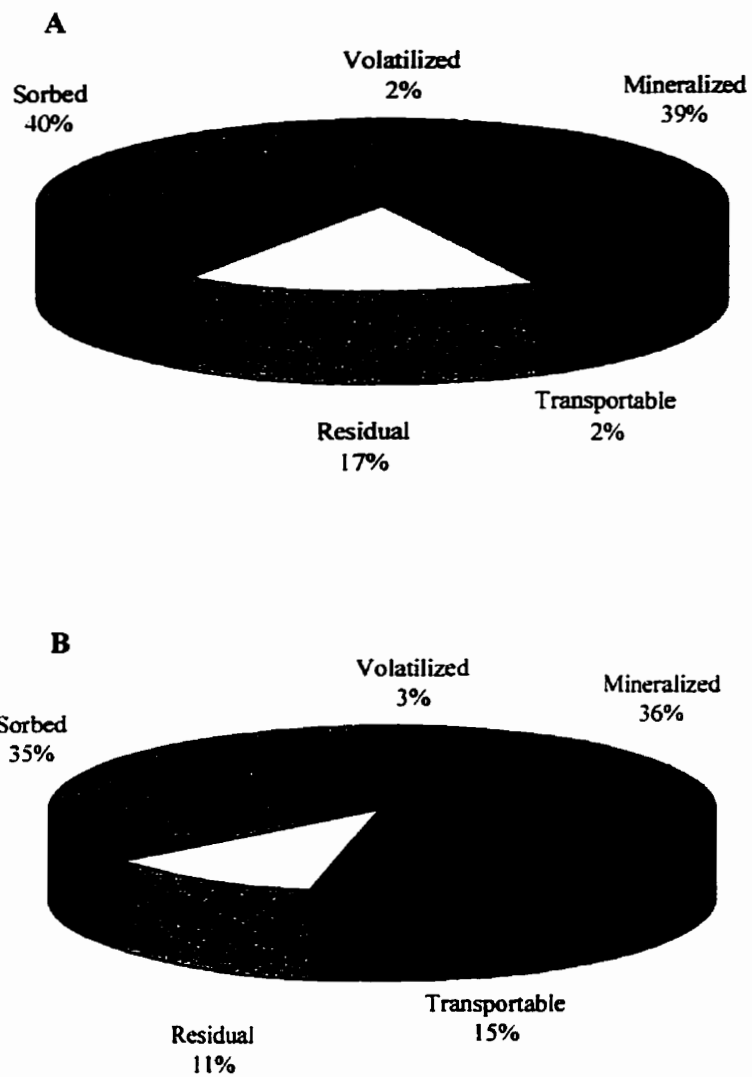
Figure 3.16 Fate of radiolabeled phenanthrene in soil microcosms with previous
contamination of hydrocarbons. (A = surface, B = subsurface)

3.5.3 Drying of Soil and Further Addition of ^{14}C Phenanthrene

After 259 days of incubation with phenanthrene the soils were split into 2 portions of approximately 30g. One set was allowed to remain unaltered while the other was dried, ground and wetted to determine the potential increase in mineralization as a result of disturbance. After further incubation (36 days) the soils were again spiked with ^{14}C phenanthrene in diesel and monitored for mineralization to determine the effects of prior exposure and prolonged incubation on microorganism properties (total of 343 days).

3.5.3.1 Continuously Wet Phenanthrene Mineralization The mineralization of the second phenanthrene addition was minimal (Table 3.21). After about 12 weeks from the initial end of the experiment, all of the continuous wet soils had less than 5% of the phenanthrene mineralized. The readdition of 5000 ppm diesel after 36 days from the start of the second experiment caused little mineralization (day 295). After about 295 days of incubation with ^{14}C phenanthrene in diesel fuel, the second readdition had no effect. Even the sites with prior hydrocarbon exposure had little or no mineralization. Site #10 had greater than 40% mineralization in the initial experiment and less than 1% after the readdition of phenanthrene. The results may indicate the microbial populations in these microcosms after extended incubations (>1 year) start to loose the ability to degrade phenanthrene. In the case of Site #10, the initial mineralization of phenanthrene was very rapid with the majority of the mineralization occurring in the first 14 days of addition. This indicates the soil microbial biomass has probably incubated for about 40 weeks without interacting with any form of phenanthrene. In this case it would be conceivable to believe the organisms "lost" the ability to mineralize phenanthrene without the substrate present, or organisms able to degrade the compound were selected out of the microflora

due to selective advantages. This could also happen in the environment and may explain the poor mineralization of Site #8 in the previous section. The other 9 soils had poor mineralization of phenanthrene after the initial addition. This indicates the populations were unable to express the appropriate enzymes for mineralization. If phenanthrene sorbs to the soil matrix and becomes unavailable, the microbial populations may not have induced enzymes to mineralize the compound. If phenanthrene enzymes are constitutively produced then the results would indicate there were no organisms present with the potential to mineralize the compound.

3.5.3.2 Drying, Grinding and Wetting of the Soil The drying of the soil, grinding, and subsequent wetting caused a minor increase in the mineralization of phenanthrene (Table 3.19). The addition of phenanthrene after five weeks of incubation had little effect on the mineralization of phenanthrene. There was less than 1% increase in mineralization once it was added and monitored for 48 days. The results indicate there was little effect of drying for 2 days, grinding and wetting of the soil on further mineralization of phenanthrene. Amoto et al. (1983) indicated the importance of the type and extent of drying on the microbial degradation capacity. They found the greater the frequency of drying and rewetting (3 weeks wet-1 week dry) increased decomposition of plant parts compared to continuously wet soil. In this experiment the soil environment may not experience the same effects wetting and drying has if only one drying event has occurred. One drying event may not supplement the soil with added nutrients, therefore downplaying the burst of microbial activity as compared to a soil that is dried and wetted on a regular basis.

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Table 3.19 The mineralization of ^{14}C phenanthrene in 5000 ppm diesel fuel in soils maintained continuous wet and in soils dried, ground and rewetted for 84 days.

Site	%^{14}C Phenanthrene Mineralized in Continuous Wet Soil	%^{14}C Phenanthrene Mineralized in Dried and Ground Soil	%^{14}C Phenanthrene Mineralized in Continuous Wet Soil After Second Spike of Diesel	%^{14}C Phenanthrene Mineralized in Dried and Ground Soil After Second Spike of Diesel
Site #1 0-10 cm	0.24 ± 0.41	0.08 ± 0.04	0.90 ± 0.35	0.78 ± 0.07
90-100 cm	0.09 ± 0.03	0.22 ± 0.1	0.80 ± 0.54	0.76 ± 0.13
Site #2 0-10 cm	0.06 ± 0.02	0.06 ± 0.02	0.60 ± 0.1	0.78 ± 0.14
90-100 cm	0.09 ± 0.05	0.17 ± 0.08	0.90 ± 0.26	1.33 ± 0.15
Site #3 0-10 cm	0.05 ± 0.01	0.07 ± 0.01	0.66 ± 0.08	1.01 ± 0.19
90-100 cm	0.12 ± 0.11	0.16 ± 0.06	0.74 ± 0.16	1.52 ± 0.2
Site #4 0-10 cm	0.71 ± 0.43	0.12 ± 0.09	1.19 ± 0.44	1.23 ± 0.3
90-100 cm	0.23 ± 0.31	0.19 ± 0.16	0.71 ± 0.42	1.45 ± 0.5
Site #5 0-10 cm	0.04 ± 0.01	0.12 ± 0.15	4.31 ± 7.84	0.79 ± 0.4
90-100 cm	0.07 ± 0.02	0.10 ± 0.04	0.57 ± 0.02	0.95 ± 0.49
Site #6 0-10 cm	0.06 ± 0.03	0.12 ± 0.01	0.67 ± 0.15	1.02 ± 0.06
90-100 cm	0.07 ± 0.17	0.13 ± 0.03	0.86 ± 0.22	1.19 ± 0.23
Site #7 0-10 cm	0.10 ± 0.03	0.11 ± 0.02	0.71 ± 0.19	1.28 ± 0.07
90-100 cm	0.38 ± 0.45	0.18 ± 0.03	0.90 ± 0.55	1.49 ± 0.21
Site #8 0-1 m	0.41 ± 0.02	0.10 ± 0.03	0.88 ± 0.08	1.37 ± 0.26
1-2 m	0.21 ± 0.03	0.13 ± 0.06	0.60 ± 0.09	0.83 ± 0.16
Site #9 0-10 cm^P	0.05 ± 0.51	0.14 ± 0.27	4.43 ± 0.41	1.17 ± 0.2
90-100 cm^P	0.04 ± 0.02	0.07 ± 0.06	0.38 ± 0.16	0.47 ± 0.22
Site #10 0-10 cm	0.06 ± 0.72	0.07 ± 0.37	0.44 ± 0.73	0.65 ± 0.28
90-100 cm	0.15 ± 0.2	0.04 ± 0.23	0.62 ± 0.22	0.73 ± 0.15

^{P}Site #9 (no previous hydrocarbon exposure) was a control for Site #10 (previous hydrocarbon exposure)

3.6 Conclusions

Microorganisms capable of mineralization of phenanthrene were found in all soils sampled. The results tend to indicate freshly sampled soils in Manitoba without prior hydrocarbon exposure had low mineralization in soil microcosms. Where significant mineralization occurred in soils, the results tended to indicate the mineralization was a sporadic occurrence with only two sites having greater than 5% mineralization. Total phenanthrene mineralization in the surface was not significantly different from the subsurface without prior hydrocarbon exposure. This does not mean the subsurface will always have the same potential as the surface because where there was significant mineralization (>5%) the surface had a higher mineralization rate. The rates of mineralization were significantly different between the surface and subsurface. This indicates there was greater activity of the microorganisms mineralizing the available phenanthrene where organic carbon may be found.

The prior exposure of freshly sampled soil to hydrocarbons tended to increase mineralization and decrease half lives. In two soils examined with prior sensitization, one had rapid mineralization while the other had significantly less. The results indicate the occurrence of a hydrocarbon spill does not guarantee significant mineralization but may help in prior risk assessment. Other factors are involved to enhance this process.

Volatilization will be an insignificant fate of phenanthrene in the environment. It was greater in subsurface soils compared to the surface. This was most likely an effect of organic matter. Because subsurface soils have less organic matter, there will be less specific adsorption of the hydrophobic phenanthrene. With less adsorption of the molecule

there will be a tendency for it to enter the vapour phase more readily than if it were adsorbed to organic matter.

The final sequential extraction of the residual ^{14}C indicates the major fate of this molecule in the environment will be adsorption and possibly humification. This indicates that once phenanthrene was introduced it remained adsorbed preventing mineralization. The reduced bioavailability would result in phenanthrene accumulating in the environment. The inability of microorganisms to access this source of substrate could be removed by the use of surfactants desorbing phenanthrene making it more available for mineralization.

Texture, organic carbon, and metabolic diversity were poor indicators of phenanthrene mineralization. The results indicate that another factor or a combination of factors (type and richness of organic matter, presence of microbial populations etc.) influence the mineralization of phenanthrene (Smith et al. 1993). Organic carbon and metabolic diversity were good indicators of the mineralization rate. This property of the soil organic carbon and biomass may be used in prior risk assessment to determine the potential for phenanthrene mineralization in soil. Organic carbon was a good indicator of phenanthrene adsorption to soil particles. As the amount of organic carbon increased the adsorption increased and bioavailability decreased. It would be recommended to determine the organic carbon content of a spill site in prior risk assessment as it will both affect the movement and mineralization of hydrocarbons.

The handling of soil prior to and during remediation studies may not simulate *in situ* mineralization. Drying and grinding of the soil had little effect on the further mineralization of phenanthrene in diesel fuel. Though there was a marginal increase in mineralization, there was still less than 2% difference in phenanthrene mineralization.

There also appears to be an effect of prolonged incubation of soil in microcosms. The second addition of phenanthrene had little effect on the production of $^{14}CO_2$. In other words some soils (Sites #10, #4 and #9) appeared to "lose" the ability to degrade significant amounts of phenanthrene. The organisms capable of mineralization may have been selected out of the surviving biomass after one year of incubation.

CHAPTER 4

Effect of Landscape Variation on the Mineralization of Phenanthrene
in Diesel Fuel #2 Contaminated Soil.

4.1 Abstract

Cores were collected from the surface (0-50 cm) at the upper, upper-mid, lower-mid, lower, and depressional slope positions to determine the fate of phenanthrene in diesel fuel. Subsurface (50-100 cm) samples were also collected at the lower-mid and depressional positions to characterize fates in the parent material. As a result of differences in landscape positions the organic matter contents, texture and water regime of the soils sampled varied dramatically. Soil samples were also taken for a parallel microcosm experiment to compare the differences, if any, in the two experimental designs. Radiolabeled phenanthrene was added along with sufficient diesel fuel to contaminate the upper 15 cm of the core to a concentration of 51 mg of diesel fuel g^{-1} soil in order to assess the degradation potential of a severe spill ($\sim$100,000 kg ha^{-1}). Volatilization and movement of phenanthrene and diesel in the soil profile was also determined. The results indicated the major fate of phenanthrene was adsorption with mineralization comprising a small percentage (<10%). Intact soil columns had greater mineralization than corresponding microcosms indicating a possible role of soil structure and aeration in mineralization of phenanthrene. Surface soils also had greater mineralization than the

subsurface and diesel addition to soils increased respiration of the microbial biomass. The total extractable diesel fuel in the soil columns at the end of the experiment ranged between 98.3 and 74.7% recovered in the subsurface and 91.8 and 56.5 % in the surface indicating degradation of the total hydrocarbons may be a major fate.

4.2 Introduction

The contamination of soil and water with diesel fuel is a widespread problem (Keuth and Rehm 1991; McGill 1976; Atlas and Cerniglia 1995; Brodkorb and Legge 1992; Rainwater et al. 1993). Leakage of storage tanks and tankers on land and sea create an environmental catastrophe affecting wildlife and human health. It is important to be able to determine the major fate of the components of diesel fuel to prevent further exposure. To ensure public safety, the knowledge of the fate of diesel fuel when spilled into a soil landscape will enable a more cost effective remediation strategy (Atlas and Cerniglia 1995). If a spill is localized and has no potential for further contamination, *in situ* bioremediation may be the best option.

Soil is a complex system with many factors controlling the potential for bioremediation (Scow 1990; Rainwater et al. 1993). The relationship between the activity of the microbial population and availability of water is an important controlling factor in bioremediation (Alters and Bartha 1993; West et al. 1989). In a catena, slope position influences the soil water status. Hanna et al. (1982) demonstrated that depressions have more available water than upper slope positions indicating a greater potential for microbial activity. If over saturation of water occurs in the depression, aerobic activity may decrease

resulting in reduced conditions which may result in less microbial activity. Available water is influenced by the amounts of organic matter and texture of the soil. In the depression the water table is closer to the surface and can have greater amounts of clay and organic matter due to runoff. Van Kessel et al. (1993) also demonstrated various slope positions have a controlling effect on the microbial process of denitrification. The denitrification was higher in the depression (157 to 556 g N ha^{-1} d^{-1}) than on the knoll (37 to 302 g N ha^{-1} d^{-1}) signaling the effect of texture, organic matter and location of the water table in a landscape on microbial processes. In this example the availability of oxygen was a controlling factor in the microbial denitrification. Diesel fuel degradation is mainly an aerobic pathway (Atlas and Bartha 1993), therefore the depression may have significantly less bioremediation yet have the majority of the diesel present due to runoff. The gradual change in clay contents and organic matter in a catena will also affect the microbial metabolism and availability of diesel components. Increasing clay content and organic matter results in a higher potential for adsorption thus decreasing the bioavailability of hydrocarbons. Sampling the 50-100 cm depth where organic matter and nutrients are sparse, allows the determination of the fate of diesel in subsurface environments.

The method of sampling soil can influence the bioremediation potential. Grab sampling alters the soil physical properties by disrupting micro and macro pore channels. The disruption may change the remediation properties of the soil. Pivetz et al. (1996) found preferential flow columns had greater degradation of PNP (p-nitrophenol) than soils with few or no channels. Rainwater et al. (1993), Widrig et al. (1995) and Devare and Alexander (1995) also stress the importance of intact soil columns in insuring more consistent results when mimicking the environment. Intact soil columns also allow the

researcher to monitor not only mineralization but volatilization, adsorption and transport (Phelps et al. 1994). Microcosms, on the other hand, cannot measure the transport of a contaminant through a soil profile. In the end, determining the effect sampling and the changing slope positions have on the degradation of diesel fuel will be important in risk assessment in the event a spill does occur.

4.3 Objective of Study

The objective of this study was to examine the effect of landscape position on the fate of diesel fuel containing ^{14}C phenanthrene in intact soil cores.

4.4 Materials and Methods

4.4.1 Soil Landscape

The site selected for the study was located at the Manitoba Zero Till Research Farm located North of Brandon, Manitoba. The south facing catena was under conventional till at the time with the previous years crop being wheat. The soils ranged from a Rego Black at the top of the knoll to Humic Luvic Gleysol in the depression (Figure 4.1, Figure 4.2). Five sites were selected along the landscape with two subsurface samples giving a total of 7 sites. Sites were divided into 5 reps with 3 feet separating the sample positions. Cores were inserted into the ground using a tractor equipped with a front end loader. Compaction of about 10 cm was noted for each 50 cm core. The cores were excavated then sealed with plastic bags and stored in a cool place until transport back

to the lab. Along with the core samples, grab samples were also taken for microcosm studies at 0-10, 40-50, 50-60, and 90-100 cm depths. The soil was stored in plastic bags and maintained in coolers to prevent temperature changes. Once transport to the lab was complete, the soil was then stored at 15°C until the start of the experiment. This temperature was selected because it was considered the average Manitoba temperature at the depth of 50 cm (Krpan 1982). The particle size distribution and organic carbon composition are listed in Table 4.1 along with other soil properties. Table 4.2 indicates the profile characterization of the soils at each site.

Rego Black

Rego Black

Orthic Black

Location 1

Location 2   Location 3a

Orthic Black

Location 3b

Humic Luvic Gleysol

Site #5   Subsurface   Location 4

Location 5a

Location 5b

Subsurface
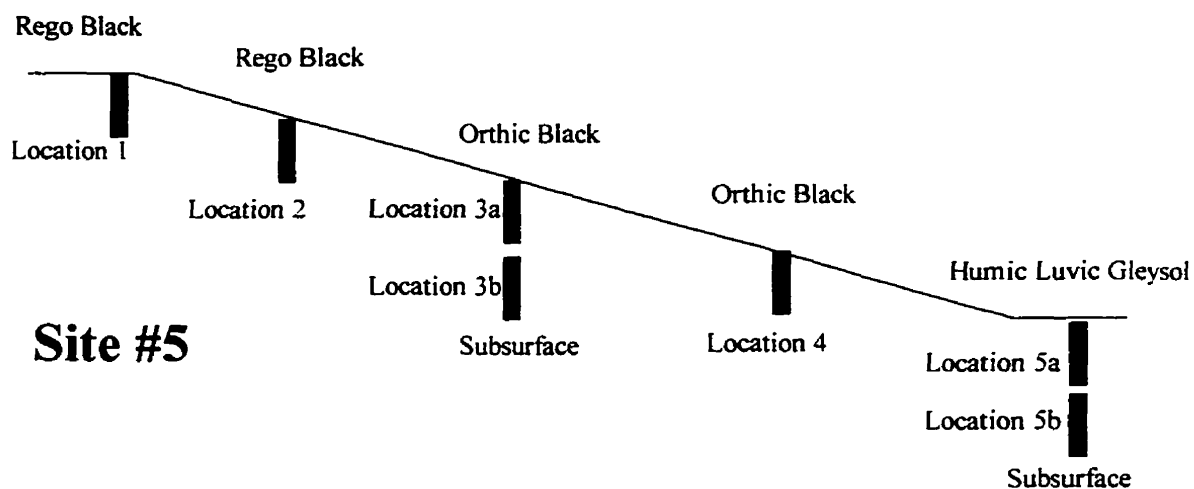
Figure 4.1   Stylized diagram of a slope transect of site where intact soil columns were sampled.

Table 4.1 Properties of Soils Studied Along Landscape.

Site	Soil Classification	Texture				Field Capacity	% Organic Carbon
		% Sand	% Silt	%Clay	Class		
Site 5-1	Rego Black	28.5	33.5	38	CL*	35.6 ± 2.1	4.3 ± 0.1
Site 5-2	Rego Black	28.5	33.5	38	CL	37.4 ± 2.2	4.6 ± 0.1
Site 5-3a	Orthic Black	28.5	33.5	38	CL	34.4 ± 2.4	5.7 ± 0.1
Site 5-3b	Subsurface	32.5	34.5	33	CL	21.9 ± 1.4	0.6 ± 0.3
Site 5-4	Orthic Black	28.5	33.5	38	CL	39.4 ± 3.1	5.7 ± 0.1
Site 5-5a	Humic Luvic Gleysol	31.5	35.5	33	CL	30.7 ± 6.4	4.7 ± 0.3
Site 5-5b	Subsurface	34.5	30.5	35	CL	29.4 ± 1.3	0.4 ± 0.2

*CL represents Clay Loam

Table 4.2 Soil Profile Characterization of Landscape.

Site	Classification	Profile	
Site 5-1	Rego Black	Ahp	0-10 cm
		ACk	10-23 cm
		Ck	23+ cm
Site 5-2	Rego Black	Ahp	0-15 cm
		ACk	15-30 cm
		Ck	30+ cm
Site 5-3a	Orthic Black	Ah	0-30 cm
		Bm	30-45 cm
		Cca	45-50 cm
Site 5-3b	Subsurface	Cca	50-55 cm
		Ck	55+ cm
Site 5-4	Orthic Black	Ah	0-45 cm
		Bm	45-100 cm
		BC	100-110 cm
		Ckg	110+ cm
		(Water table at 110 cm)	
Site 5-5a	Humic Luvic Gleysol	Ah	0-35 cm
		Bmg	35-45 cm
		Cg	45-50 cm
Site 5-5b	Subsurface	Cg	50+ cm
		(Water table at 100 cm)	
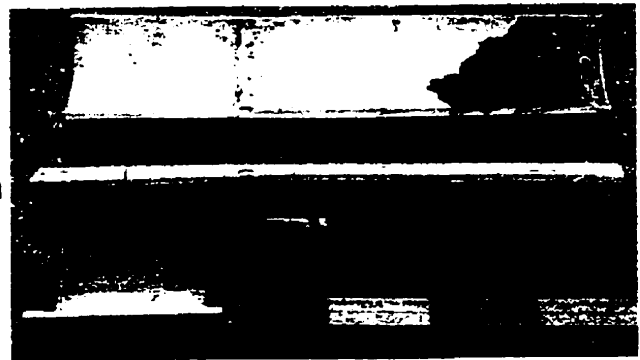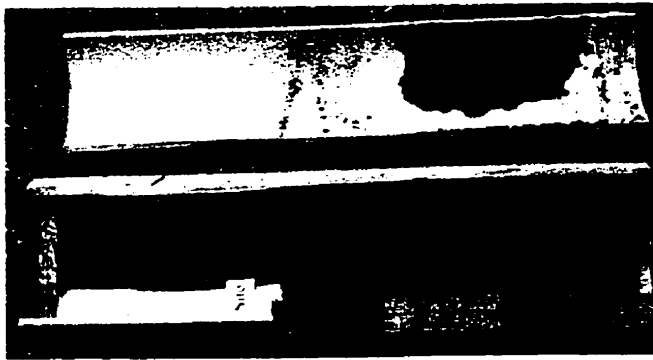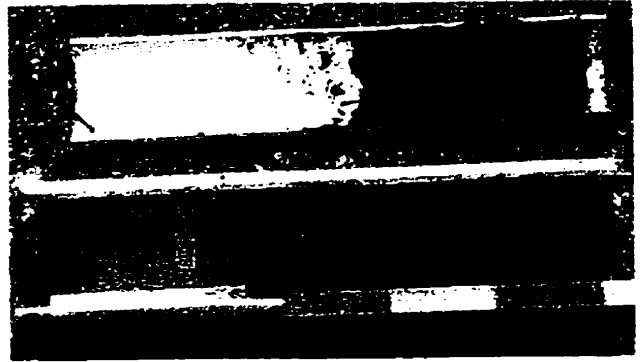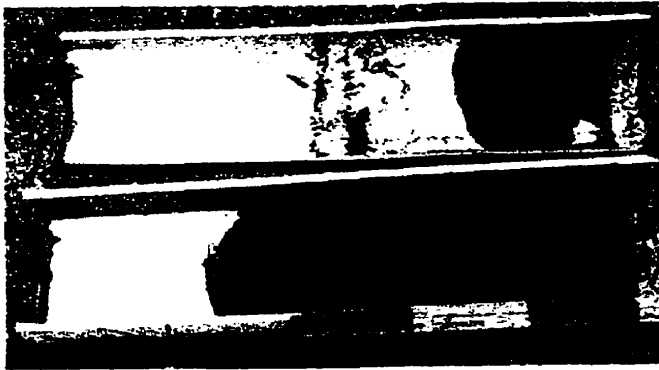
Figure 4.2. Images of intact soil columns prior to destructive sampling. (A=Site 5-1, B=Site 5-2, C=Site 5-3a, D=Site 5-3b, E=Site 5-4, F=Site 5-5a, G=Site 5-5b)

4.4.2 Soil Columns

PVC columns consisted of a 60 cm length of 15 cm diameter by 0.5 cm thick wall. The columns were cut into 60 cm lengths using a motorized hacksaw to allow a 10 cm air space on top of the core between the soil surface and the lid. The bottom edge of each core was sharpened to ease insertion into the soil. Once the soil was sampled, 5 cm of the base of the core was excavated and a septum was attached by drilling a hole about 2.5 cm from the bottom. Once the septum was glued in place the remainder of the base was filled with coarse sand to allow sampling of any liquids reaching the bottom. The bottom was then sealed air tight with a 20 x 20 cm piece of plexiglas (0.5 cm thickness). To the top of the core a plexiglas lid was attached and sealed air tight with glue and silicone. The lid consisted of an inlet and outlet for air to enter and leave and a septum containing a Suba Seal #29 to allow water and diesel fuel addition via a syringe (Figure 4.3). A fish tank pump delivered air to the cores for 343 days at a rate of 3.2 L/hr and changed the headspace of the core about twice an hour. Prior to entering the core the air was bubbled through water to humidify and prevent core drying. Outflow air was then passed through a tube containing a polyurethane foam (PUF) plug to trap any ^{14}C-phenanthrene (EPA §796.3400). At various times a 1cc sample of the air inside the core was analyzed by GC to make sure the soil environment was aerobic. The columns were then allowed to incubate for one week at 15 ± 2°C prior to addition of the diesel. At day 63, a 1.27 cm (230ml/core) simulated rainfall was applied to the soil with distilled water in order to check whether this would stimulate mineralization.
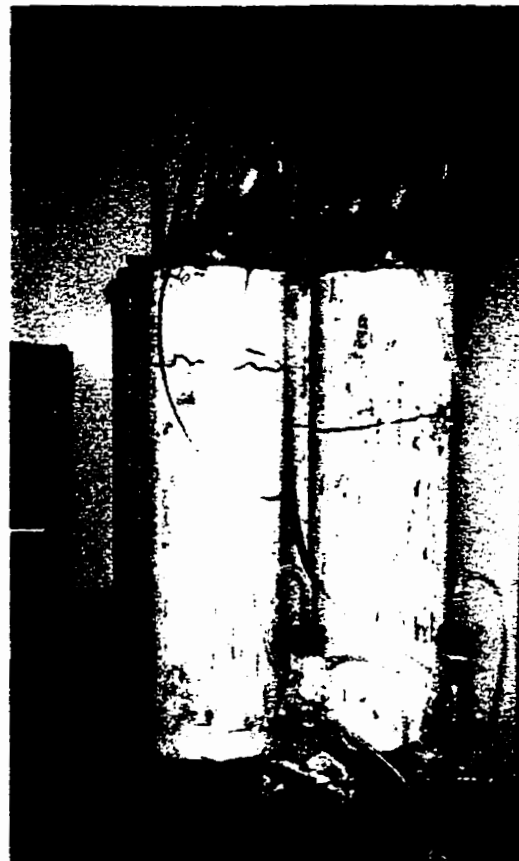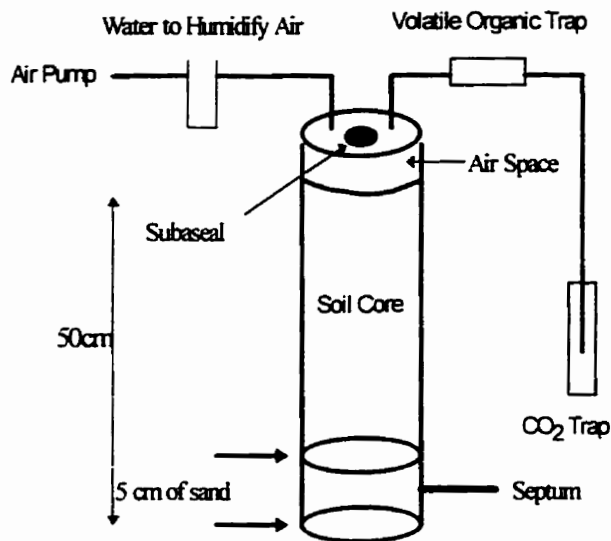
Figure 4.3 Intact Soil Column used in diesel fuel remediation study.

4.4.3 Soil Microcosms

Microcosms were purchased from Richards Packaging (Winnipeg, MB) as 500 mL glass jars with sealed metal lids. To the internal base of each microcosm a 3 cm length of polyvinylchloride (PVC) was attached using silicone to hold a 7 mL scintillation vial for $^{14}CO_2$ trapping. A polyurethane foam plug (PUF) was added to trap any volatile phenanthrene and a 20 mL scintillation vial containing 10 mL water (pH 3) was included to humidify the air. The lid of the microcosm also contained a sampling port (Rubber Suba Seal #9) to allow the sampling of gas. At various times a 1cc sample of the air inside the microcosm was analyzed by GC to make sure the soil environment was aerobic. The field moist soil equivalent to 40 g oven dry mass was added to 50 mL glass beakers then

brought to field capacity. The beaker was inserted into a microcosm and incubated at 15 ±

2°C for one week to allow equilibration of the microbial biomass.

4.4.4 ^{14}C Phenanthrene and Diesel Fuel

^{14}C Phenanthrene was purchased from the Sigma Chemical Co. (St. Louis, MO) as

Phenanthrene-9-^{14}C (8.3 nCi/mmol) (Figure 4.4). Stock solutions were first made up in

hexane then transferred to diesel fuel #2 for addition to soil. At the commencement of

each experiment 202 mL or 171.7 g of diesel-^{14}C Phenanthrene mixture was added to each

core with approximately 1.1 µCi of activity (4 cores with diesel and one control per site).

The microcosms each received 2.4 mL or 2.04 g of stock diesel with 0.05µCi of activity.

Stock diesel fuel added contained about 7.34 µg unlabelled and 0.609 µg of labeled

phenanthrene per gram of soil. The addition of diesel fuel was to mimic what would be

categorized as a severe spill (McGill 1976). The concentration of diesel fuel added to the

soil was 50 000 µg/g soil (100 000 kg/ha in the top 15 cm of the core).
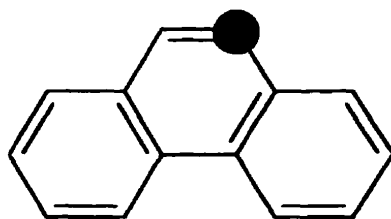
Figure 4.4 ^{14}C Phenanthrene added to intact soil columns and soil microcosms.

4.4.5 ^{14}CO$_2$ Microcosm Traps

Radioactive carbon dioxide was trapped with 0.5 mL (+/-)-α-phenylethylamine and

0.5 mL methanol in a 7 mL scintillation vial (the methanol prevents crystallization).

During the experiment, traps were changed at weekly intervals until there was no longer

any significant radioactivity recovered (<0.1% of ^{14}C added). At the end of the experiment 5 g of soil was removed and combined with 20 mL 1M HCl in a 50 mL beaker and placed in a separate microcosm. A 1 mL phenylethylamine-methanol CO_2 trap was included to trap any residual ^{14}C remaining in the soil as carbonates. The trap was then counted in the scintillation counter and added on to the overall mineralization value.

4.4.6 Volatilization

When the experiment was completed, the polyurethane foam plugs were removed from the apparatus and placed in 100 mL French Square Bottles (EPA §796.3400). To these bottles 20 mL Methanol was added, making sure 5 mL was in excess, for total extraction of the phenanthrene. Bottles were then stopperd and shaken on a lateral shaker for 2 minutes. The foam plugs were then removed and placed in a 50 mL syringe to extract all of the methanol. From the extract, a 1 mL subsample was collected and counted by the scintillation counter.

4.4.7 $^{14}CO_2$ and Total CO_2 Traps for Cores

The out flowing air stream bubbled in 30 mL of 2M NaOH placed in a glass 50 mL test tube. These tubes trapped not only radioactive CO_2 but total CO_2 produced in the cores. At weekly intervals the traps were changed and 0.3 mL was removed and placed in a 20 mL scintillation vial with 15 mL scintillation fluid and counted. The remaining NaOH (29.7 mL) was placed in a 100 mL titrator cup with 10 mL of 15% $BaCl_2$. $BaCl_2$ reacts with the remaining carbonates in solution forming a precipitate (Anderson 1982). The following equation demonstrates the reaction process:

$$CO_2 + H_2O \longleftrightarrow H_2CO_3 \longleftrightarrow HCO_3^- + H^+ \longleftrightarrow CO_3^{-2} + 2H^+$$
$$BaCl_2 + CO_3^{-2} \longrightarrow BaCO_{3(ppt)} + 2Cl^-$$
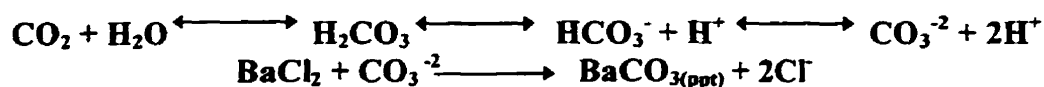
Equation 4.1

The remaining base is then titrated to pH 8.5 with 1 M HCl to determine the amount of CO_2 introduced into solution.

(Titer of Blank) - (Titer of Sample) = (OH⁻ Neutralized by H⁺ from CO_2 Introduction)

Equation 4.2

The amount of CO_2 production from microbial activity is then calculated by subtracting the amount of CO_2 that would be trapped from atmospheric origin and not respiration. Atmospheric carbon dioxide was calculated by running a separate air pump without soil.

(OH⁻ Neutralized by H⁺ from CO_2 Introduction) - (Titer of Air Blank) = (Titer of Respiration)

Equation 4.3

The amount of carbon evolved from the soil is then calculated by:

mg C = (Titer of Respiration) * [mol/L (HCl) * 6 mg C/mmol H⁺]
mg C = mL Titre * mmoles H⁺/mL * 12 mgC/2 mmoles H⁺

Equation 4.4

To determine the efficiency of radioactive and total CO_2 production, a NaOH tube attached to a core was sealed and a second air line was run to another trap. This was allowed to bubble for a week and sampled. The result indicated a 95% efficiency of trapping of total CO_2.

At the end of the experiment the residual ^{14}C remaining in the soil as carbonates was determined. 10 g of soil was removed from every 10 cm interval of the core and combined with 20 mL 1M HCl in a 50 mL beaker and placed in a separate microcosm. A 1 mL phenylethylamine-methanol CO_2 trap was used to collect the CO_2 released. The trap

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was then counted in the scintillation counter and added on to the overall mineralization value at the end of the experiment.

4.4.8 Liquid Scintillation Counting

Each 1 mL CO_2 trap removed from the microcosm was combined with 5 mL Ecolite (+) Liquid Scintillation Fluid (ICN Biochemicals Inc. Aurora, OH). The 0.3 mL NaOH sampled from the cores was placed in a 20 mL scintillation vial and combined with 15 mL Ecolume Liquid Scintillation Fluid (ICN Biochemicals Inc. Aurora, OH). The cocktails were then allowed to equilibrate in the dark for 24 hours before counting to prevent any chemiluminescence from ion interaction with the scintillation fluid. A Beckman LS 7500 scintillation counter was implemented with quench curve correction to give final results of disintegrations per minute (DPM). Final DPMs were corrected for background and controls then related to the original radioactivity added to each apparatus to give the percent mineralization of phenanthrene in diesel.

4.4.9 Core Destructive Sampling

At the end of the experiment, the air stream was shut off and the lids and bases were removed. The 5 cm of sand on the bottom of the cores was bagged and stored. The bottom and top of the core were then plugged to prevent disruption and cut lengthwise (vertically) with a skillsaw exposing the soil profile (Figure 4.5). The soil was then sampled every 10 cm and bagged for later analysis.

Figure 4.5 Destructive sampling of cores after 343 day experiment.

4.4.10 Movement of Phenanthrene

To determine whether preferential flow along the sides of the core had occurred, the bottom 10cm (30-40cm or 40-50cm depths) of 12 cores were sampled prior to destructive sampling using a 20cm by 2.5cm diameter PVC tube. The sampling device was inserted with a hammer at the center of the core and at the edge along the core wall (Figure 4.6). This soil was sealed in a plastic bag and incubated at 15°C until analysis. The analysis of ^{14}C was conducted using a wet digestion technique (Voroney et al. 1991). 0.8 g of soil was placed in a wet digestion tube along with 6 mL of digestion solution (189.4 g CrO_3 in 250 mL 14.7M H_3PO_4 and 500 mL 18M H_2SO_4). A glass rod stand to hold 2 mL of 2M NaOH in a 6 mL glass scintillation vial (NaOH traps $^{14}CO_2$ liberated from oxidation of organic carbon) was also added. The tube was then sealed with a #49 Rubber Suba Seal and placed in a digestion block and heated at 145°C for 1 hour. The $^{14}CO_2$ was then trapped for 24 hours after which a 0.3 mL subsample was removed and combined with scintillation fluid and analyzed for radioactivity. The center (x) and side (y)

results were then plotted against each other and an equation of a line was determined. If the slope was much greater than 1, preferential flow occurred. If the slope was equal to one, uniform transport would be assumed.
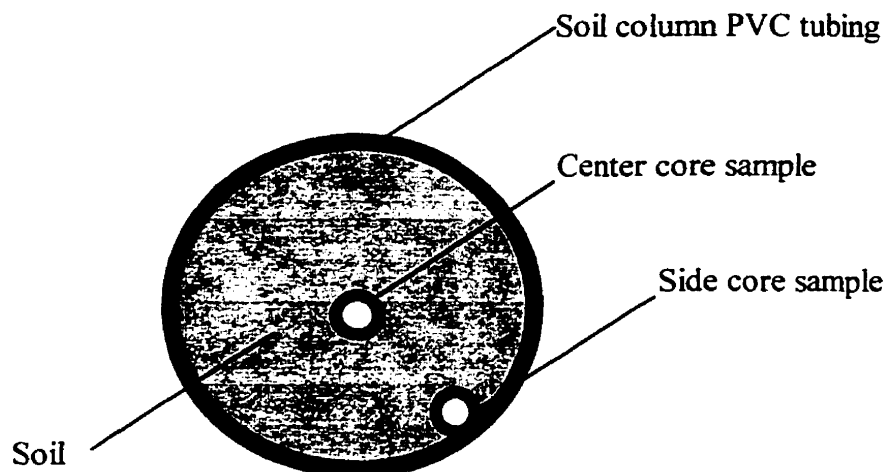
Figure 4.6 Soil sampling in the determination of preferential flow of ^{14}C in intact soil columns. Samples were taken at the center and side of the PVC tubing.

4.4.11 Kinetic Analysis of $^{14}CO_2$ Evolution.

The first order rate model was used to determine the rate of mineralization of phenanthrene in diesel #2. The calculation describes the reaction over time as a single first order component:

$$P = A[1-e^{(-kt)}]$$

Equation 4.5

P is the cumulative percent phenanthrene mineralized at time t, A is the percent of compound evolved as CO_2 at t = ∞, and k is the rate constant for $^{14}CO_2$ evolution (day^{-1}). Once the rate constant was determined a mineralization half life can be calculated using the following equation:

$$t_{1/2} = \ln 2 \, / \, k$$

<div align="right">**Equation 4.6**</div>

The first order decay rate was determined for each replicate and statistical analysis performed on the parameter estimates.

4.4.12 Sequential Extraction of ^{14}C from Soil

Residual ^{14}C was extracted to determine the properties of the remaining fractions. A step-wise extraction using water, methanol, and dichloromethane (soxhlet) followed by total digestion of the organic carbon was used to recover any radioactivity remaining in the soil. The water extractable ^{14}C would indicate the possible free phenanthrene and degradation products in soil. These fractions would then be considered in the mobile phase. The methanol extract would contain the weakly associated phenanthrene and degradation products sorbed to soil. This fraction would be considered no longer bioavailable and have some potential for desorption. The soxhlet extractable fraction would contain the highly sorbed and stable ^{14}C. Wet digestible ^{14}C would be considered residual and may be in the humified fraction in the soil. At the end of the experiment, 10g of oven dry equivalent soil was removed from each depth of the sampled cores (including the sand fraction) and microcosms and placed in 50 mL acid washed Teflon tubes (Knaebel et al. 1994). To this tube 25 mL of 10 mM NaN$_3$ (2.5:1 ratio of soil to sterilisant) was added along with 5 glass beads to aid in agitation. The tubes were then vortexed for 10 seconds and placed on a lateral shaker for 24 hours. The next day the tubes were centrifuged at 12,000 xg for 15 minutes then the supernatant was removed and a 1 mL subsample was analyzed in a scintillation counter. The remaining pellet was then combined with 25 mL of methanol, and subjected to the same procedure as the above.

After pouring off the methanol, the pellet was allowed to air dry to aid in the transfer of the soil into cellulose extraction thimbles (EPA Method 3540A). The thimble containing soil was placed in a soxhlet extraction apparatus and extracted for 24 hours with 100 mL methylene chloride and 3 boiling chips. After the 24 hour extraction, the solvent was removed from the extraction vessel and a 1 mL subsample was counted in a liquid scintillation counter. Again the soil was allowed to air dry to aid in transfer of 0.8 g of soil into a wet digestion tube (Voroney et al. 1991). The $^{14}CO_2$ was then trapped for 24 hours after which a 0.3 mL subsample was removed and combined with scintillation fluid and analyzed for radioactivity. The above procedure was also run on controls using a spiked sample of ^{14}C phenanthrene to determine the efficiency of extraction. The controls were run in triplicate and consisted of a surface soil and a sand sample much the same as what was placed in base of the cores.

4.4.13 Total Extractable Hydrocarbons

The Total extractable hydrocarbon (TEH) content was determined for each sample depth of the core experiment including the sand at the base of the core. 10g of oven dry equivalent soil was removed from each 10 cm depth of the core and placed in 50 mL acid washed Teflon tubes (Knaebel et al. 1994). To these tubes 10 g of sodium sulfate was added to prevent clumping and aide in extraction of the diesel. Next, 25 mL of dichloromethane (DCM) was added and vortexed for 30 seconds then shaken on a lateral shaker for 2 hours. After 2 hours, the test tubes were allowed to sit for 30 minutes to allow the soil to settle. A 1 mL subsample was removed and placed in a 2 mL glass screw cap vial with a Teflon lined septum. The samples were then stored at 4°C for no more than a week prior to GC analysis.

The analysis of the extract was performed using a Varian Star 3400Cx with a temperature programmed GC-FID. The sample vials were allowed to reach room temperature then placed in a 48 vial Varian 8200Cx Autosampler. A 1 μL sample was automatically drawn with an upper and lower air gap from each vial with a 10 μL syringe then injected into the GC inlet (Injector = 200°C, Detector = 300°C). The sample syringe was then washed with DCM for 10 seconds after each sampling event to prevent contamination of runs. A Clot (Carbon Layer Open Tubular) column with a 30 m length, 320μm diameter, and 0.25μm thickness (Serial #41597-6) was used to analyze the hydrocarbons. Compressed air (300 mL/min), the carrier gas (prepurified helium 1.2 mL/min), and the fuel (prepurified hydrogen 3 mL/min) were also used in the analysis. Each injection was run on a preset program for one hour using the Star Chromatography Software (Copyright® 1989-1995, Varian Associates, Inc., v. 4.02). The column was set to begin at 50°C and held for two minutes. The rate increased 5°C/min from 50-250°C (at 250°C held for 5 minutes), 3°C/min from 250-280°C until 60 minutes was up. The area under the chromatogram was determined and reported for each DCM extract and standard analyzed. Standards were run in quadruplicate and ranged between 1 and 35μg/μL injection (Table 4.3). A standard curve was plotted (r^2 = 0.9809) and an equation of a line was determined ($y = 15301x^{1.5161}$) (Figure 4.7). Uncontaminated surface and subsurface soils were also run to determine the "background" areas. These values were averaged and subtracted from the areas of the unknown soil extracts to determine the total extractable hydrocarbons.

Table 4.3 Standard curve determined for total extractable hydrocarbon analysis using a Varian Star 3400Cx GC. One μL standards were injected using a Varian 8200Cx Autosampler.

Diesel Injection (μg Diesel / μL Injection)	Concentration (mg/L)	Total Area Under the Chromatogram Curve
1	1000	17222.3 ± 5163.7
5	5000	168682.7 ± 74689.4
10	10000	480286.8 ± 141712.5
20	20000	1550293.8 ± 2198179.2
35	35000	3463466.8 ± 130758.6
Soil Sample Extract		
Soil (Surface)	---	11749.3 ± 12813.9
Soil (Subsurface)	---	723 ± 585

$$y = 15301x^{1.5161}$$
$$R^2 = 0.9809$$
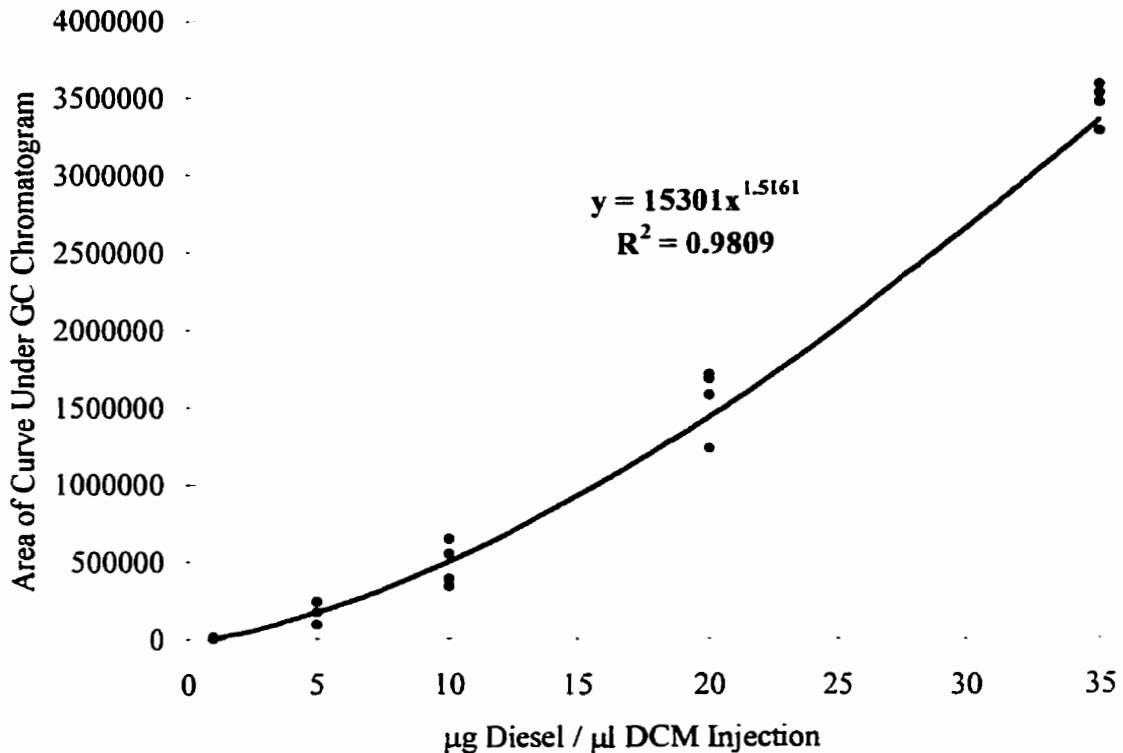
Figure 4.7 Standard curve determined for the analysis of total extractable hydrocarbons in the soils studied.

4.4.14 Statistical Analysis

Single factor ANOVAs and Duncan's New Multiple Range Tests were conducted using SuperANOVA (Abacus Concepts Inc., Berkley, CA). Treatments included site,

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depth and site by depth properties at the 5% significance level. First order curve fitting of

the mineralization data was performed using JMPIN (SAS Institute Inc., Cary, NC).

4.5 Results And Discussion

4.5.1 Phenanthrene Fate in Intact Soil Columns

The mineralization of radiolabeled phenanthrene in diesel fuel was monitored in

intact soil columns along a landscape. The soil columns preserved the physical properties

of the soil profile while providing a method to determine the potential mineralization in

surface and subsurface soils.

4.5.1.1 Phenanthrene Volatilization in Cores Based on the low vapour pressure (0.113

Pa at 25°C) and high log K_{ow} (4.53 at 26°C) (Piatt et al. 1996), the volatility of

phenanthrene in soil was considered to be low (<1%). The total percent ^{14}C phenanthrene

evolved after 343 days from each site is listed in Table 4.4. The data seems to indicate

almost no transfer into the gaseous phase which is consistent with the properties of this

compound. Landscape had no effect on the volatilization of phenanthrene because the

sites were not significantly different from each other. Without volatilization as a fate there

may be a greater potential for mineralization, adsorption or off-site movement via surface

flow or transport through the soil profile.

Table 4.4 ^{14}C Phenanthrene volatilization over the course of the intact soil column experiment.

Site	% ^{14}C Volatilized[f]
Site 5-1	0.008 ± 0.001 a
Site 5-2	0.004 ± 0.003 a
Site 5-3a	0.007 ± 0.002 a
Site 5-3b	0.008 ± 0.005 a
Site 5-4	0.009 ± 0.003 a
Site 5-5a	0.004 ± 0.003 a
Site 5-5b	0.007 ± 0.003 a
ANOVA	
Site	ns

[f]Average of 4 replicates. Means followed by a common letter are not significantly different at the 5% level using Duncan New Multiple Range Test.
***-highly significant, **-significant, *-low significance, ns-not significant ($\alpha = 0.05$)

4.5.1.2 ^{14}C Phenanthrene Mineralization in Cores The ^{14}CO$_2$ production did not vary greatly between the sites sampled (Table 4.5, Figure 4.8, Figure 4.9). The results indicate landscape had no effect on the mineralization of phenanthrene in the environment due to little significant differences between surface sites. Surface cores (0-50 cm) were not significantly different from the subsurface (50-100cm) at the two positions in the landscape (Table 4.6).

In most cases the rate of mineralization is relatively consistent with the literature. Broadkorb and Legge (1992) found 37.7% phenanthrene mineralized in a 15mg/kg soil slurry, while Møller and Ingvorsen (1993) concluded up to 12% phenanthrene was degraded in 1mg/kg of soil. Berry (1995) studied the mineralization of phenanthrene in diesel and found only 6.2% of this compound was mineralized in 1.41mg/kg soil in diesel fuel at a concentration of 10,000mg/kg soil. Though the mineralization levels are fairly low, there is no need to supplement the soil with hydrocarbon degrading organisms due to

the presence of intrinsic organisms capable of degradation *in situ*. Further research should be done dealing with methods of enhancing this degradation process.

Site 5-3b (subsurface) was significantly different from Site 5-5b, also a subsurface soil. This indicates differences in the mineralization potential in some subsurface sites. Site 5-5b was not significantly different from the surface of Sites 5-1 and 5-3a indicating this subsurface soil profile may have the same potential for mineralization as the surface. Table 4.6 indicates there was no differences between the mineralization of phenanthrene in the subsurface and surface sites sampled at those positions in the landscape. The data indicates subsurface (50-100cm) environments can have the same potential for mineralization as surface (0-50cm) soils.

Figure 4.8 indicates there was a two phase mineralization process. From 0 to 50 days there was a minor amount of mineralization which proceeded to slow down until about day 98. The second mineralization interval seem to proceed until day 200 where the rate again trailed off. Table 4.5 displays the subsequent modeling of the data after separation of the two mineralization intervals. The two phase mineralization scheme may have been due to the addition of water at day 63. Up to this point there was almost no significant mineralization, therefore water was added to simulate a half inch (1.27 cm; 230ml per core) rainfall. It is not known if this was the driving force for the second phase of mineralization, though it is a reasonable guess.

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Table 4.5 ^{14}C Phenanthrene mineralization over the course of the intact soil column experiment.

Site	% ^{14}C Phenanthrene Mineralized (A) (0-98 days)	k_1 (0-98 days) (day^{-1})	$t_{1/2}$ (days)	% ^{14}C Phenanthrene Mineralized (A) (98-343 days)[‘]	k_2 (98-343 days) (day^{-1})	$t_{1/2}$ (days)
Site 5-1	2.2 ± 1.6	0.02 ± 0.014	29	7.2 ± 0.09 ab	0.01 ± 0.004	52
Site 5-2	1.6 ± 0.3	0.01 ± 0.002	50	7.9 ± 3.0 b	0.02 ± 0.005	36
Site 5-3a	1.5 ± 0.3	0.01 ± 0.006	49	6.5 ± 0.8 ab	0.02 ± 0.003	42
Site 5-3b	0.5 ± 0.2	0.02 ± 0.008	30	3.6 ± 0.7 a	0.01 ± 0.002	53
Site 5-4	1.6 ± 1.2	0.01 ± 0.005	58	7.9 ± 0.7 b	0.02 ± 0.009	33
Site 5-5a	3.4 ± 5.6	0.02 ± 0.013	38	8.1 ± 6.1 b	0.02 ± 0.012	31
Site 5-5b	0.2 ± 0.05	0.02 ± 0.006	41	5.5 ± 0.6 ab	0.01 ± 0.002	71
ANOVA						
Site				***		

[‘]Average of 4 replicates. Means followed by a common letter are not significantly different at the 5% level using Duncan New Multiple Range Test.

***-highly significant, **-significant, *-low significance, ns-not significant ($\alpha = 0.05$)

Table 4.6 Site and depth means for ^{14}C Phenanthrene mineralization at the end of the intact soil column experiment.

	% ^{14}C Mineralized After 343 days
Site Comparisons[‘]	
Site 5-3a and 5-3b	4.6 a
Site 5-5a and 5-5b	5.9 a
Depth Comparison	
0-50 cm	6.6 a
50-100 cm	3.8 a
ANOVA	
Site	ns
Depth	ns
Site x Depth	ns

[‘]Average of 8 replicates. Means followed by a common letter are not significantly different at the 5% level using Duncan New Multiple Range Test.

***-highly significant, **-significant, *-low significance, ns-not significant ($\alpha = 0.05$)
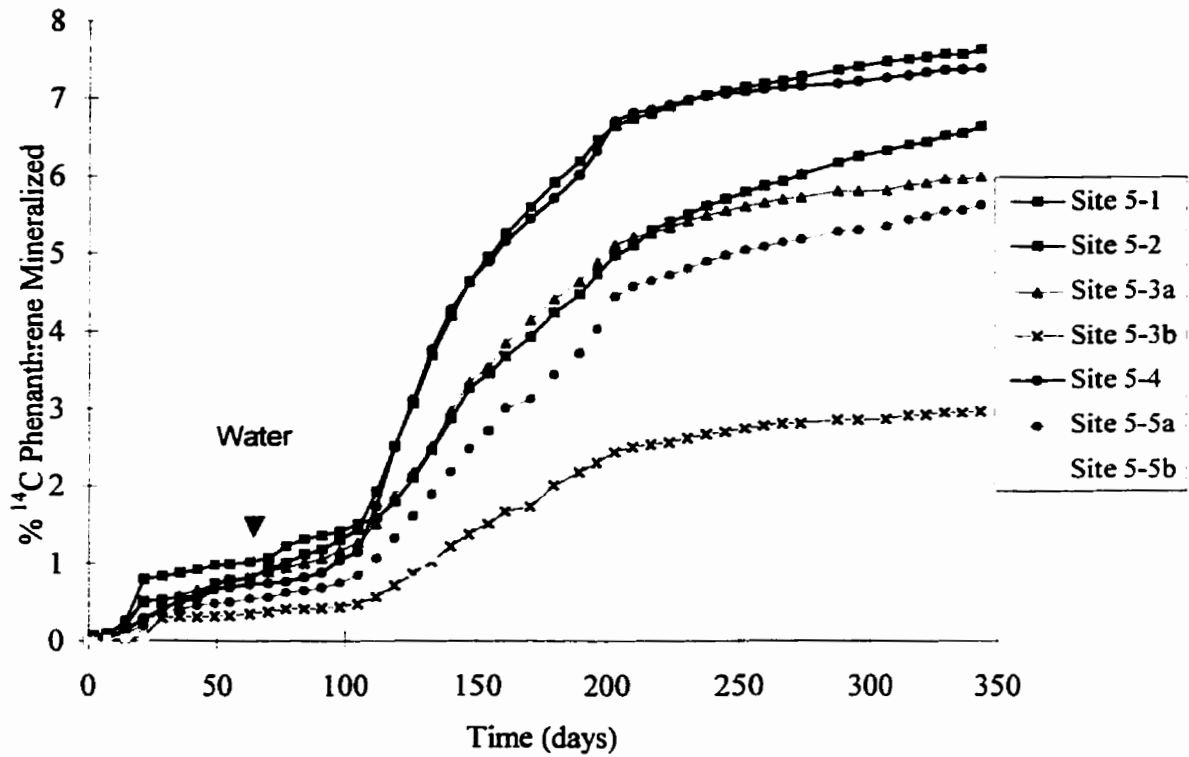
Figure 4.8 Mineralization of ^{14}C Phenanthrene in intact soil columns sampled at each site located along a slope transect. Each site had 4 reps which were average and plotted.
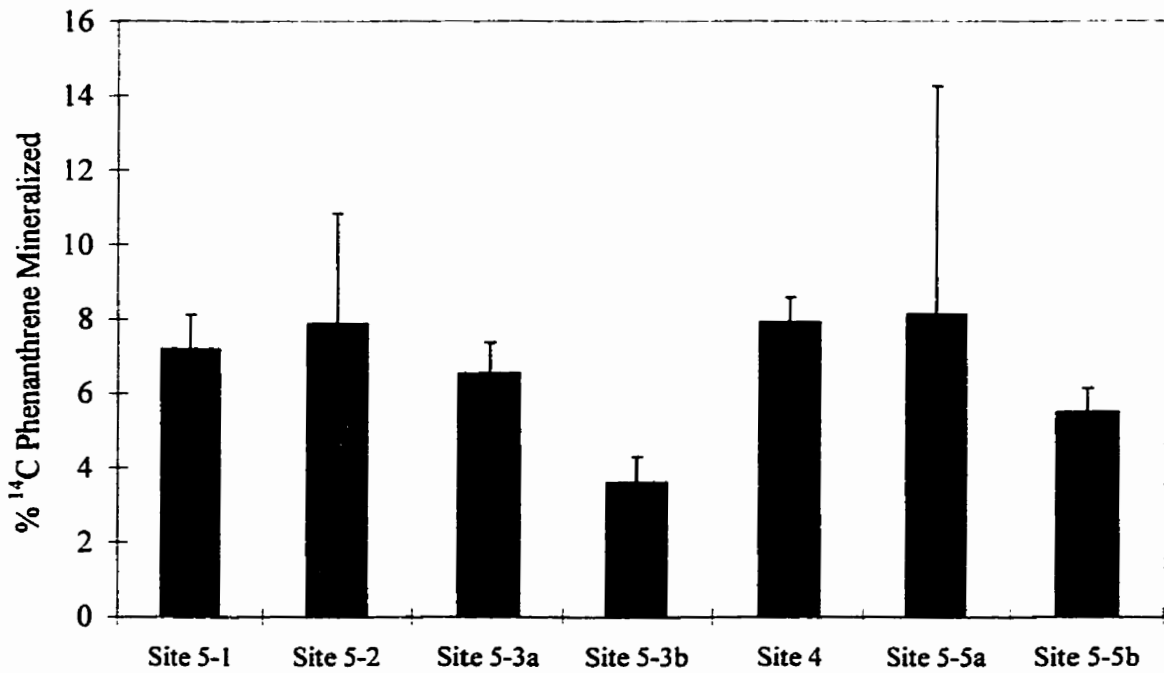
Figure 4.9 Mineralization of ^{14}C Phenanthrene in intact soil columns sampled along a sloping landscape.

4.5.1.3 Total CO_2 Production in Intact Soil Columns The total respiration data is

listed in Table 4.7. Surface soils had significantly higher rates of respiration with diesel

addition than the subsurface soils of Sites 5-3a and 5-5b. This may be due to increased

microbial activity in surface soils due to organic matter (Smith et al. 1993). Organic

matter contains many substrates and cofactors that enhance microbial activity. Of the

surface soils, Site 5-5a in the depression had the least amount of diesel induced microbial

activity. This may be a problem in the environment because the depression may be

supplemented with the greatest amount of diesel due to surface runoff and may not have as

much of a microbial potential (respiration) to degrade the contaminant. The remaining

surface soils of Sites 5-1, 5-2, 5-3, and 5-4 had greater than 10 g of carbon evolved from

respiration and were significantly different from Sites 5-3b, 5-5a, and 5-5b (Table 4.7,

Table 4.8). Thought there were greater respiration rates with diesel as a substrate, there

was less than 3% of the total mineralized to CO_2. This indicates a small percent of the

diesel was degraded to this fate.

Control soils with no diesel addition had less respiration than test soils. Figures

4.10 and 4.11 indicate the increased respiration of cores up and above the controls. It is

important to note that at day 150 there appears to be a decrease in respiration, but this was

not the case. The control core began to increase in respiration therefore resulting in the

apparent decrease in microbial activity (Figure 4.12). Even in the subsurface there was an

increase in CO_2 production over no diesel addition. This was most likely due to the

supplementation of a suitable substrate found in diesel (Widrig and Manning 1995). These

substrates could be mineralized to CO_2 resulting in higher recoveries than control soils.

Phelps et al. (1994) monitored the CO_2 evolution from similar soil columns. They found

between 23 and 6 mg C was produced per day at 23°C in 7cm diameter by 1 m long columns. The 23 mg/day evolution of carbon dioxide was obtained from the addition of water, air, nutrients and microbes while the 6 mg/day was found using water and air only. The differences between this data and Phelps et al. (1994) could be explained because different soils may have different respiration due to temperature and richness of substrate in the soil. The data also suggests two phases of respiration. There was an initial period of CO_2 evolution between 0 and 98 days followed by a second, more rapid respiration phase between 98 and 343 days (Figure 4.12). The shift in respiration may have been due to the water addition at day 63, but it is unknown at this time why all cores including the controls acted in this manner.

Table 4.7 Total carbon evolved as CO_2 over 343 days in cores saturated with diesel fuel and ^{14}C phenanthrene. Sites were run in quadruplicate with one controL

Site	Total g Carbon Evolved as CO_2[t]	Rate of Carbon Evolution (0-98 days) (mg/day)	Rate of Carbon Evolution (98-343 days) (mg/day)	% Total Diesel Recovered As CO_2[A]
Site 5-1	10.8 ± 0.6 b	21.7 ± 5.3	37.0 ± 0.4	1.8 ± 0.3
Site 5-1 Control	7.7	2.2	32.8	-----
Site 5-2	10.2 ± 0.3 b	23.3 ± 1.1	34.0 ± 5.7	3.1 ± 1.0
Site 5-2 Control	4.8	6.0	17.0	-----
Site 5-3a	10.7 ± 0.3 b	21.1 ± 3.0	36.3 ± 1.2	1.9 ± 0.3
Site 5-3a Control	7.4	12.9	25.2	-----
Site 5-3b (Subsurface)	8.1 ± 0.2 a	11.6 ± 4.3	29.7 ± 3.4	0.3 ± 0.7
Site 5-3b Control	7.5	9.5	29.2	-----
Site 5-4	10.1 ± 1.2 b	20.2 ± 2.6	35.2 ± 1.1	2.0 ± 0.1
Site 5-4 Control	6.7	5.3	26.3	-----
Site 5-5a	8.4 ± 5.6 a	14.5 ± 7.8	29.9 ± 4.3	0.8 ± 0.7
Site 5-5a Control	7.5	16.9	25.7	-----
Site 5-5b (Subsurface)	6.9 ± 0.5 a	4.5 ± 3.1	28.2 ± 2.6	1.0 ± 0.4
Site 5-5b Control	5.1	2.5	21.6	-----
ANOVA				
Site	***			

[t] Average of 4 replicates. Means followed by a common letter are not significantly different at the 5% level using Duncan New Multiple Range Test.
[A] Total = Average CO_2 minus the control core divided by the total diesel added (171g).
***-highly significant, **-significant, *-low significance, ns-not significant ($\alpha = 0.05$)

Table 4.8 Site and depth means for CO_2 production over the course of the intact soil column experiment.

	Total g Carbon Evolved as CO_2
<u>Site Comparisons</u>[t]	
Site 5-3a and 5-3b	9.4 b
Site 5-5a and 5-5b	7.6 a
<u>Depth Comparison</u>	
0-50 cm	9.5 b
50-100 cm	7.4 a
<u>ANOVA</u>	
Site	**
Depth	***
Site x Depth	ns

[t]Average of 8 replicates. Means followed by a common letter are not significantly different at the 5% level using Duncan New Multiple Range Test.
***-highly significant, **-significant, *-low significance, ns-not significant ($\alpha = 0.05$)

Figure 4.10 Respiration of Site 5-1 with diesel addition up and above the controls with no diesel added (Replications - Control).

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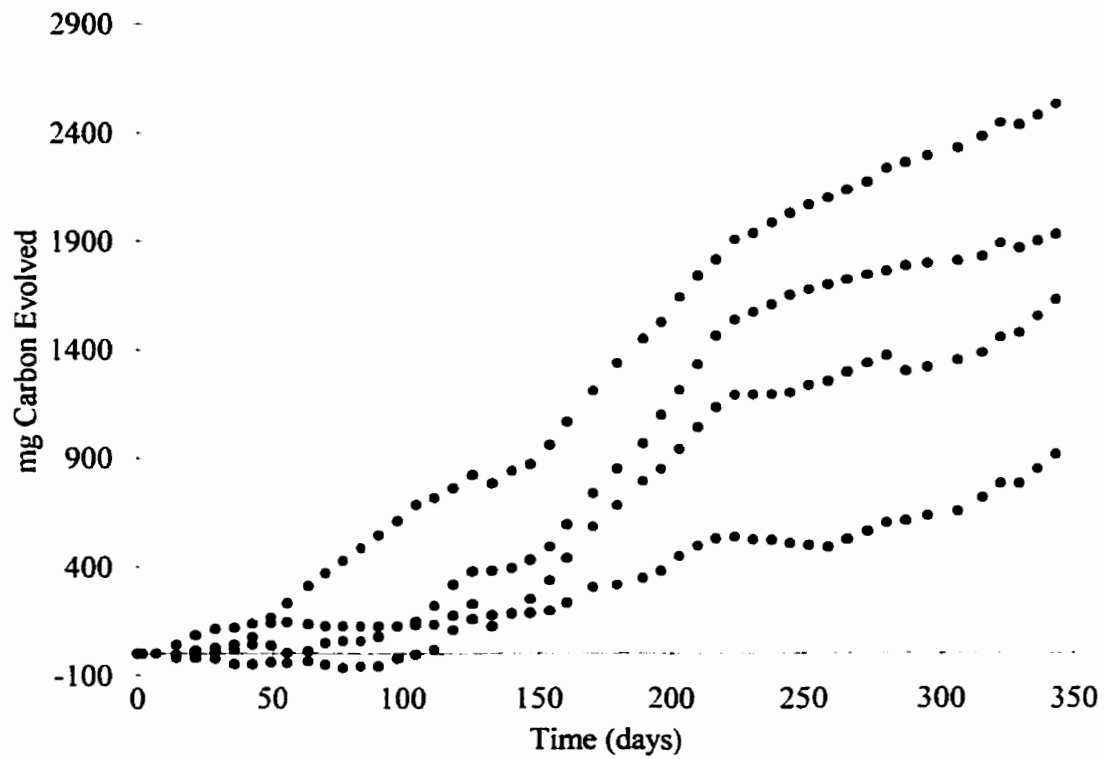
Figure 4.11 Respiration of Site 5-5b with diesel addition up and above the controls with no diesel added (Replications - Control).
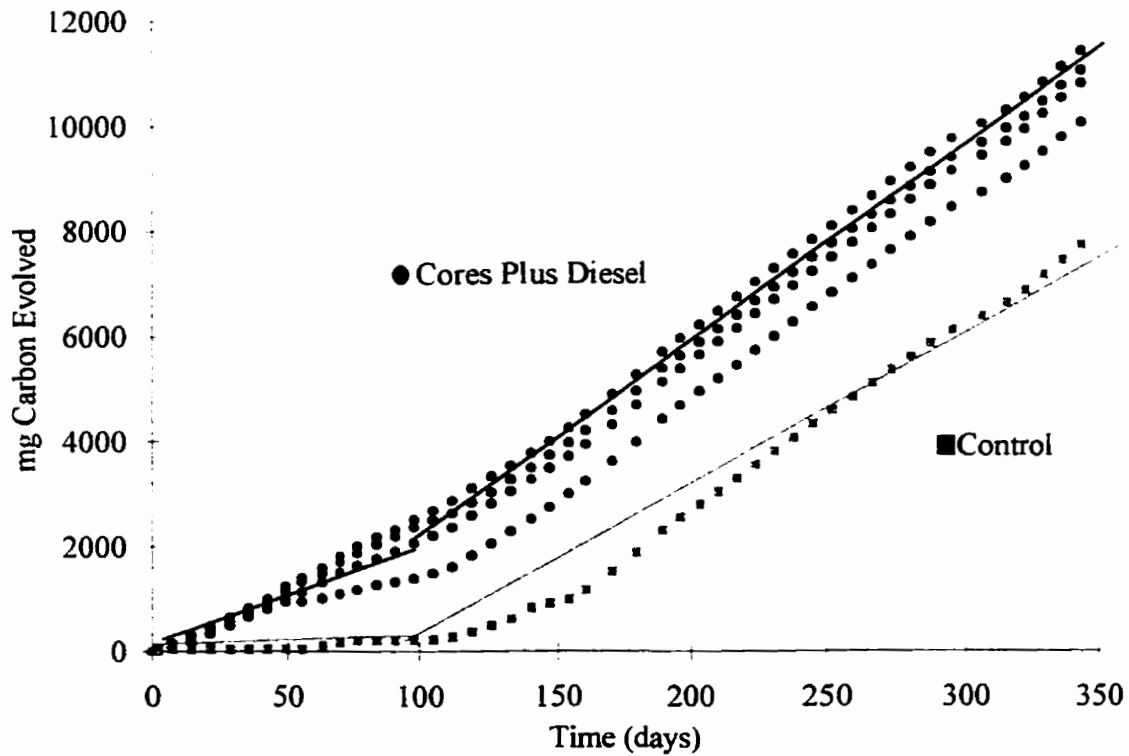
Figure 4.12 Microbial respiration in the surface (0-50cm) at Site 5-1 in an intact soil column sampled along a landscape.

4.5.1.4 Sequential Extraction of Cores It appears that the ^{14}C equally partitioned between the extractable fractions in all treatments with similar results obtained in all fractions of water, methanol, soxhlet and wet digestion (Table 4.9). Sites 5-3b and 5-5b had significantly different water extractable ^{14}C as high as 31% of the total radioactivity added to the soil while the surface soils at these sites had much lower results. This may indicate that there was less adsorption of the radioactive compound in low organic soils (Schwartzenbach 1993) resulting in phenanthrene or its degradative products being more available for microbial degradation or transport off site. Degradation intermediates would become more polar as phenanthrene is mineralized, therefore, more water extractable ^{14}C may occur due to the change in hydrophobicity. The potential for up to 30% of this compound being water soluble after 343 days of incubation raises concerns surrounding contamination of the nearby environment.

The methanol and soxhlet extractions had no significant differences between sites. The subsurface total extracted ^{14}C appears to be higher than the corresponding surface soils with some significant differences. This indicates the radioactivity from ^{14}C phenanthrene remained in soil to a greater extent in intact soil columns in the subsurface or the efficiency of extraction was poor in the surface.

The controls were fresh soil samples spiked with 51,000 μg/g diesel fuel-^{14}C phenanthrene mixture then extracted (Table 4.9). In this example the methanol phase had more extractable ^{14}C than all other phases. The total efficiency of extraction was between 85 and 89% in both the surface soil and the sand sample.

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Table 4.9 Sequential extraction of ^{14}C from soil at the end of the intact soil column experiment.

Site	% ^{14}C Water Extracted	% ^{14}C Methanol Extracted	% ^{14}C Soxhlet Extracted	% ^{14}C Wet Digested	Total % ^{14}C Extracted
Site 5-1[t]	15.2 ± 6.6 b	13.7 ± 3.8 a	17.0 ± 1.6 a	20.2 ± 5.2 d	66.2 ± 9.2 c
Site 5-2	6.0 ± 2.8 a	12.2 ± 4.2 a	13.1 ± 6.3 a	18.0 ± 4.1 cd	49.3 ± 9.0 ab
Site 5-3a	8.2 ± 2.6 a	12.4 ± 3.4 a	12.7 ± 3.3 a	13.3 ± 1.6 bc	46.6 ± 9.8 c
Site 5-3b	31.0 ± 6.7 d	15.5 ± 2.6 a	6.5 ± 2.5 a	13.2 ± 3.9 bc	66.2 ± 7.9 a
Site 5-4	5.2 ± 0.8 a	10.8 ± 1.4 a	17.8 ± 4.8 a	10.0 ± 2.5 ab	43.8 ± 7.8 a
Site 5-5a	7.4 ± 3.3 a	11.5 ± 2.0 a	23.9 ± 12.5 a	10.9 ± 3.8 ab	53.6 ± 5.3 ab
Site 5-5b	22.9 ± 4.4 c	11.0 ± 2.2 a	23.0 ± 11.6 a	7.2 ± 1.0 a	64.1 ± 8.8 bc
Control Soil	7.3 ± 2.0	67.2 ± 4.1	8.7 ± 1.4	2.6 ± 0.1	85.8 ± 4.5
Control Sand	16.1 ± 2.4	68.9 ± 10.4	4.9 ± 0.1	0.0 ± 0.0	89.9 ± 10.4
ANOVA					
Site	***	ns	ns	***	***

[t]Average of 4 replicates. Means followed by a common letter are not significantly different at the 5% level using Duncan New Multiple Range Test.
***-highly significant, **-significant, *-low significance, ns-not significant (α = 0.05)

Table 4.10 Site and Depth means for ^{14}C Extracted in each solvent phase.

	% ^{14}C Water Extracted	% ^{14}C Methanol Extracted	% ^{14}C Soxhlet Extracted	% ^{14}C Wet Digested	Total % ^{14}C Extracted
Site Comparisons[t]					
Site 5-3a and 5-3b	19.6 a	11.2 a	9.6 a	13.2 b	56.4 a
Site 5-5a and 5-5b	15.1 a	13.9 a	17.6 a	9.1 a	53.0 a
Depth Comparison					
0-50 cm	7.8 a	11.9 a	15.3 a	12.1 a	47.1 a
50-100 cm	27.0 b	13.2 a	11.9 a	10.2 a	62.3 b
ANOVA					
Site	ns	ns	ns	*	ns
Depth	***	ns	ns	ns	***
Site x Depth	ns	ns	ns	ns	ns

[t]Average of 8 replicates. Means followed by a common letter are not significantly different at the 5% level using Duncan New Multiple Range Test.
***-highly significant, **-significant, *-low significance, ns-not significant (α = 0.05)

All of the soils investigated had less than 15% of the radioactivity transported into the sand fraction at the bottom of the core after the 343 day experiment (Figure 4.13).

The majority of the soils had the highest recoveries of phenanthrene between 0-20 cm in the surface soils and 50-70 cm in the subsurface. The subsurface soils had greater amounts of phenanthrene enter the sand fraction at the bottom of the core. This increased transport was probably due to less phenanthrene-organic carbon adsorption in these sites resulting in more water soluble ^{14}C. The recovery of phenanthrene in the sand fraction indicates transport off-site into water tables and aquifers may be a factor in the fate in the environment.

4.5.1.5   Movement of Phenanthrene   The movement of ^{14}C phenanthrene was not controlled by preferential flow (Figure 4.14). The amounts of radioactivity sampled in the center of the core was similar to the amounts sampled next to the PVC column. This indicates there was equal movement throughout the soil profile and transport was not affected by the edges of the column. The results confirm the idea that transport may be a significant fate of phenanthrene or its degradative products in the environment.

4.5.1.6   Total Extractable Hydrocarbons   There was up to 50% reduction in hydrocarbons in the surface Site 5-5a and as little as 10.6% reduction in the subsurface of Site 5-3b (Table 4.11). The surface of Site 5-1 also had large recoveries of hydrocarbons with only 16.4% reduction in total extractable hydrocarbons. The subsurface of Site 5-5b was not significantly different from the surface Sites 5-1 and 5-2 indicating the subsurface may have as much potential for "degradation" of hydrocarbons as the surface depending on the conditions of the soil (Table 4.11).
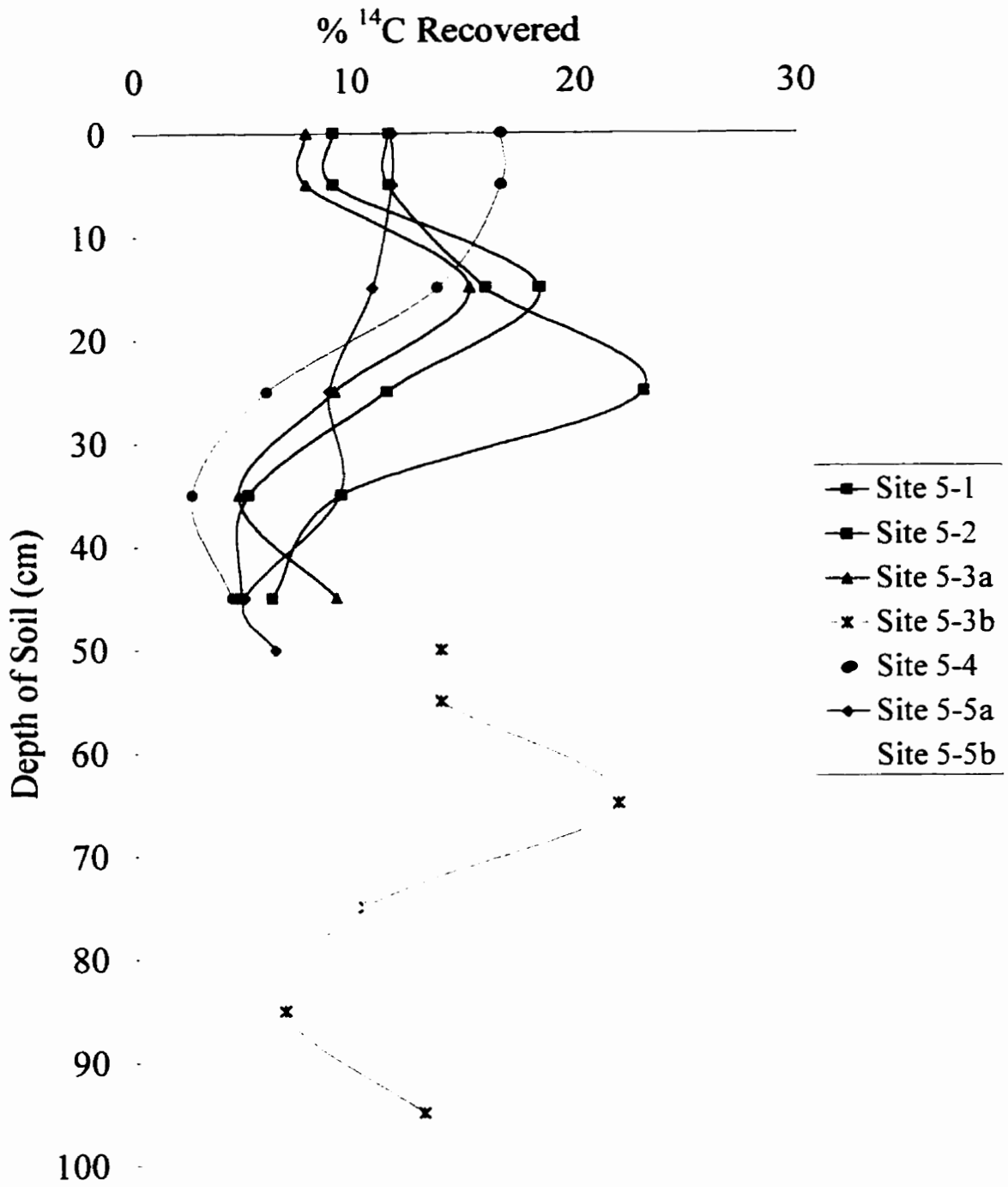
Figure 4.13 Location of ^{14}C at the end of the experiment (343 days) in each soil profile sampled in the landscape.
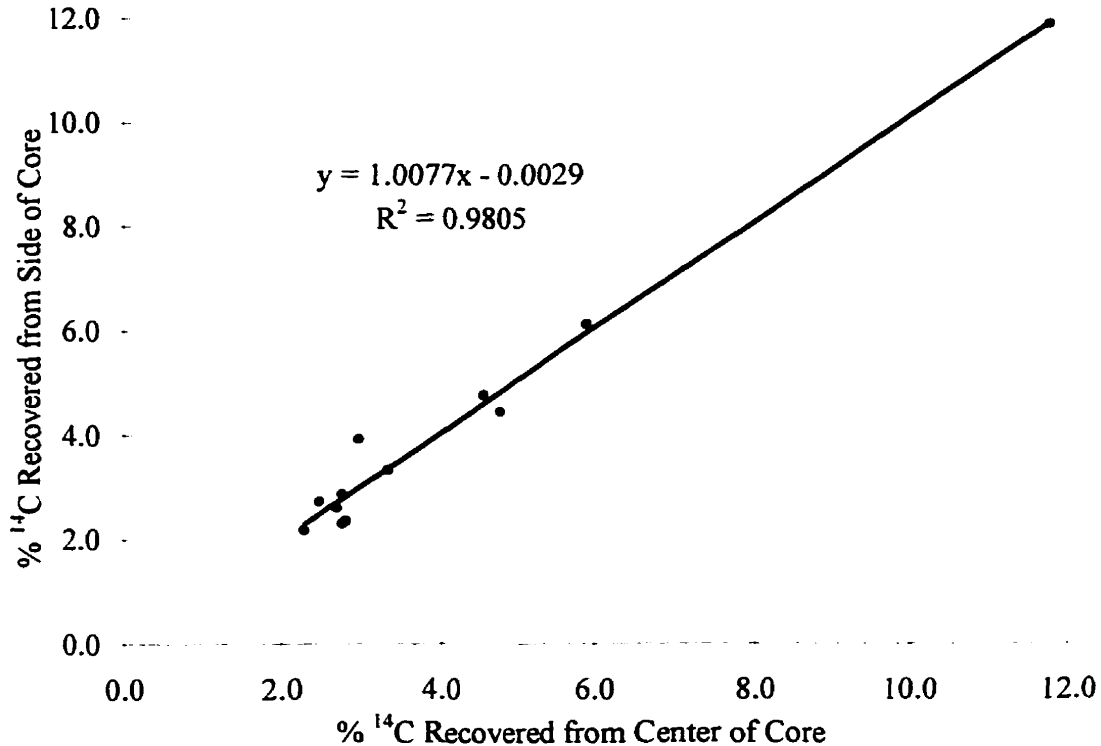
Figure 4.14 Comparison of ^{14}C in 12 cores sampled at the side along the PVC column and at the center of the core. The equation of the line yielded a slope of 1, therefore there was no preferential flow.

Starting with the knoll (Site 5-1) and ending in the depression (Site 5-5a) there appears to be a decrease in the hydrocarbons recovered in the landscape. This indicates there could have been more microbial activity in the depression compared to the upper slope positions resulting in greater mineralization. The respiration data indicates there was less diesel induced microbial activity in the depression compared to the upper slope positions. The data may in fact suggest there was greater adsorption and incorporation into the humic material in the depression resulting in less hydrocarbons extracted. Table 4.12 indicates there was significant differences between the surface and subsurface sites examined. The subsurface had significantly less unrecoverable hydrocarbons than the

surface soils studied. The greater unrecoverable hydrocarbons in the surface can indicate two things. First, there may have been greater microbial activity in the surface due to the presence of organic matter. The greater the microbial activity indicates a greater potential for mineralization. The second possibility may be due to the adsorption of hydrocarbons therefore decreasing the total extractable diesel. This is also due to the accumulation of organic matter in the surface allowing a greater potential for adsorption and humification. At the end of the experiment there was between 56 and 100% of the hydrocarbons remaining in the soil after 343 days of incubation. If this was a site in the environment, a potential for movement off-site in the years to come would pose a threat to the surrounding environment if this fraction was mobile.

Widrig and Manning (1995) and Phelps et al. (1994) also ran a similar column study. In the first study, Widrig and Manning (1995) constructed soil columns contained 6200 ppm of diesel fuel from which various nutrient and water additions took place. They found over 50% reduction in the TPH in the soils receiving these treatments. The control core, on the other hand, had no water and nutrient addition and had similar experimental conditions to this study. The total diesel fuel reduced in this soil was 16%. The results in this chapter indicated up to 50% reduction in diesel fuel. Phelps et al. (1994) demonstrated the degradation of petroleum hydrocarbons in previously contaminated soil at 23°C. They subjected the soil to various treatments of water, nutrients and bacteria and included control cores with no treatments. Treatment soils had between 8 and 68% more degradation of the diesel fuel than in the control, though the majority of the data was below 20% degradation. The researchers did not indicate how much diesel fuel was

initially in the soil but did indicate the control core had little degradation. It appears that even with the addition of nutrients, water and bacteria degradation may not be rapid.

The movement of hydrocarbons through the soil profiles indicates there will be hydrocarbons past the 50 cm depth in almost all cores (Figure 4.15). A significant fraction (between 10 and 40%) of the diesel seemed to remain in the top 10 cm of each core. The sand fraction at the bottom of the core had between 3 and 31% of the diesel pooled in this area. The transport of hydrocarbons in the surface appears to increase as it enters the depression. It appears that the subsurface cores also had increased transport due to the greater amounts of diesel recovered at the bottom of the cores. The results again indicate the transport of hydrocarbons will be a significant fate *in situ*. The increased transport results in the accumulation of hydrocarbons below the surface where degradation may be inhibited due to the absence of microorganisms, nutrients, and oxygen.

Table 4.11 The total extractable hydrocarbons recovered from the soil in the sites sampled in the landscape. Initial diesel fuel added was 171.7g.

	Hydrocarbons Recovered (g)	% of Total Hydrocarbons Remaining in Soil	% Hydrocarbons Unrecoverable[t]
Site 5-1	135.8 ± 28.4	79.1 ± 16.5	16.4 ± 20.9 ab
Site 5-2	100.1 ± 20.0	58.3 ± 11.7	41.7 ± 11.7 bc
Site 5-3a	95.3 ± 30.2	55.5 ± 17.6	44.5 ± 17.6 c
Site 5-3b	144.6 ± 11.9	84.2 ± 6.9	10.6 ± 12.3 a
Site 5-4	104.7 ± 29.0	61.0 ± 16.9	39.0 ± 16.9 c
Site 5-5a	88.0 ± 22.3	51.2 ± 13.0	48.8 ± 13.0 c
Site 5-5b	111.0 ± 32.0	64.6 ± 18.6	35.4 ± 18.6 bc
ANOVA			
Site			**

[t]Average of 4 replicates. Means followed by a common letter are not significantly different at the 5% level using Duncan New Multiple Range Test.
***-highly significant, **-significant, *-low significance, ns-not significant ($\alpha = 0.05$)

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Table 4.12 Site and depth means for total extractable hydrocarbons in each site in the landscape.

	% Hydrocarbons Unrecoverable
Site Comparisons[t]	
Site 5-3a and 5-3b	27.6 a
Site 5-5a and 5-5b	42.1 b
Depth Comparison	
0-50 cm	46.6 b
50-100 cm	23.0 a
ANOVA	
Site	*
Depth	**
Site x Depth	ns

[t]Average of 8 replicates. Means followed by a common letter are not significantly different at the 5% level using Duncan New Multiple Range Test.

***-highly significant, **-significant, *-low significance, ns-not significant ($\alpha = 0.05$)

Phenanthrene was a poor indicator of the movement of diesel through the soil (Figure 4.16). The phenanthrene was about 10% higher than the amount of diesel at each depth in the soil profile. This is not surprising because diesel fuel contains hundreds of unique compound with different chemical properties (Widrig and Manning 1995).

It is these properties which determine their individual fate in the environment.
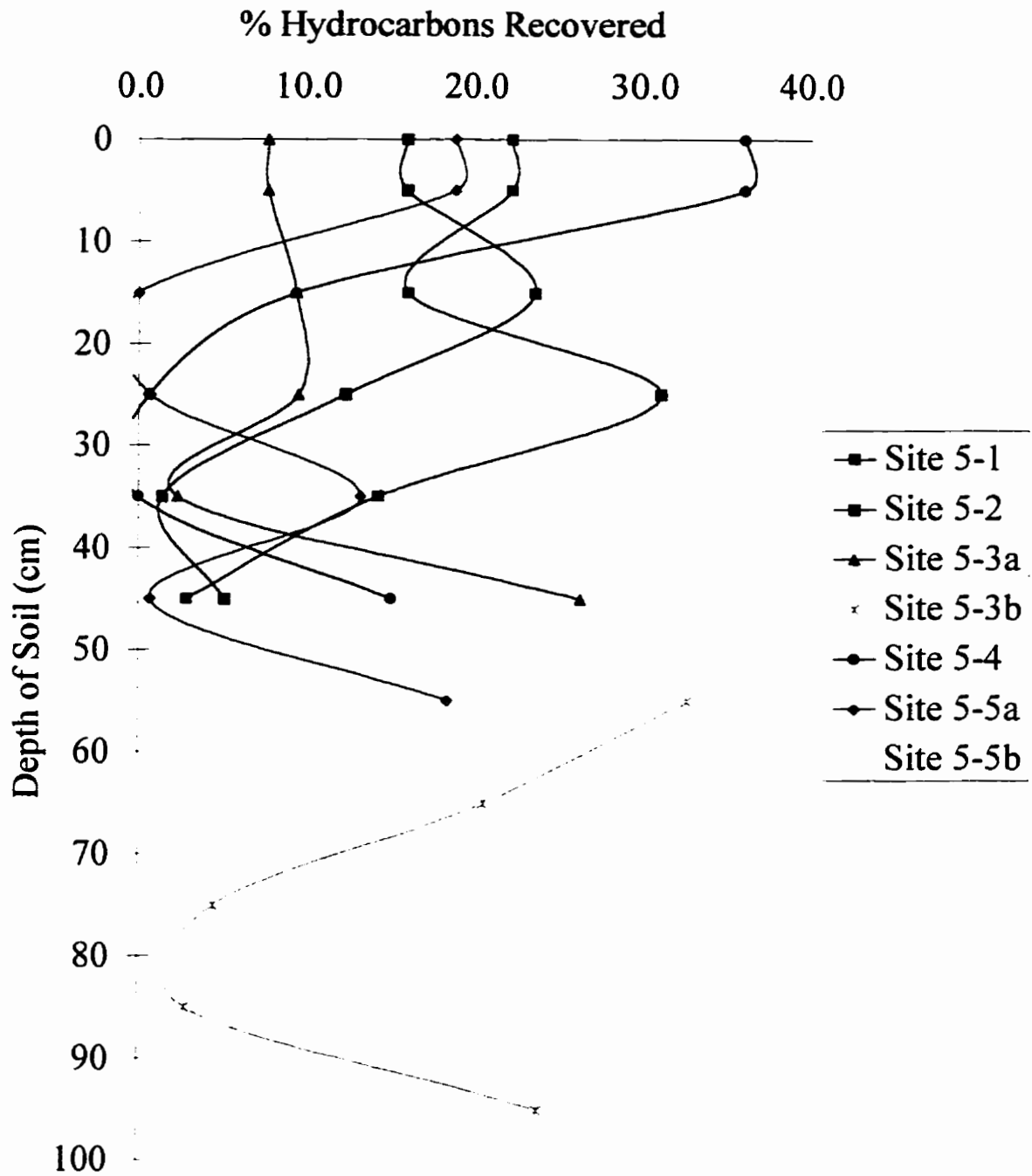
Figure 4.15 Location of the total extractable hydrocarbons at the end of the experiment (343 days) in each soil profile sampled in the landscape. The amounts of diesel are as follows: Total Added = 171.7g (202ml); 5% = 8.6g (10.1ml); 10% = 17.2g (20.2 mL); 20% = 34.3g (40.4ml); 30% = 51.5g (60.6ml); 40% = 68g (80.8ml).
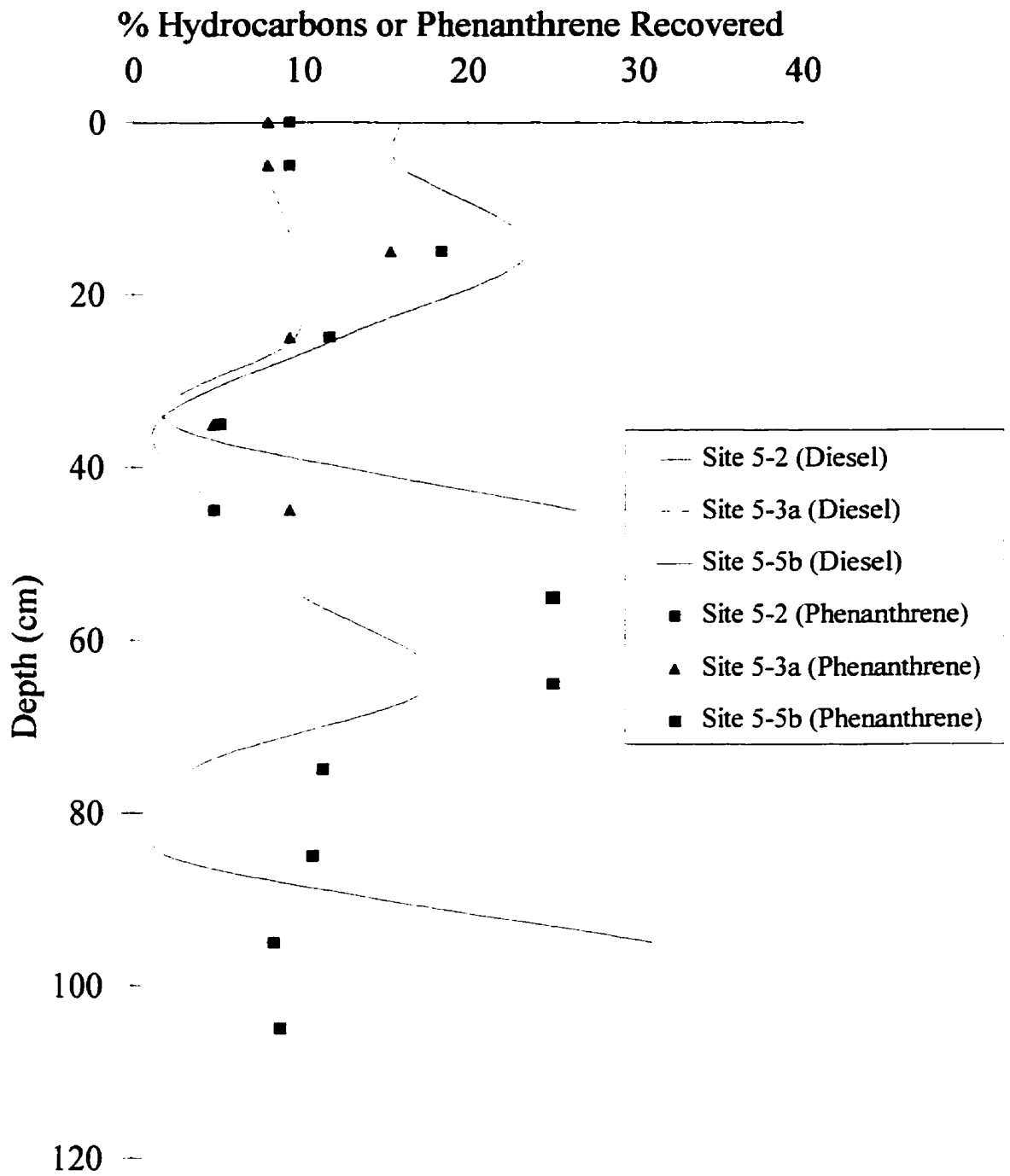
Figure 4.16 Location of phenanthrene and total extractable hydrocarbons at the end of the experiment (343 days) in each soil profile sampled in the landscape.

4.5.1.7 Fate of Phenanthrene The implications of this data for *in situ* remediation are great. Volatilization was extremely low, a common characteristic of this compound, and would contribute to almost no loss of phenanthrene from the system (Table 4.13). Intrinsic microbial populations were present but mineralization in this soil (3.6 to 8.1%) was a minor factor involved in the overall fate. Sorbed [14]C (methanol and soxhlet extractable) had values up to 66% indicating the majority of the radioactivity remained in the soil columns. The amounts of phenanthrene adsorbed in the soil columns (i.e. methanol + soxhlet extractions) ranged between 5 and 35% and contributed to the major fate. The partitioning of the labeled ^{14}C into this phase indicates there may be a decrease in the bioavailability of our compound which affects mineralization. Residual or possibly humified ^{14}C resulted in recoveries up to 20%. This indicates the partitioning of phenanthrene and its degradation products into a residual state may be a significant fate *in situ*. With water extractable ^{14}C ranging between 5 and 30%, it can be concluded transport through the soil profile to the water table will also be a significant fate in the environment.

Comparing the average results associated with the fates in the environment for the surface (Sites 5-1, 5-2, 5-3a, 5-4, and 5-5a) and subsurface (Sites 5-3b and Sites 5-5b) we can get a general idea of what phenanthrene will do in the environment (Figure 4.17). Phenanthrene will be adsorbed (methanol and soxhlet extractable ^{14}C) and form a residual fraction (wet digestible ^{14}C) readily in soils if it is not mineralized by the microbial populations. The remainder of this compound at these concentrations will partition into the transportable, mineralized and volatilized fractions.

Table 4.13 Comparison of the various fates of phenanthrene in the soils sampled in the landscape.

Site	% ^{14}C Volatilized	% ^{14}C Phenanthrene Mineralized (343 days)	% ^{14}C Phenanthrene Available for Further Transport*	% ^{14}C Adsorbed [t]	% ^{14}C Residual [A]
Site 5-1	0.008 ± 0.001	7.2 ± 0.09	15.2 ± 6.6	30.8 ± 5.3	20.2 ± 5.2
Site 5-2	0.004 ± 0.003	7.9 ± 3.0	6.0 ± 2.8	25.3 ± 9.9	18.0 ± 4.1
Site 5-3a	0.007 ± 0.002	6.5 ± 0.8	8.2 ± 2.6	25.1 ± 2.3	13.3 ± 1.6
Site 5-3b	0.008 ± 0.005	3.6 ± 0.7	31.0 ± 6.7	22.0 ± 2.1	13.2 ± 3.9
Site 5-4	0.009 ± 0.003	7.9 ± 0.7	5.2 ± 0.8	28.6 ± 6.0	10.0 ± 2.5
Site 5-5a	0.004 ± 0.003	8.1 ± 6.1	7.4 ± 3.3	35.3 ± 11.5	10.9 ± 3.8
Site 5-5b	0.007 ± 0.003	5.5 ± 0.6	22.9 ± 4.4	34.0 ± 9.5	7.2 ± 1.0

* Water Extractable ^{14}C
[t] Methanol + Soxhlet Extractions
[A] Wet Digestible Fraction

The transport of this compound will be a significant fate in the subsurface with almost half of the recoverable compound being in a water soluble phase. It is important to note the data used in Figure 4.17 is calculated by averaging of all sites in the surface and subsurface then dividing by the total recovered ^{14}C and not the total added to the cores.

A

B
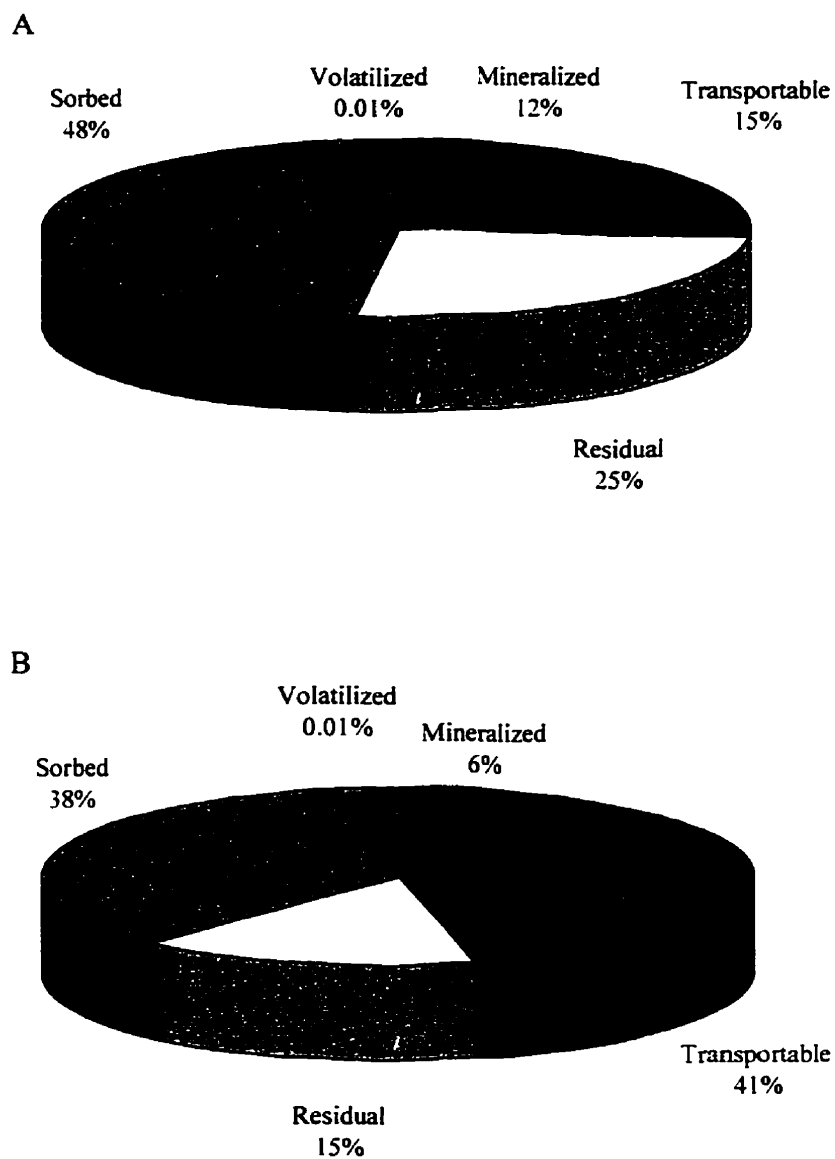
Figure 4.17 Fate of radiolabeled phenanthrene in intact soil columns in the surface (A) and subsurface (B) environments.

4.5.1.8 Mass Balance of Intact Soil Column Study The mass balance of radioactivity added to the soil is listed in Table 4.14. The summing of mineralization. volatilization, and residual ^{14}C results in recoveries ranging from 51.7% in Site 5-4 to 73.4% in Site 5-1. Low overall recoveries may be due to the extraction procedure. The heterogeneity of these large samples (up to 1.5 kg obtained after the core was destructively sampled every 10cm) may have constituted to the discrepancies in the mass balance because only 10g was extracted. The data still indicate the majority of the ^{14}C phenanthrene introduced into the cores remains in the soil as phenanthrene or degradation products.

Table 4.14 Mass balance of total ^{14}C phenanthrene in diesel fuel added to intact soil columns.

Site	% ^{14}C Volatilized	% ^{14}C Phenanthrene Mineralized	% ^{14}C Extracted	Total ^{14}C Recovered[f]
Site 5-1	0.008 ± 0.001	7.2 ± 0.09	66.2 ± 9.2	73.4 ± 9.9 b
Site 5-2	0.004 ± 0.003	7.9 ± 3.0	49.3 ± 9.0	57.2 ± 6.2 a
Site 5-3a	0.007 ± 0.002	6.5 ± 0.8	46.6 ± 9.8	53.1 ± 4.9 a
Site 5-3b	0.008 ± 0.005	3.6 ± 0.7	66.2 ± 7.9	69.8 ± 7.9 b
Site 5-4	0.009 ± 0.003	7.9 ± 0.7	43.8 ± 7.8	51.7 ± 7.8 a
Site 5-5a	0.004 ± 0.003	8.1 ± 6.1	53.6 ± 5.3	61.8 ± 8.8 a
Site 5-5b	0.007 ± 0.003	5.5 ± 0.6	64.1 ± 8.8	69.7 ± 8.9 ab
ANOVA				
Site				**

[f]Average of 4 replicates. Means followed by a common letter are not significantly different at the 5% level using Duncan New Multiple Range Test.
***-highly significant, **-significant, *-low significance, ns-not significant ($\alpha = 0.05$)

4.5.2 Fate of Phenanthrene in Soil Microcosms

The mineralization of radiolabeled phenanthrene in diesel fuel was studied in soil microcosms. The results in this section will be compared to the results of the intact soil columns to determine their ability to predict degradation potentials.

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4.5.2.1 ¹⁴C Phenanthrene Volatilization in Microcosms The volatilization of

phenanthrene inside the soil microcosms was extremely low (<1%) (Table 4.15). As seen

in the cores, minimal phenanthrene was sampled in the PUF plugs signaling a low potential

of transport into the vapour phase inside a microcosm. The implications for *in situ* fate

again demonstrate almost all of the phenanthrene will remain in the soil and not be

removed due to volatilization.

4.5.2.2 ¹⁴C Phenanthrene Mineralization in Soil Microcosms The mineralization of

phenanthrene in diesel fuel was extremely low (<1%) in the sites sampled in the landscape

(Table 4.15, Table 4.16). Surface soils had the same poor mineralization as subsurface,

with no more than 1% mineralization. Whether or not the soils were located on the knoll

(Site 5-1) or in the depression (Site 5-5a), poor mineralization occurred throughout the

landscape inside the microcosms. The radiochemical purity of the phenanthrene was 98 ±

2%. The data recovered was less than 1%, therefore mineralization in these soils cannot

be unequivocally demonstrated because the impurities may be the only compounds

mineralized in the soil. The results are somewhat puzzling because intrinsic

microorganisms (organisms already present in natural soil environments) from the same

site were found to degrade phenanthrene in the previous section. The differences between

the experimental apparatuses (flow through core system vs. static microcosms) or toxicity

may have been the deciding factors in determining mineralization of this compound. The

intact soil columns were probably more aerated than the static microcosms resulting in

greater mineralization. Also, the effective concentrations per gram of soil were higher in

the microcosms because in the columns the diesel was allowed to move down the soil

profile.

Table 4.15 [14]C Phenanthrene volatilization and mineralization over the course of the soil microcosm experiment.

Sites	% [14]C Volatilized	% [14]C Phenanthrene Mineralized (A)	Rate Constant (k) (day⁻¹)	Mineralization Half Life (days)
Site 5-1 0-10cm	0.2 ± 0.02	0.6 ± 0.1	0.018 ± 0.003	39
Site 5-1 40-50cm	0.4 ± 0.2	0.5 ± 0.1	0.004 ± 0.002	173
Site 5-2 0-10cm	0.3 ± 0.1	0.6 ± 0.1	0.017 ± 0.003	41
Site 5-2 40-50cm	0.2 ± 0.1	0.7 ± 0.2	0.004 ± 0.002	189
Site 5-3a 0-10cm	0.2 ± 0.1	0.5 ± 0.1	0.015 ± 0.004	45
Site 5-3a 40-50cm	0.4 ± 0.05	0.7 ± 0.3	0.004 ± 0.003	160
Site 5-3b 50-60cm	0.3 ± 0.1	0.5 ± 0.1	0.004 ± 0.001	173
Site 5-3b 90-100cm	0.5 ± 0.1	0.5 ± 0.1	0.004 ± 0.002	160
Site 5-4 0-10cm	0.5 ± 0.2	0.6 ± 0.02	0.017 ± 0.002	41
Site 5-4 40-50cm	0.4 ± 0.04	0.5 ± 0.1	0.008 ± 0.002	83
Site 5-5a 0-10cm	0.2 ± 0.2	0.5 ± 0.1	0.018 ± 0.003	38
Site 5-5a 40-50cm	0.3 ± 0.2	0.5 ± 0.1	0.003 ± 0.001	260
Site 5-5b 50-60cm	0.3 ± 0.1	0.5 ± 0.1	0.004 ± 0.002	189
Site 5-5b 90-100cm	0.2 ± 0.1	0.5 ± 0.004	0.005 ± 0.001	139

Table 4.16 Site and depth means for ^{14}C Phenanthrene volatilization and mineralization over the course of the microcosm experiment.

	% ^{14}C Volatilized	% ^{14}C Phenanthrene Mineralized
Site Comparisons[t]		
Site 5-1	0.30 abc	0.35 bc
Site 5-2	0.23 a	0.35 bc
Site 5-3a	0.31 abc	0.39 c
Site 5-3b	0.4 bc	0.3 ab
Site 5-4	0.43 c	0.45 d
Site 5-5a	0.26 ab	0.35 bc
Site 5-5b	0.26 ab	0.38 a
Depth Comparison		
0-10 cm[x]	0.3 a	0.45 b
40-100 cm[y]	0.3 a	0.3 a
ANOVA		
Site	*	**
Depth	ns	***
Site x Depth	ns	ns

[t]Average of 6 replicates.
[x]Average of 15 replicates
[y]Average of 27 Replicates
Means followed by a common letter are not significantly different at the 5% level using Duncan New Multiple Range Test.
***-highly significant, **-significant, *-low significance, ns-not significant ($\alpha = 0.05$)

4.5.2.3 Sequential Extraction of Soil Microcosms The results of the extraction of the remaining ^{14}C in the soil after the experiment are listed in Table 4.17. The majority of the radioactivity was extracted from the soil indicating this would be the major fate *in situ*. Most sites, if not all, had greater than 55% of the radioactivity recovered in the methanol extract. Site 5-1 and Site 5-3a in the subsurface had greater than 80% of the total added radioactivity in a methanol extractable phase, much greater than any other phase. Brodkorb and Legge (1992) found similar results with the addition of phenanthrene to soil. They concluded after 21 days of incubation 55% of the total added ^{14}C phenanthrene was

extracted from the soil after 24 hours with a 50:50 mixture of methanol and methylene chloride.

The water extractable ^{14}C was higher in the subsurface soils compared to the surface (Table 4.18). This result compares the 0-10 cm soil samples to all other samples (40-50cm, 50-60cm and 90-100cm) in the landscape. This may be due to the fact that less organic matter was in the subsurface resulting in less adsorbed [14]C (Schwartzenbach 1993). When considering the methanol extraction, the subsurface soils had greater ^{14}C extracted than the surface in most sites investigated. This may be due to the fact that methanol is weakly hydrophobic yet is unable to remove all of the ^{14}C associated with organic matter in the surface. The soxhlet extraction is more exhaustive and yielded less ^{14}C extracted in the subsurface compared to the surface. This indicates there was a greater portion of ^{14}C associated with a more resistant fraction in the soil. Finally in the wet digestible experiment, all sites had more ^{14}C extracted in the surface compared to the subsurface, therefore the phenanthrene is held in more resistant forms in the surface soils and is released primarily by soxhlet extraction and complete digestion. The final extraction procedure removed highly recalcitrant radioactivity that may have been incorporated into the organic matter. Because the surface has more organic matter, there was a significant difference between the subsurface soil. The average total recoverable ^{14}C in the subsurface was greater than the surface for all sites examined regardless of the position in the landscape yet there was no significant differences between sites.

The results indicate under these conditions phenanthrene will be adsorbed in a methanol extractable form and will be relatively inaccessible to microorganisms and not available for transport. The results contradict the findings in the previous experiment

because more water extractable ^{14}C was recovered in intact soil columns. The differences between a core and microcosm apparatus may have contributed to the discrepancies. The results remain the same because adsorption was still the major fate in the experiment.

The control, a sample where diesel and phenanthrene were added just prior to extraction to determine the efficiency of extraction, is also listed in Table 4.17. In this example the majority of the ^{14}C was found in the methanol phase. This result is consistent with the experimental data seen in most of the sites examined. The efficiency of extraction in the control was high with total extracted ^{14}C around 91% and a standard deviation of 8.2.

Table 4.17 Sequential extraction of ^{14}C from soil at the end of the soil microcosm experiment.

Site	% ^{14}C Water Extracted	% ^{14}C Methanol Extracted	% ^{14}C Soxhlet Extracted	% ^{14}C Wet Digested	Total % ^{14}C Extracted
Site 5-1 0-10cm	8.0 ± 0.3	62.7 ± 5.0	4.9 ± 2.0	3.5 ± 2.0	79.1 ± 5.7
Site 5-1 40-50cm	4.1 ± 1.6	80.6 ± 10.5	2.6 ± 1.8	3.8 ± 1.1	91.1 ± 13.9
Site 5-2 0-10cm	7.8 ± 2.9	65.4 ± 5.6	4.2 ± 1.6	4.1 ± 1.5	81.5 ± 6.8
Site 5-2 40-50cm	7.2 ± 0.2	75.3 ± 2.8	2.9 ± 0.4	2.8 ± 0.8	88.2 ± 2.1
Site 5-3a 0-10cm	1.3 ± 1.2	47.8 ± 12.4	3.1 ± 2.2	6.3 ± 4.6	58.5 ± 19.8
Site 5-3a 40-50cm	14.4 ± 4.4	84.1 ± 21.2	3.1 ± 0.7	3.2 ± 1.3	104.9 ± 25.8
Site 5-3b 50-60cm	8.4 ± 0.5	77.7 ± 1.0	3.2 ± 1.3	2.0 ± 1.9	91.3 ± 1.5
Site 5-3b 90-100cm	5.7 ± 0.5	69.5 ± 8.2	3.6 ± 0.3	8.6 ± 12.7	87.3 ± 9.9
Site 5-4 0-10cm	7.0 ± 1.4	55.7 ± 3.1	6.7 ± 1.0	18.4 ± 25.2	87.8 ± 28.2
Site 5-4 40-50cm	15.1 ± 3.5	60.4 ± 5.2	3.0 ± 0.3	20.2 ± 23.7	98.7 ± 18.1
Site 5-5a 0-10cm	5.1 ± 1.1	60.8 ± 2.9	4.4 ± 0.6	6.2 ± 1.7	76.5 ± 5.5
Site 5-5a 40-50cm	9.2 ± 4.2	65.7 ± 5.8	3.6 ± 0.4	2.1 ± 0.6	80.6 ± 10.3
Site 5-5b 50-60cm	5.4 ± 1.3	62.0 ± 10.9	2.6 ± 0.8	3.1 ± 0.3	73.1 ± 12.4
Site 5-5b 90-100cm	8.4 ± 1.9	78.7 ± 3.6	4.4 ± 0.2	2.4 ± 1.3	94.0 ± 2.0
Control Soil	8.4 ± 3.2	56.9 ± 4.2	8.7 ± 0.3	16.9 ± 2.8	90.9 ± 8.2

4.5.2.4 Mass Balance of Soil Microcosm Study and Fate of Phenanthrene The mass balance of radioactivity added to the soil is listed in Table 4.19. The summing of mineralization, volatilization, and residual ^{14}C results in recoveries ranging from 59.2% in

Site 5-3a at the surface to 106.0% in Site 5-3a in the subsurface. With the exception of

Site 5-3a, recoveries of radioactivity were high. There were no significant differences

between sites for the total recovered radioactivity yet there was a significant difference

between depths (Table 4.20).

Table 4.18 Site and depth means for ^{14}C Phenanthrene extraction over the course of the microcosm experiment.

	% ^{14}C Water Extracted	% ^{14}C Methanol Extracted	% ^{14}C Soxhlet Extracted	% ^{14}C Wet Digested	Total % ^{14}C Extracted
Site Comparisons[t]					
Site 5-1	6.0 a	71.6 a	3.7 a	3.7 a	85.1 a
Site 5-2	7.5 a	70.3 a	3.5 a	3.4 a	84.8 a
Site 5-3a	7.8 a	66.0 a	3.1 a	4.8 a	81.7 a
Site 5-3b	7.0 a	73.6 a	3.4 a	5.3 a	89.3 a
Site 5-4	11.0 b	58.1 a	4.8 a	19.3 a	93.2 a
Site 5-5a	7.1 a	63.3 a	4.0 a	4.2 a	78.5 a
Site 5-5b	6.9 a	70.4 a	3.5 a	2.8 a	83.5 a
Depth Comparison					
0-10 cm[x]	5.8 a	58.5 a	4.7 b	7.7 a	76.7 a
40-100 cm[y]	8.6 b	72.7 b	3.2 a	5.4 a	90.0 b
ANOVA					
Site	**	ns	ns	ns	ns
Depth	***	***	***	ns	**
Site x Depth	***	*	ns	ns	ns

[t]Average of 6 replicates.
[x]Average of 15 replicates
[y]Average of 27 Replicates
Means followed by a common letter are not significantly different at the 5% level using Duncan New Multiple Range Test.
***-highly significant, **-significant, *-low significance, ns-not significant ($\alpha = 0.05$)

The potential for *in situ* remediation of phenanthrene in the soil microcosm study

can be summed up by looking at the various fates. Volatilization and mineralization were

very low indicating most if not all of the phenanthrene remained in the soil matrix.

Extractable or residual ^{14}C had values up to 104.9% indicating the majority of the

radioactivity remained in the soil and was not removed by volatilization or mineralization.

The results of this portion of the study indicate *in situ* remediation would be a very poor strategy for clean up. The lack of mineralization and volatilization indicates the phenanthrene would remain in the soil as itself or degradation intermediates, and have a potential for off-site movement contaminating the environment further.

Table 4.19 Mass balance of total ^{14}C phenanthrene in diesel fuel added to soil microcosms.

Site	% ^{14}C Volatilized	% ^{14}C Phenanthrene Mineralized	% ^{14}C Extracted	Total ^{14}C Recovered
Site 5-1 0-10cm	0.2 ± 0.02	0.6 ± 0.1	79.1 ± 5.7	79.9 ± 5.7
Site 5-1 40-50cm	0.4 ± 0.2	0.5 ± 0.1	91.1 ± 13.9	92.0 ± 14.0
Site 5-2 0-10cm	0.3 ± 0.1	0.6 ± 0.1	81.5 ± 6.8	82.4 ± 6.7
Site 5-2 40-50cm	0.2 ± 0.1	0.7 ± 0.2	88.2 ± 2.1	89.1 ± 1.9
Site 5-3a 0-10cm	0.2 ± 0.1	0.5 ± 0.1	58.5 ± 19.8	59.2 ± 19.8
Site 5-3a 40-50cm	0.4 ± 0.05	0.7 ± 0.3	104.9 ± 25.8	106.0 ± 25.5
Site 5-3b 50-60cm	0.3 ± 0.1	0.5 ± 0.1	91.3 ± 1.5	92.1 ± 1.5
Site 5-3b 90-100cm	0.5 ± 0.1	0.5 ± 0.1	87.3 ± 9.9	88.3 ± 10.0
Site 5-4 0-10cm	0.5 ± 0.2	0.6 ± 0.02	87.8 ± 28.2	88.9 ± 28.4
Site 5-4 40-50cm	0.4 ± 0.04	0.5 ± 0.1	98.7 ± 18.1	99.6 ± 18.2
Site 5-5a 0-10cm	0.2 ± 0.2	0.5 ± 0.1	76.5 ± 5.5	77.2 ± 5.5
Site 5-5a 40-50cm	0.3 ± 0.2	0.5 ± 0.1	80.6 ± 10.3	81.3 ± 10.4
Site 5-5b 50-60cm	0.3 ± 0.1	0.5 ± 0.1	73.1 ± 12.4	73.8 ± 12.2
Site 5-5b 90-100cm	0.2 ± 0.1	0.5 ± 0.004	94.0 ± 2.0	94.7 ± 2.0

The phenanthrene and its degradation intermediates in microcosms is largely associated with the adsorbed state (Figure 4.18). Mineralization was very poor with as little as 0.3% mineralized while volatilization will also be low *in situ*. The transportable phenanthrene or degradative products can be a significant fate in the surface and subsurface therefore must be monitored to prevent further contamination of the site. Adsorption is higher in the surface while volatilization and water soluble phenanthrene are lower indicating organic carbon may play a large role in the fate of phenanthrene in the environment. It is important to note the data used in the Figure 4.14 is calculated by

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averaging of all sites in the surface and subsurface then dividing by the total recovered ^{14}C and not the total added to the cores.

Table 4.20 Site and depth means for total ^{14}C recovered for the soil microcosm experiment.

	Total ^{14}C Recovered[t]
<u>Site Comparisons[t]</u>	
Site 5-1	85.8 a
Site 5-2	85.5 a
Site 5-3a	82.5 a
Site 5-3b	90.1 a
Site 5-4	94.1 a
Site 5-5a	79.2 a
Site 5-5b	84.2 a
<u>Depth Comparison</u>	
0-10 cm[x]	77.4 a
40-100 cm[y]	90.6 b
<u>ANOVA</u>	
Site	ns
Depth	**
Site x Depth	ns

[t]Average of 6 replicates.

[x]Average of 15 replicates

[y]Average of 27 Replicates

Means followed by a common letter are not significantly different at the 5% level using Duncan New Multiple Range Test.

***-highly significant, **-significant, *-low significance, ns-not significant ($\alpha = 0.05$)

Figure 4.18 Fate of radiolabeled phenanthrene in soil microcosms in the surface (A) and subsurface (B) environments.

4.6 Conclusion

Intact soil columns and soil microcosms had low rates of mineralization of the phenanthrene in diesel fuel. The ability of the microbial populations to degrade phenanthrene in the cores indicates the mineralization potential is low but not nonexistent in this landscape. Mineralization of phenanthrene was essentially the same in the surface and subsurface. This indicates the subsurface may have the same potential for mineralization as some surface landscape positions. The respiration data indicates there was more microbial activity when a suitable substrate like diesel was added. The cores with diesel addition had greater total respiration and respiration rates than the control cores without diesel. Also the total extractable hydrocarbons indicated that there was a significant reduction in the hydrocarbons at some sites in the landscape. It can be concluded phenanthrene mineralization was low yet total diesel reduction was significant.

The intact soil columns demonstrated greater mineralization of the phenanthrene than microcosms. One factor neglected by static microcosms is aeration. The role of oxygen in the enhanced mineralization of hydrocarbons would be lost when soil becomes anaerobic. The results indicate static microcosms may provide a poor estimate of mineralization of phenanthrene in the environment. Toxicity may have been a factor because cores were contaminated with diesel fuel on a dry weight basis of the upper 15 cm depth while the microcosms were contaminated on a dry weight basis of the total soil added. The diesel would then be able to distribute throughout the core reducing the effective concentration (toxicity) in the upper 15cm.

There was no effect of landscape on the mineralization of phenanthrene. Mineralization in the surface was essentially the same throughout the catena. Total extractable hydrocarbons decreased in the lower slope positions indicating there was either significant degradation or adsorption at these positions in the landscape. This is important because if a diesel spill was to occur in the environment the majority of the diesel would end up in the depression where, in this example, the greatest potential for degradation may occur.

The volatilization of phenanthrene was low in the microcosms and cores studied. The data seemed to indicate very little tendency for the transfer into the gaseous phase. The poor volatilization may be due to the low vapour pressure, high molecular weight and high K_{oc} of this compound. The combination of these and other properties of phenanthrene reduce the likelihood of volatilization from soil *in situ*. Extraction of the residual ^{14}C remaining in the microcosms and cores indicate the major fate of the radioactivity added was to remain in the soil adsorbed or in a water soluble form. The amounts of ^{14}C extracted from methanol and more exhaustive methods indicates bioavailability may be a controlling factor in mineralization *in situ*. Though the cores had as much as 30% of the ^{14}C in a water soluble form, this fraction was not mineralized and has the potential for transport through the soil profile to environments less conducive to degradation. The movement of phenanthrene, degradation products, and other hydrocarbons was greater in the subsurface cores indicating these environments are less likely to hinder transport than the surface.

CHAPTER 5

The Effect of Wet-Dry Cycles on the Mineralization of a) Glucose and b) Phenanthrene in Diesel Fuel in a Soil Landscape

5.1 Abstract

The mineralization potential of phenanthrene in diesel fuel was undertaken to understand the effects of landscape and moisture on this process. The organic matter contents, texture and water regime varied dramatically between the slope positions sampled. Soil was collected from the surface (0-10 cm) at the upper-mid and depression slope positions. The first experiment examined the addition of 1000μg of Glucose-C per gram of soil. The second trial used radiolabeled phenanthrene with 5000 μg of diesel fuel g^{-1} soil in order to assess the mineralization potential of a Dark Grey soil sampled near Rossburn, Manitoba. Volatilization and the assimilation by the microbial biomass was also examined in the experiment. The mineralization of glucose was rapid in the soils studied. Overall, the upper-mid slope had greater mineralization (36.8%) compared to the depression (31.8%) and continuously wet treatments (53.9%) were greater than wet-dry (48.4%). The assimilation into the biomass had the opposite effect with the greatest amounts of ^{14}C found in the depression (19.3%) over the upper-mid slope (14.1%) and the wet-dry treatment (18.9%) over continuously wet (15.6%). In the end there were no differences between the landscape positions and moisture treatments. The mineralization

of phenanthrene in 5000 ppm diesel fuel was also rapid had the same effect. The rapid mineralization may have been due to the forced aeration system. Though there were no differences between landscape positions and moisture treatments, the upper-mid slope had the greatest mineralization under continuously wet conditions (45.8%) while the depression had the highest mineralization under wet-dry conditions (46.4%).

5.2 Introduction

The mineralization of complex compounds is dependent on many physical, chemical and biological properties of the soil. Characteristics such as water availability (Rainwater et al. 1993), nutrient status (Widrig and Manning 1995), and microbial populations (Atlas and Cerniglia 1995) all control the factors involved in optimum biodegradation. The understanding of how these factors affect the overall degradation of compounds in the environment may allow proper treatment to enhance bioremediation.

One property of the environment that has not been studied extensively in the literature is the effect of wetting and drying on the mineralization of hydrocarbons. The majority of the studies involved looking at the degradation potential under constant moisture conditions (Phelps et al. 1994; Tatarko and Bumpas 1993). The potential for enhanced degradation under wet-dry conditions was looked at by Widrig and Manning (1995). They found enhanced mineralization of diesel fuel when they periodically added water and nutrients (~70% reduction) compared to a continuous flood (~50% reduction). They cited the enhanced biodegradation was due to increased air flow and hydraulic

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conductivity throughout the column allowing aerobic degradation and better movement of the nutrients when the soil began to dry.

The relationship between microbial populations and availability of water is a controlling factor in degradation of diesel fuel (Alters and Bartha 1993; West et al. 1989). In a catena various proportions of water are seen at each slope position. Though there are few examples of the effect landscape has on diesel fuel degradation, Hanna et al. (1982) demonstrated that depressions have more available water than upper slope positions indicating a greater potential for microbial activity. Available water can be controlled by organic matter and texture found in a landscape. Depressions are usually closer to the water table and have greater amounts of clay and organic matter to hold water. The depression would also be subject to a continuous supply of water while upper slope positions would be controlled by wet-dry conditions when rainfall and subsequent drying occurs. Van Kessel et al. (1993) also demonstrated various slope positions have a controlling effect on the microbial process of denitrification. On June 4th, 1991 denitrification was higher in the depression (157 to 556 g N ha^{-1} d^{-1}) than on the knoll (37 to 302 g N ha^{-1} d^{-1}) as a result of the influence of landscape on texture, organic matter and location of the water table on microbial processes.

Wetting and drying the soil is a natural process creating soil fertility. When soil dries, fractures form in aggregates exposing substrates usually inaccessible to the biomass (Van Gestel et al. 1991). As the moisture potential drops, microbial populations unable to survive the moisture stress lyse and supplement the soil with utilizable substrates (Sommers et al. 1981). Upon wetting of the soil, available substrates can be utilized causing a flush of microbial respiration and can create the opportunity for the

cometabolism of recalcitrant compounds. The literature indicates various results concerning the mineralization of compounds in soil when subjected to wetting and drying. Sorensen (1974) concluded increased degradation of plant material under fluctuating water content (30.1%) relative to soils maintained in a continually moist condition (13.9%) over a 284 day experiment. Shelton et al. (1995) concluded adsorption of atrazine increased during drying (extraction efficiency dropped by 22% when soil was dried). This corresponds to less bioavailability which in turn affects degradation if a soil is maintained at low water potentials.

In the landscape various moisture conditions occur leading to the adaptation and evolution of different microbial populations and soil physical properties. The upper-mid slope would be subject to wet-dry conditions due to runoff and decreased water infiltration. The depression would receive the water from the upper slope and be subject to continuous moist conditions depending on the water table. The determination of the positive or negative effect wetting and drying has on the bioremediation potential in a landscape will aid in determining the fate of contaminants in the environment.

5.3 Objectives of Study

The objective of this study was to examine the effects of drying and wetting on the mineralization of glucose and phenanthrene in a soil collected from i) a typically moist environment and ii) a soil naturally subjected to dry-wet cycles.

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5.4 Materials and Methods

5.4.1 Soil Landscape

The site selected for the study was North of Rossburn, Manitoba (Site #2 from Chapter 3). The south facing catena was under conventional till with wheat as the crop at the time of sampling. The upper-mid slope position was classified as an Orthic Dark Grey while in the depression the soil was a Rego Humic Gleysol (Figure 5.1). Sites were divided into 3 reps with 10 feet separating the sample positions. 0-10 cm of soil was removed using Dutch Augers and placed in air tight plastic bags. Soil was then placed in coolers and transported back to the lab and stored at 4°C until processing. Reps were then air dried and ground, then stored in bags at room temperature until use (approximately 6 months). The soil was then weighed into microcosm jars (50 g oven dry equivalent) and wet up to field capacity. The jars were then incubated at room temperature (22 ± 2°C) for one week prior to the experiment.

The particle size distribution and organic carbon contents of the soils are listed in Table 5.1 along with other soil properties including the profile characterization of the soils at each site.
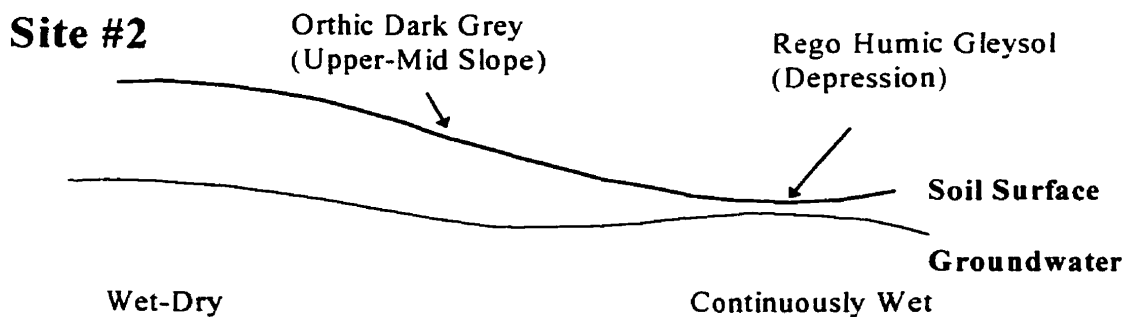
Figure 5.1 Stylized diagram of a slope transect of Dark Grey site where soil was sampled

Table 5.1 Texture, physical properties and soil profile characterization of soils studied along landscape.

Site (Site #2)	Soil	Profile		Air Dry Moisture Content (0-10 cm)	Field Capacity (0-10 cm)	% Organic Carbon (0-10 cm)	Texture (0-10 cm)			
							% Sand	% Silt	%Clay	Class
Upper-Mid Slope	Orthic Dark Grey	Ap	0-15 cm	3.0 ± 0.05	26.8 ± 4.1	2.5 ± 0.2	43.5	29	27.5	CL*
		Btj	15-38 cm							
		BC	38-45 cm							
		Cca	45-70 cm							
		Ck	70-80 cm							
Depression	Rego Humic Gleysol	Apk	0-15 cm	4.8 ± 0.6	29.3 ± 5.4	3.6 ± 0.1	40	16	44	CL
		Ahk	15-30 cm							
		Ahkg	30-50 cm							
		Ckg	50+ cm							

*CL represents Clay Loam

5.4.2 Experimental Apparatus

The soil was placed in 500 mL glass Microcosms purchased from Richards Packaging (Winnipeg, MB). To each microcosm rubber tubing delivering atmospheric air had been attached to the lid (Sorensen 1974). A Gast diaphragm vacuum pump/compressor (Gast Manufacturing Corp., Benton Harbor, MI) ran continuously pumping air at a rate of 3.2 L/hr replacing the jar headspace about 6 times per hour. Exhaust air was passed through a volatile trap and NaOH solution to trap volatiles and $^{14}CO_2$. The volatiles were trapped using a 13cm by 1.5cm diameter dessicator tube containing a polyurethane foam plug. Radioactive carbon dioxide was trapped by bubbling the exiting air stream through 30 mL of 2M NaOH. At various times a sample of the air inside the microcosm was analyzed by GC to make sure the soil environment was aerobic. The air flow system allowed the study of volatilization, wetting and drying treatments and mineralization at $22 \pm 2°C$ (Figure 5.2).

5.4.3 Soil Treatments

Three experimental treatments were imposed on the soil relating to changes in soil water content. The treatments were: wet (humidified air stream), wet-dry (dry air stream with periodic water addition) and dry (dry air stream and no water addition). In the continuously wet example the microcosm soil was wetted to field capacity with distilled water. Then to maintain field capacity, the air delivered to the microcosms was passed through 200 mL distilled water in an Erlenmeyer flask. Under these conditions the soil could remain at field capacity for one week before water had to be added (usually 0.1 mL). The wet dry experiment was conducted in much the same manner as the wet except the air was run through a 26cm by 4cm diameter desiccator column containing Drierite

(Anhydrous Calcium Sulfate, 8 mesh) to dehumidify the air. The soil was wetted to field capacity then allowed to dry to air dry conditions over a one week interval (Figure 5.3) from which it was wetted again to field capacity and repeated throughout the experiment. The final treatment was to maintain the soil at air dry conditions. This was done by running a continuous stream of dehumidified air over the soil without water addition. After an extended period of time (21 days for glucose, 77 days for phenanthrene), the air dry soil was then subjected to the wet-dry cycle to determine if the prolonged drying had any effect on mineralization. The phenanthrene experiment also had the continuous wet condition subjected to wet-dry conditions after 77 days.
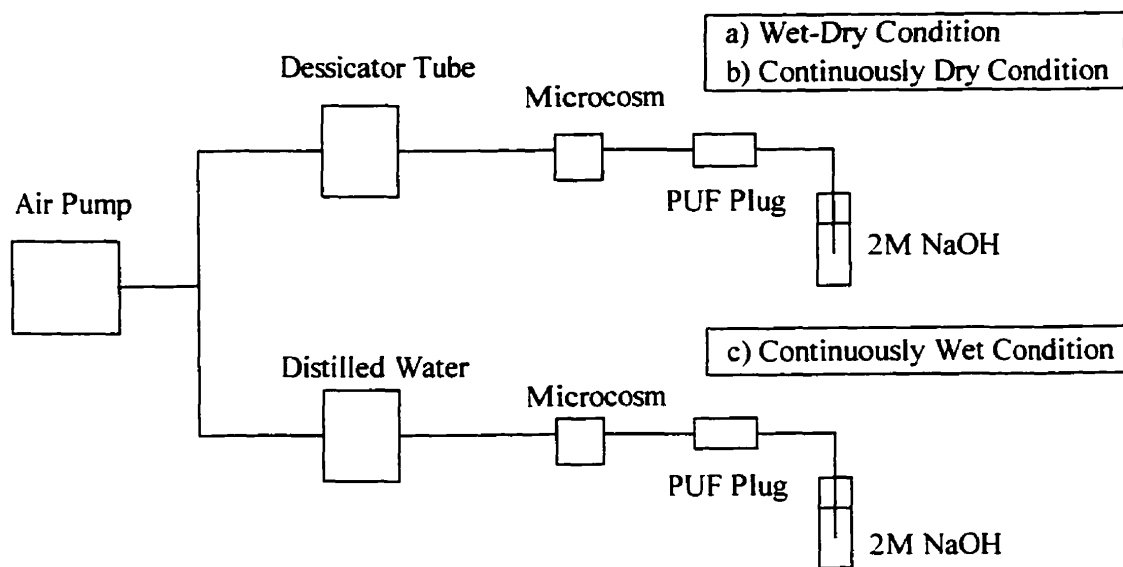
Figure 5.2 Experimental apparatus used to determine the phenanthrene mineralization potential within a Dark Grey Soil (Site #2).

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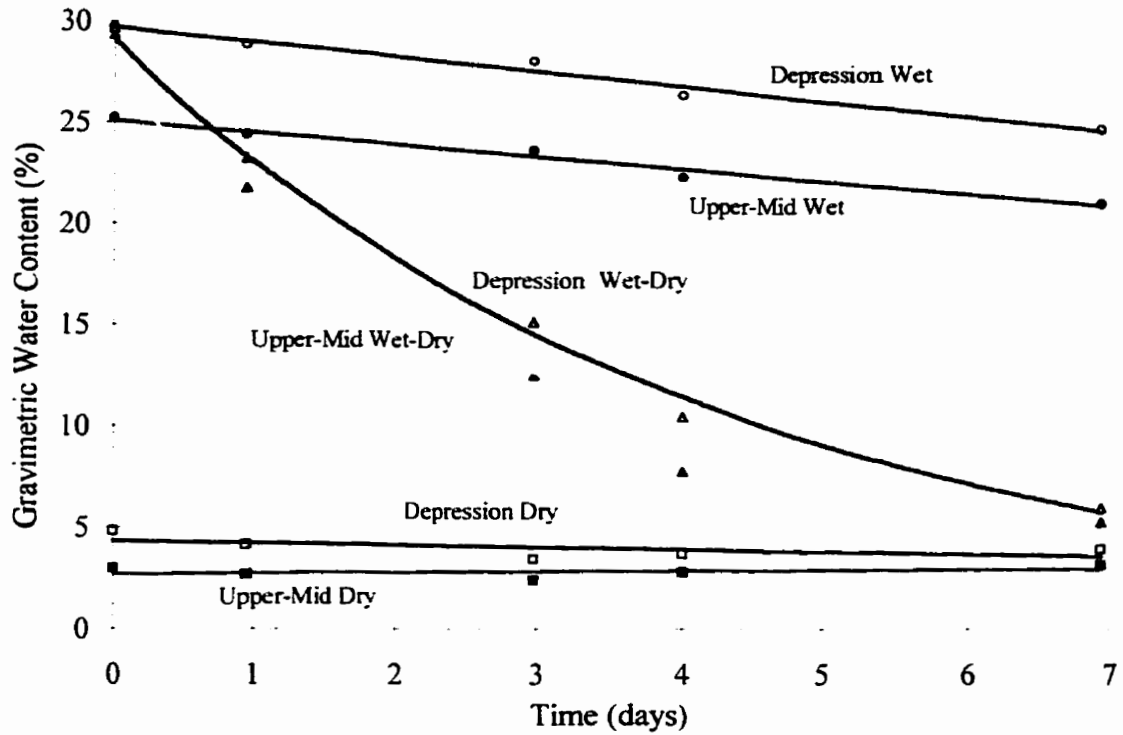
Figure 5.3 Representative change in water content during one week of incubation.

5.4.4 ^{14}C Glucose

D-Glucose-UL-^{14}C was purchased from Sigma Chemical Co. (St. Louis, MO)(10 nCi/mmol). Stock solution were made up in distilled water then diluted. At the beginning of the experiment 3 mL of stock solution containing 1000 µg Glucose-C per g of soil was dispensed evenly over the surface of the soil (0.1 g glucose with 0.4 µCi of activity per flask).
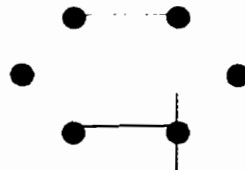
Figure 5.4 Radioactive glucose added to soil microcosms.

5.4.5 ^{14}C Phenanthrene and Diesel Fuel

^{14}C Phenanthrene was purchased from the Sigma Chemical Co. (St. Louis, MO) as Phenanthrene-9-^{14}C (8.3 nCi/mmol) (Figure 5.5). Stock solutions were first made up in hexane then transferred to diesel fuel #2 for addition to soil. At the commencement of each experiment 5000 ppm (5000 µg/g soil) of diesel-^{14}C Phenanthrene mixture was added with approximately 0.3 µCi of activity. Stock diesel fuel added contained about 0.7059µg unlabelled and 0.0586µg of labeled phenanthrene per gram of soil.
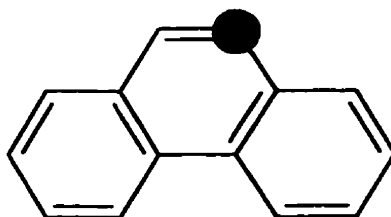
Figure 5.5 ^{14}C Phenanthrene added to soil microcosms.

5.4.6 Volatilization

When the experiment was completed, the polyurethane foam plugs were removed from the apparatus and placed in 100 mL French Square Bottles. To these bottles 20 mL Methanol was added, making sure 5 mL was in excess, for total extraction of glucose or phenanthrene. Bottles were then sealed and shaken on a lateral shaker for 2 minutes. The foam plugs were then removed and placed in a 50 mL syringe to extract all of the Methanol. From the extract, a 1 mL subsample was collected and counted by the scintillation counter.

5.4.7 ^{14}CO$_2$ Traps

The out flowing air stream was bubbled through 30 mL of 2M NaOH placed in a glass 50 mL test tube. The alkali traps were changed on a weekly basis and replaced with

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fresh NaOH. Following removal of the 30 mL NaOH trap from the air stream, 0.3 mL was removed and placed in a 20 mL scintillation vial with 15 mL scintillation fluid and counted. Trap changes occurred until there was no longer any significant radioactivity recovered (<0.1% of ^{14}C added).

5.4.8 Biomass Determination

The chloroform fumigation-extraction technique was used in the determination of the incorporation of ^{14}C into the microbial biomass (Voroney et al. 1993). At the end of the experiment the soil in each microcosm was removed and 20 g (oven dry equivalent) was placed into two French Square Bottles. Each pair was then split up and placed into a desiccation chamber containing a moist paper towel to maintain a humid environment. In the desiccator containing the soil to be fumigated, a beaker containing 50 mL of chloroform and boiling chips was placed in the center of the bottles. The non fumigated control did not receive any chloroform. The desiccators were sealed and the air was pumped out until the chloroform began to boil (about one minute) and replaced the atmosphere. The pump was then shut off and the desiccator was left to incubate for 72 hours. The control was placed at 4°C for the same amount of time. After 3 days the chloroform was removed and 50 mL of 0.5 M K_2SO_4 was added to each soil sample. The bottles were then stoppered and shaken on a lateral shaker for one hour to extract the organic carbon. Samples were then emptied into a Whatman #5 filter paper to filter the particulate matter. 0.1 mL of the filtrate was then combined with scintillation fluid and counted for radioactivity. The values obtained from the non fumigated samples were subtracted from the fumigated to give the amounts of extractable ^{14}C in the soil. The previous calculation was combined with a correction factor (K_{ec}) of 0.25 (Voroney et al.

1993) to correct for unrecoverable biomass carbon and give the resulting total biomass carbon.

$$\text{Biomass Carbon} = \frac{\text{(Fumigated Soil - Non Fumigated Soil)}}{K_{ec}}$$

Equation 5.1

5.4.9 Total Organic ^{14}C Remaining in Soil After Phenanthrene Experiment

In order to determine the residual radioactivity in the soil a wet digestion experiment was implemented in the phenanthrene in diesel fuel experiment. At the end of incubation the soil was air dried and stored at 4°C. At the time of determination, 0.8 g of soil was placed into a wet digestion tube (Voroney et al. 1991). To this tube 6 mL of digestion solution (189.4 g CrO_3 in 250 mL 14.7M H_3PO_4 and 500 mL 18M H_2SO_4) was added to the soil along with a glass rod stand to hold 2 mL of 2M NaOH in a 6 mL glass scintillation vial (NaOH traps $^{14}CO_2$ liberated from oxidation of organic carbon). The tube was then sealed with a #49 Rubber Suba Seal and placed in a digestion block and heated at 145°C for 1 hour. The $^{14}CO_2$ was then trapped for 24 hours after which a 0.3 mL subsample was removed and combined with scintillation fluid and analyzed for radioactivity. The above procedure was also run on a control soil using a spiked sample of ^{14}C phenanthrene to determine the efficiency of digestion.

5.4.10 Liquid Scintillation Counting

When counting the NaOH for radioactive CO_2, 0.3 mL was placed in a 20 mL scintillation vial and combined with 15 mL Ecolume Liquid Scintillation Fluid (ICN Biochemicals Inc. Aurora, OH). In the case of volatilization and biomass the 1 mL of methanol and 0.1 mL of K_2SO_4, respectfully, was added to a 7 mL scintillation vial with 5

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mL of cocktail. In all cases, the samples were allowed to equilibrate in the dark for 24 hours before counting to prevent any erroneous readings from ion interaction with the scintillation fluid. A Beckman LS 7500 scintillation counter was implemented with quench curve correction to give final results of disintegrations per minute (DPM). Final DPMs were corrected for background and controls then related to the original radioactivity added to each apparatus to give the percent mineralization of phenanthrene in diesel.

5.4.11 Kinetic Analysis of $^{14}CO_2$ Evolution

The first order rate model was used to determine the rate of mineralization of glucose or Phenanthrene in diesel #2. The calculation describes the reaction over time as a single first order component. The cumulative CO_2 produced (P) over time (t) is expressed as:

$$P = A[1-e^{(-kt)}]$$

Equation 5.2

where A is the percent of compound evolved as CO_2 at $t = \infty$, and k is the rate constant for $^{14}CO_2$ evolution (day^{-1}). Once the rate constant was determined a mineralization half life can be calculated using:

$$t_{1/2} = \ln2 / k$$

Equation 5.3

A rate constant was determined from the cumulative CO_2 evolved from each replicate and the estimated rate constant (k) and pool size (A) analyzed statistically.

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5.4.12 Statistical Analysis

Single factor ANOVAs and Duncan's New Multiple Range Tests were conducted using SuperANOVA (Abacus Concepts Inc., Berkley, CA). Treatments were analyzed at the 5% significance level. First order curve fitting of the mineralization data was performed using JMPIN (SAS Institute Inc., Cary, NC).

5.5 Results And Discussion

5.5.1 Fate of ^{14}C Glucose

The effects of moisture potentials on microbial mineralization was determined using as easily degradable substrate. Because it is easily mineralized, glucose can be compared to phenanthrene, a compound found to accumulate in the environment. The mineralization of radiolabeled glucose was monitored in soils sampled at two positions in the landscape. The first position was at the upper-mid slope where soil is regularly under wet-dry conditions in the environment. The second position was found in the depression where the soil is moist throughout the year. The soils were then subjected to wet, wet-dry and dry moisture conditions to determine their effect on mineralization. The soil microcosms allowed the simultaneous study of mineralization, volatilization, and incorporation into the microbial biomass throughout a 62 day experiment.

5.5.1.1 ^{14}C Glucose Volatilization No ^{14}C was detected in the PUF traps for the glucose amended soil (Table 5.2).

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Table 5.2 Volatilization of glucose under various moisture conditions.

Sample	% ^{14}C Glucose Volatilized
Upper-Mid Dry	nr
Upper-Mid Wet	nr
Upper-Mid Wet-Dry	nr
*Upper-Mid Dry Then Wetted	nr
Depression Dry	nr
Depression Wet	nr
Depression Wet-Dry	nr
*Depression Dry Then Wetted	nr

*Air Dry soil subjected to wetting and drying at day 21 for Glucose.
nr = none recovered

5.5.1.2 Mineralization of ^{14}C Glucose The mineralization of glucose occurred very rapidly in the microcosms. The majority of the ^{14}C was released within the first day of the experiment with the exception of the air dry treatment (Table 5.3, Figure 5.6). This indicates the wet-dry treatment would have had little effect on the mineralization because all of the available ^{14}C substrate was used before soils could dry and be wetted again (7 day cycle). After 21 days of air dry conditions before being wetted, less total mineralization occurred compared to continuous wet and wet dry treatments (Figure 5.7). There also appears to be a slower rate of mineralization after wetting the dry soil. A compound when introduced into dry soil may tend to adsorb to soil constituents more readily than if it were in solution, effectively reducing bioavailability (Shelton et al. 1995; Shelton and Parkin 1991). Because the soil remained at air dry conditions for 21 days, there would be enhanced adsorption which may not be reversible during the wet-dry treatments. Table 5.4 indicates the significant differences between sites and treatments. The upper-mid slope and depression were significantly different with greater mineralization in upper-mid slope. The treatments were also significantly different indicating an effect of wetting and drying on the mineralization of glucose.
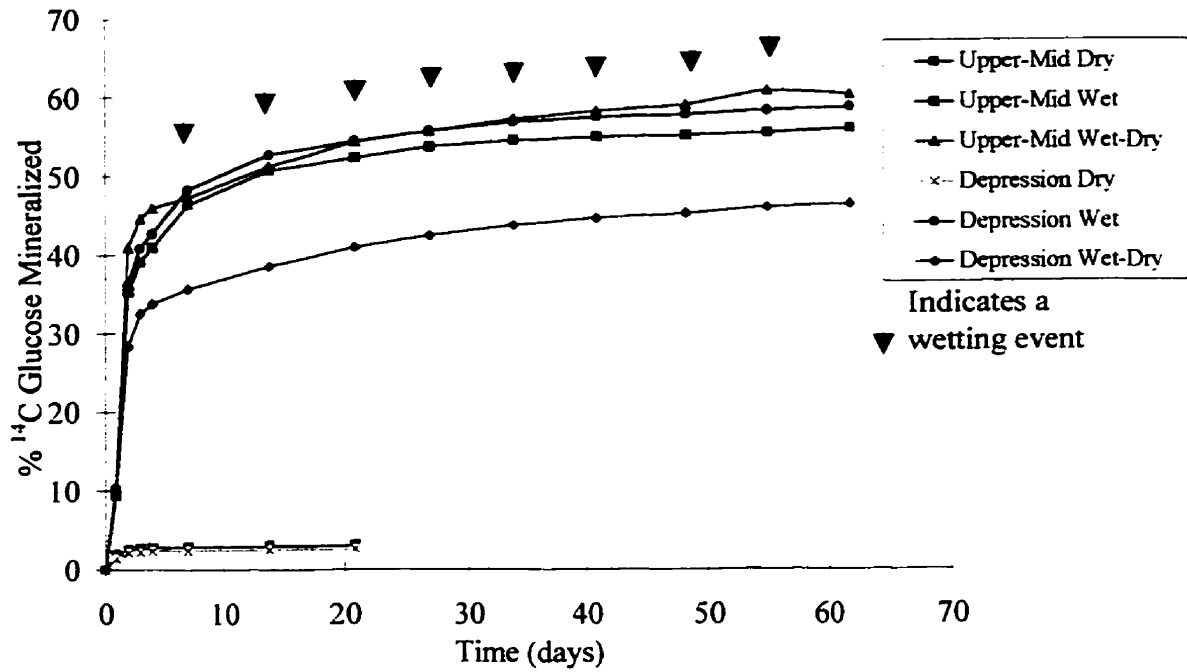
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Figure 5.6 Mineralization of ^{14}C Glucose under air dry, field capacity, and wet-dry conditions in a soil sampled along a landscape.
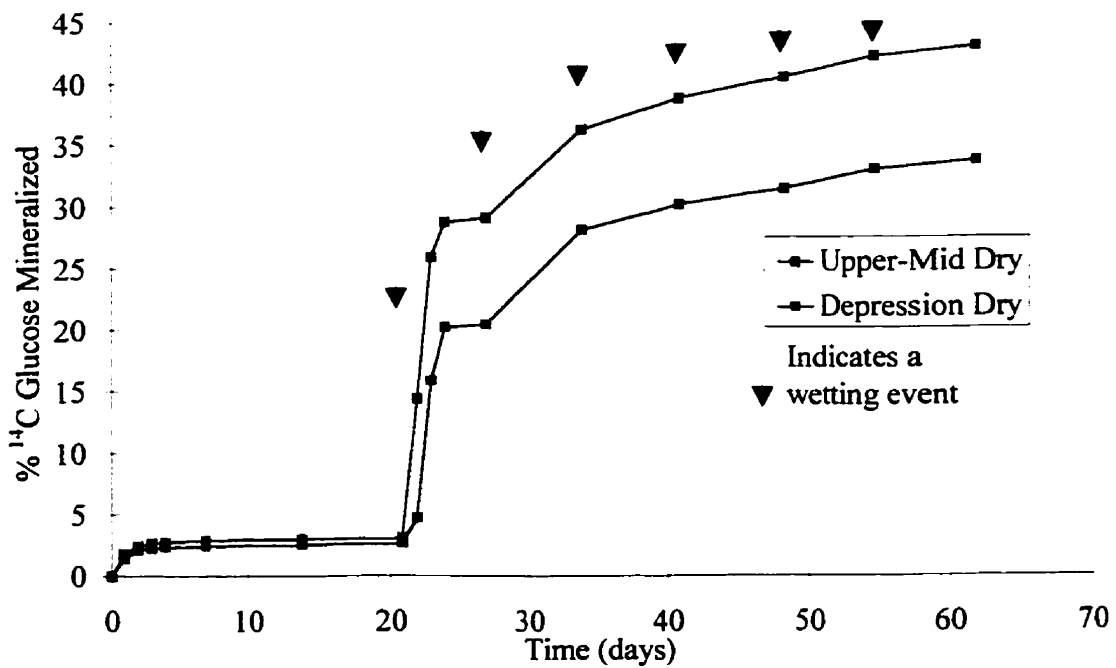

Figure 5.7 Mineralization of ^{14}C Glucose in soils which were air dried for 21 days then subjected to wet-dry conditions

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Table 5.3 Mineralization of ^{14}C glucose in the upper-mid and depression of a slope transect. Continuous dry, continuous wet, wet-dry and wetting of prolonged dry soil were moisture conditions subjected to the soil.

Soil	% ^{14}C Glucose Mineralized (A)	Mineralization Rate (k) (day^{-1})	Mineralization Half Life (days)
Upper-Mid Dry	2.3 ± 0.4	1.7 ± 0.2	0.4
Upper-Mid Wet	52.7 ± 2.9	0.4 ± 0.04	1.6
Upper-Mid Wet-Dry	55.1 ± 2.1	0.5 ± 0.05	1.4
Upper-Mid Air Dry Then Wetted	37.0 ± 3.6	0.3 ± 0.1	2.0
Depression Dry	1.9 ± 1.2	2.4 ± 1.8	0.3
Depression Wet	55.1 ± 0.8	0.4 ± 0.02	1.6
Depression Wet-Dry	41.7 ± 0.2	0.5 ± 0.01	1.5
Depression Air Dry Then Wetted	28.5 ± 5.7	0.2 ± 0.1	3.2

Table 5.4 Duncan New Multiple Range Test for comparing the sites for ^{14}C Glucose mineralization over the course of the wet-dry experiment.

	% ^{14}C Glucose Mineralized
Site Comparisons[x]	
Upper-Mid	36.8 b
Depression	31.8 a
Treatment Comparison[t]	
Dry	2.1 a
Wet	53.9 d
Wet-Dry	48.4 c
Dry then Wet	32.8 b
ANOVA	
Site	***
Treatment	***
Site x Treatment	***

[x]Average of 12 replicates.
[t]Average of 6 replicates.
Means followed by a common letter are not significantly different at the 5% level.
***-highly significant, **-significant, *-low significance, ns-not significant ($\alpha = 0.05$)

The upper-mid slope under wet-dry conditions and the depression under continuous wet conditions had the greatest overall mineralization of the glucose at about 55%. This may be explained by looking at the properties of the soil in the environment. The upper-mid slope in the landscape would be subjected to wet-dry conditions

throughout the year. When rainfall occurs there would be some infiltration but the majority would run off and end up in the depression. Because this site receives less water and can be further away from the water table, the soil dries out more readily than a depression soil. Microbial populations in the soil would then be more adapted to these conditions, therefore degrading substrate efficiently. The same can be said about the depression. This soil would be under continuous wet conditions with organisms adapted to these conditions. The results must be treated with some caution because the majority of the mineralization occurred in the first week of application of the glucose. The least amount of mineralization was seen in the depression after prolonged dry soil was subjected to wet-dry conditions. The upper-mid soil also had significantly less mineralization but was higher than the depression. These results may be attributed to the fact more organic matter would be found in the depression creating a greater chance for adsorption and decrease bioavailability of the compound resulting in less mineralization. Manilal and Alexander (1991) found a soil with 36.7% organic matter had 42% degradation of phenanthrene while a soil with 5.9% organic matter had 46% after 20 days.

The depression had less mineralization than the upper-mid slope in almost all cases. This may be a bit deceiving because greater microbial activity would be expected where there was greater organic matter for substrate and nutrients (Smith et al. 1993). This may indicate a possible role of organic matter in the decreased bioavailability of substrates in the depression.

5.5.1.3 Incorporation of ^{14}C Glucose into the Microbial Biomass Incorporation of glucose into the biomass is another method of determining the fate (degradation) of compounds in the environment. The incorporation of ^{14}C into biomass is listed in Table

5.5. Glucose is a readily degradable substrate and was easily converted to microbial biomass. Location in the landscape significantly affects incorporation into the biomass. The upper-mid slope had less incorporation of ^{14}C than the depression. Also, incorporation into the biomass under wet-dry conditions in the depression and upper-mid slope was significantly higher than other treatments for glucose (Table 5.6). Van Veen et al. (1985) concluded wet-dry treatments significantly decreased the incorporation of ^{14}C plant material and glucose (~25%) into the microbial biomass compared to continuously moist (~37%). The results concluded in this experiment could be due to the different incubation techniques (continuous air stream as opposed to a static microcosm) and microbial populations sampled in the environment. Incorporation into the biomass was least favored on the upper-mid slope under wet and delayed wet dry conditions. The upper-mid slope when under continuously wet conditions does not favor incorporation into the biomass when compared to the depression. This may be due to the fact organisms in the environment are not subjected to these conditions therefore may not biosynthesize compounds as they normally would.

Table 5.5 Incorporation of ^{14}C glucose into the microbial biomass.

Sample	^{14}C Incorporated Into Biomass From Glucose ($K_{ec} = 0.25$)
Upper-Mid Dry	*Not Determined
Upper-Mid Wet	12.6 ± 2.6
Upper-Mid Wet-Dry	15.7 ± 2.7
Upper-Mid Dry Then Wetted	13.9 ± 0.9
Depression Dry	Not Determined
Depression Wet	18.6 ± 2.2
Depression Wet-Dry	22.1 ± 0.8
Depression Dry Then Wetted	17.3 ± 0.3

*Air dry soils were wetted during the experiment therefore no data could be analyzed because biomass was determined when the experiment was completed.

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Table 5.6 Duncan New Multiple Range Test for comparing the sites for ^{14}C incorporation into the biomass over the course of the wet-dry experiment.

	^{14}C Incorporated Into Biomass From Glucose
Site Comparisons[x]	
Upper-Mid	14.1 a
Depression	19.3 b
Treatment Comparison[t]	
Wet	15.6 a
Wet-Dry	18.9 b
Dry then Wet	15.6 a
ANOVA	
Site	***
Treatment	*
Site x Treatment	ns

[x]Average of 12 replicates.
[t]Average of 6 replicates.
Means followed by a common letter are not significantly different at the 5% level.
***-highly significant, **-significant, *-low significance, ns-not significant ($\alpha = 0.05$)

5.5.1.4 Mass Balance of the ^{14}C Glucose Experiment The mass balance of radioactivity

added to the soil is listed in Table 5.7. The summing of mineralization, volatilization, and

biomass ^{14}C results in recoveries of 45.8 to 73.6%. The remaining ^{14}C (between 50 and

25%) was unrecoverable and could have been found in the soil if a wet digestion had been

performed. The data indicates the majority of the ^{14}C glucose introduced into the soil was

mineralized to CO_2 because glucose and glucose like substrates can be found in almost all

soil environments. Incorporation into the microbial biomass was also a factor in the

disappearance of glucose with values of 12 to 22%. Volatilization of glucose was zero

indicating the fate was dependent on the soil and microbial properties. The dry then

wetted treatments had poor total recoveries and were significantly different from the other

treatments (Table 5.8). This may be due to the fact that a larger amount of glucose may

have been incorporated into the soil by adsorption or humification decreasing

bioavailability. Sorption may have occurred during the 21 day dry treatment. The time prior to water addition would favor sorption to particles and organic matter (Carmichael et al. 1997) rendering the compound unavailable to microbial attack even with wetting and drying of the soil.

The results indicate there was no effect of landscape or moisture on the total mineralization and incorporation into tne biomass (Table 5.7). The mass balance indicates there were no significant differences between wetting and drying and continuously wet treatments. There were also no differences in the landscape studied.

Table 5.7 Mass balance of the ^{14}C glucose added to the soil under various moisture conditions.

Sample	% ^{14}C Volatilized	% ^{14}C Mineralized	% ^{14}C Incorporated Into Biomass	Total ^{14}C Recovered
Upper-Mid Dry	nr	2.3 ± 0.4	*Not Determined	2.3 ± 0.4
Upper-Mid Wet	nr	52.7 ± 2.9	12.6 ± 2.6	65.4 ± 2.0
Upper-Mid Wet-Dry	nr	55.1 ± 2.1	15.7 ± 2.7	70.8 ± 1.4
Upper-Mid Dry Then Wetted	nr	37.0 ± 3.6	13.9 ± 0.9	50.9 ± 0.9
Depression Dry	nr	1.9 ± 1.2	Not Determined	1.9 ± 1.2
Depression Wet	nr	55.1 ± 0.8	18.6 ± 2.2	73.6 ± 1.8
Depression Wet-Dry	nr	41.7 ± 0.2	22.1 ± 0.8	63.8 ± 0.3
Depression Dry Then Wetted	nr	28.5 ± 5.7	17.3 ± 0.3	45.8 ± 5.8

*Air dry soils were wetted during the experiment therefore no data could be analyzed because biomass was determined when the experiment was completed.
nr = none recovered

Table 5.8 Duncan New Multiple Range Test for comparing the sites for total ^{14}C recovered at the end of the wet-dry experiment.

	Total ^{14}C Recovered
__Site Comparisons__[x]	
Upper-Mid	47.3 a
Depression	46.3 a
__Treatment Comparison__[t]	
Dry	2.1 a
Wet	69.5 c
Wet-Dry	67.3 c
Dry then Wet	48.4 b
__ANOVA__	
Site	ns
Treatment	***
Site x Treatment	***

[x]Average of 12 replicates.
[t]Average of 6 replicates.
Means followed by a common letter are not significantly different at the 5% level.
***-highly significant, **-significant, *-low significance, ns-not significant ($\alpha = 0.05$)

5.5.2 Fate of ^{14}C Phenanthrene in 5000 ppm Diesel Fuel

The mineralization of radiolabeled phenanthrene in diesel fuel was monitored in soils sampled at two positions in the landscape. Soil microcosms allowed the simultaneous study of mineralization, volatilization, and incorporation into the microbial biomass throughout a 126 day experiment.

5.5.2.1 ^{14}C Phenanthrene Volatilization The data indicates little transfer into the gaseous phase which is consistent with its chemical properties (vapour pressure 0.113 Pa at 25°C and log K_{ow} 4.53 at 26°C (Piatt et al. 1996)) (Table 5.9). There were no significant differences in volatilization between sites or treatments in this investigation (Table 5.10).

Table 5.9 Volatilization of phenanthrene in 5000 ppm of diesel fuel under various moisture conditions.

Sample	% ^{14}C Phenanthrene Volatilized
Upper-Mid Dry	0.1 ± 0.1
Upper-Mid Wet	0.1 ± 0.1
Upper-Mid Wet-Dry	0.3 ± 0.3
*Upper-Mid Dry Then Wetted	0.1 ± 0.1
Depression Dry	0.1 ± 0.1
Depression Wet	0.2 ± 0.2
Depression Wet-Dry	0.1 ± 0.04
*Depression Dry Then Wetted	0.1 ± 0.1

*Air Dry soil subjected to wetting and drying at day 77 for Phenanthrene

Table 5.10 Duncan New Multiple Range Test for comparing the sites for ^{14}C Phenanthrene volatilization over the course of the wet-dry experiment.

	% ^{14}C Phenanthrene Volatilized
Site Comparisons^x	
Upper-Mid	0.2 a
Depression	0.1 a
Treatment Comparison^t	
Dry	0.1 a
Wet	0.2 a
Wet-Dry	0.2 a
Dry then Wet	0.1 a
ANOVA	
Site	ns
Treatment	ns
Site x Treatment	ns

^{x}Average of 12 replicates.
^{t}Average of 6 replicates.
Means followed by a common letter are not significantly different at the 5% level.
***-highly significant, **-significant, *-low significance, ns-not significant (α = 0.05)

5.5.2.2 Mineralization of ^{14}C Phenanthrene in 5000 ppm Diesel Fuel The rate and extent of phenanthrene mineralization can be seen in Table 5.11. The mineralization of phenanthrene was inhibited in air dry soil. This is due to the intense moisture stress subjected to the microorganisms rendering them inactive (Wilson and Griffin 1975). The addition of water causes a burst of mineralization. The end result indicated there was less

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mineralized compounds in this treatment compared to the other two conditions. The mineralization half life was shorter after prolonged dry periods but the total mineralized phenanthrene was less than continuously wet and wet-dry conditions. This indicates less phenanthrene was bioavailable after the drying treatment. A compound when introduced into dry soil may tend to adsorb to soil constituents more readily than if it were in solution, effectively reducing bioavailability Shelton et al. (1995) found atrazine decreased in bioavailability by up to 22% when the soil was dried and rewetted. Because the soil remained at air dry conditions for 77 days, there would be enhanced adsorption which may not be reversible during the wet-dry treatments.

Soils held at continuous wet conditions mineralized about 45% of the phenanthrene while the wet-dry cycle resulted in the mineralization of 42 and 46% of the added phenanthrene. The mineralization of ^{14}C phenanthrene under prolonged drying of the soil is significantly different from the amount mineralized under the continuously wet treatment but not under the wet dry condition (Table 5.12). Prolonged drying, though, appears to have some effect on the mineralization of phenanthrene in the depression by decreasing the half life. The soil in the depression had mineralization up to 42% with a half life of 11 days. The continuous wet and wet dry conditions have half lives of 16 and 46 days respectfully. The upper-mid slope does not have as great of a difference, but the prolonged drying had a shorter half life than the wet dry treatment.

The upper-mid slope under continuous wet conditions had the greatest mineralization at this site. The microbial populations able to mineralize phenanthrene favor continuous wet conditions over wet-dry. Van Veen et al. (1985) found wet treatments had up to 65% production of $^{14}CO_2$ from glucose-ammonium compared to 63%

production in wet-dry treatments. The data indicates an important role of the properties of

the compound and microflora and not the moisture treatments. Widrig and Manning

(1995) subjected soil columns containing diesel fuel to wet-dry conditions. They found

enhanced degradation of diesel components (~70% reduction) over continuously flooded

systems (~50% reduction). These soils were constructed artificially with mixtures of sand

silt and clays and subjected to water mixtures containing nutrients. Though the

experimental methods differ, the results indicate wetting and drying can have an effect on

mineralization of components in soil.

Table 5.11 Mineralization of ^{14}C phenanthrene in 5000 ppm diesel fuel in the upper-mid and depression of a slope transect. Continuous dry, continuous wet, wet-dry and wetting of prolonged dry soil were moisture conditions subjected to the soil.

Soil	% ^{14}C Phenanthrene Mineralized (A)	Mineralization Rate (k) (day^{-1})	Mineralization Half Life (days)
Upper-Mid Dry	0.2 ± 0.2	0.03 ± 0.03	23
Upper-Mid Wet	45.8 ± 9.3	0.05 ± 0.01	14
Upper-Mid Wet-Dry	42.7 ± 4.4	0.03 ± 0.01	23
Upper-Mid Dry Then Wetted	36.2 ± 24.7	0.04 ± 0.01	18
Depression Dry	0.2 ± 0.2	0.04 ± 0.02	16
Depression Wet	44.2 ± 8.4	0.04 ± 0.004	16
Depression Wet-Dry	46.4 ± 8.5	0.02 ± 0.01	46
Depression Dry Then Wetted	41.9 ± 7.4	0.06 ± 0.01	11

Table 5.12 Duncan New Multiple Range Test for comparing the sites for ^{14}C Phenanthrene mineralization over the course of the wet-dry experiment.

	% ^{14}C Phenanthrene Mineralized
Site Comparisons[x]	
Upper-Mid	28.8 a
Depression	30.5 a
Treatment Comparison[t]	
Dry	0.2 a
Wet	46.3 c
Wet-Dry	38.3 bc
Dry then Wet	33.8 b
ANOVA	
Site	ns
Treatment	***
Site x Treatment	ns

[x]Average of 12 replicates.
[t]Average of 6 replicates.
Means followed by a common letter are not significantly different at the 5% level.
***-highly significant, **-significant, *-low significance, ns-not significant ($\alpha = 0.05$)

Figure 5.8 and 5.9 indicate mineralization occurred fairly rapidly in the soil with the majority of the mineralization occurring in the first 20 days after the addition of the phenanthrene. When soil was air dry for 77 days then rewetted there was quick mineralization within the first 25 days. When the continuous wet soil was subjected to wet-dry conditions at day 77, a slight increase in mineralization occurred in the upper-mid slope but very little occurred in the depression. The increase in mineralization may be due to the release of phenanthrene from aggregates and organic matter or the release of other substrates that may be used in cometabolism (Lebedjantzev 1924; Van Gestel et al. 1991). It appears after the addition of phenanthrene, the wet soils had a large flush of mineralization between 7 and 14 days. This flush subsequently trailed off with little mineralization afterwards. On the other hand, wet-dry conditions did not show much

mineralization until the 21 to 28 day interval. From here a much more steady rate of mineralization occurred after this point compared to wet conditions. Reasons for this slight lag before mineralization may be just the adaptation of the populations to these conditions. Also if the mineralization of phenanthrene was not immediately after the addition of the compound, the populations began to degrade and soon were subjected to moisture stress. At this point the growth and mineralization would soon trail off until the addition of water again. Wetting of prolonged dry soil had some mineralization about 14 days after addition of the water, with the majority occurring at the 21 to 28 day interval.
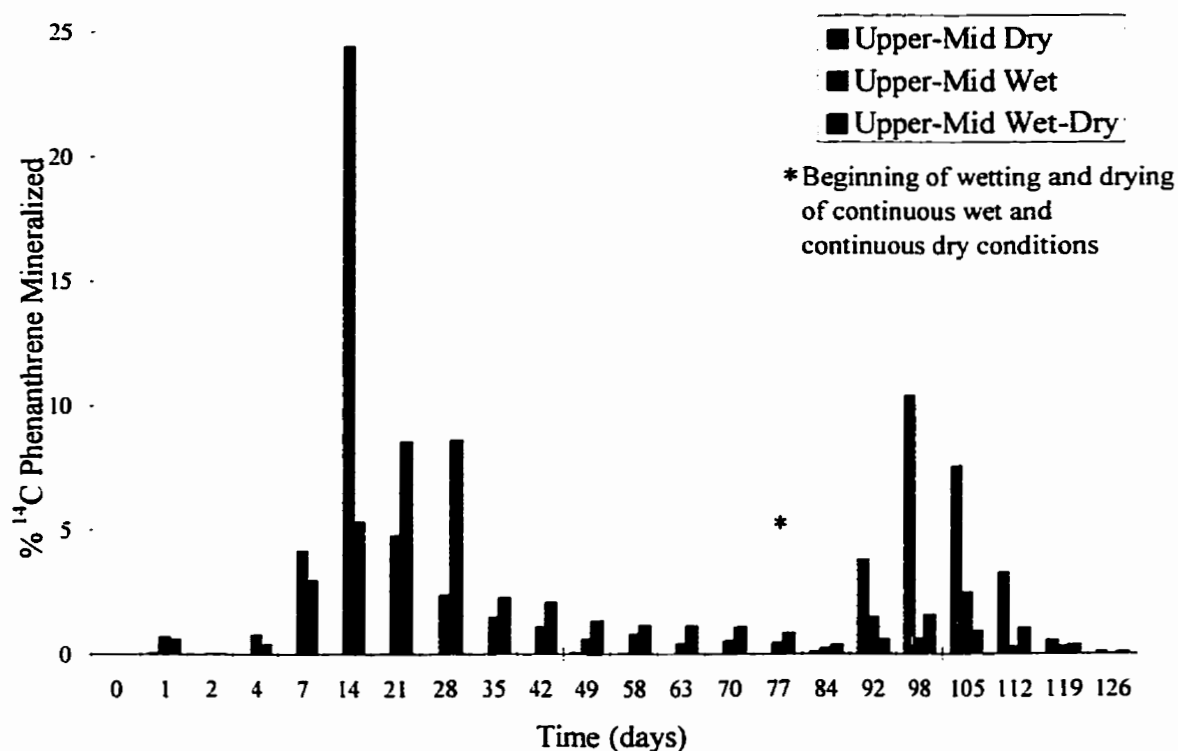
Figure 5.8 Temporal variation in the production of $^{14}CO_2$ in the upper-mid slope under continuous dry, continuous wet, and wet-dry conditions. The continuous dry and continuous wet conditions were subjected to wetting and drying at the 77 day interval.
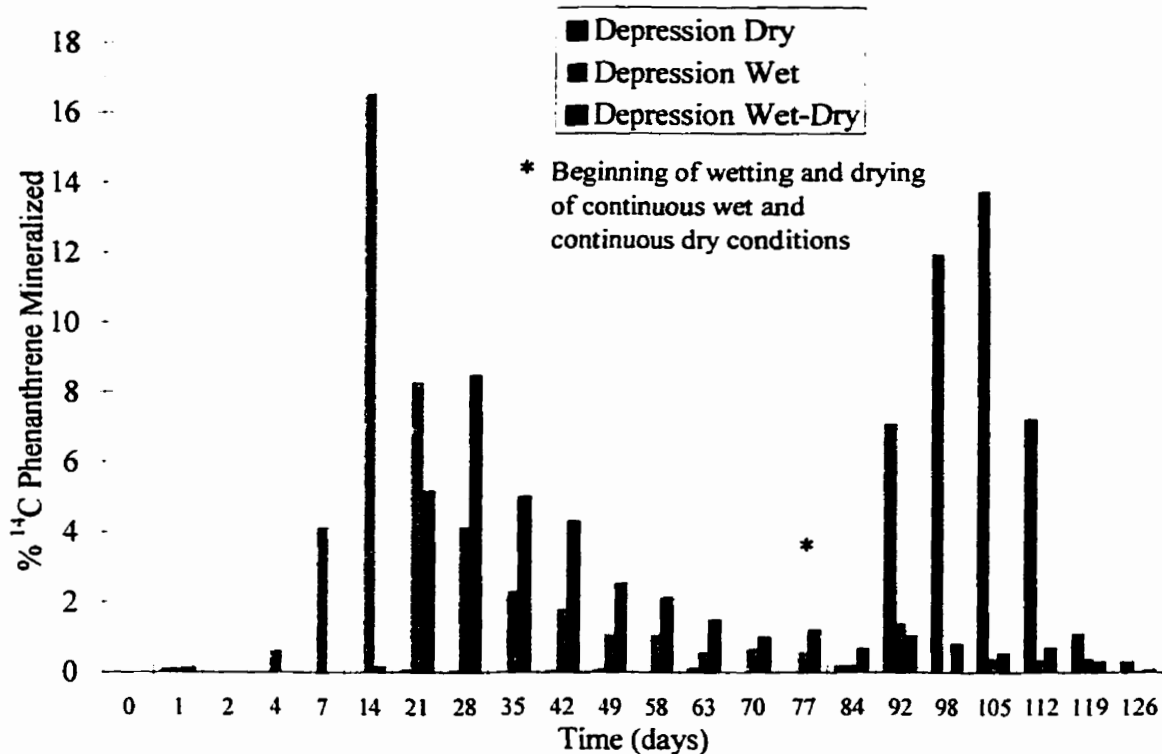
Figure 5.9 Temporal variation in the production of $^{14}CO_2$ in the depression under continuous dry, continuous wet, and wet-dry conditions. The continuous dry and continuous wet conditions were subjected to wetting and drying at the 77 day interval.

5.5.2.3 Incorporation of ^{14}C Phenanthrene into the Microbial Biomass When considering the properties of the biomass for phenanthrene, incorporation of the 9-^{14}C carbon is not favored (Table 5.13). There were no significant differences between sites or treatments for all properties of this experiment (Table 5.14). Both sites under all conditions had less than 2% of the total radioactivity added to the soil in the biomass. This was most likely due to the complexity of the degradation pathway (Figure 2.7). The location of phenanthrene-9-^{14}C may dictate the release of radioactivity at the end of degradation and result in $^{14}CO_2$ production and not biosynthesis.

Table 5.13 Incorporation of ^{14}C phenanthrene into the microbial biomass.

Sample	^{14}C Incorporated Into Biomass From Phenanthrene ($K_{ec} = 0.25$)
Upper-Mid Dry	Not Determined
Upper-Mid Wet	1.7 ± 0.9
Upper-Mid Wet-Dry	1.2 ± 0.9
Upper-Mid Dry Then Wetted	1.4 ± 0.9
Depression Dry	Not Determined
Depression Wet	0.8 ± 0.4
Depression Wet-Dry	1.9 ± 0.9
Depression Dry Then Wetted	1.0 ± 0.9

*Air dry soils were wetted during the experiment therefore no data could be analyzed because biomass was determined when the experiment was completed.

Table 5.14 Duncan New Multiple Range Test for comparing the sites for ^{14}C incorporation into the microbial biomass over the course of the wet-dry experiment.

	^{14}C Incorporated Into Biomass From Phenanthrene
Site Comparisons[x]	
Upper-Mid	1.1 a
Depression	1.5 a
Treatment Comparison[t]	
Wet	1.4 a
Wet-Dry	1.5 a
Dry then Wet	1.1 a
ANOVA	
Site	ns
Treatment	ns
Site x Treatment	ns

[x]Average of 12 replicates.
[t]Average of 6 replicates.
Means followed by a common letter are not significantly different at the 5% level.
***-highly significant, **-significant, *-low significance, ns-not significant ($\alpha = 0.05$)

5.5.2.4 Total Organic ^{14}C Remaining in Soil After Phenanthrene Experiment The

wet digestion of the soil in the phenanthrene experiment resulted in the recovery of 9.4 to

19.2% of the added radioactivity (Table 5.15). The upper-mid slope soil sample had the

least amount of ^{14}C remaining in the soil, while the depression that was dried for 77 days

then wetted had the most. The data seems to indicate drying of the soil favors adsorption and decreased bioavailability yet there was no significant differences between treatments (Table 5.16). Shelton et al. (1995) concluded drying of the soil decreases the bioavailability of atrazine by 22%. In this example, drying of the soil increased the amounts of phenanthrene recovered yet it was not significantly different from the wet conditions. It appears that a certain fraction of the ^{14}C from phenanthrene is incorporated in the soil and becomes stable, therefore resistant to mineralization and volatilization.

Table 5.15 Amount of ^{14}C liberated from the wet digested phenanthrene contaminated soil.

Sample	% ^{14}C Liberated From the Soil After Wet Digestion
Upper-Mid Dry	*Not Determined
Upper-Mid Wet	9.4 ± 4.8
Upper-Mid Wet-Dry	13.9 ± 14.0
Upper-Mid Dry Then Wetted	12.0 ± 3.3
Depression Dry	Not Determined
Depression Wet	16.9 ± 0.8
Depression Wet-Dry	13.4 ± 7.4
Depression Dry Then Wetted	19.2 ± 11.2

*Air dry soils were wetted during the experiment therefore no data could be analyzed because wet digestion was determined when the experiment was completed.

Table 5.16   Duncan New Multiple Range Test for comparing the sites for ^{14}C liberated from the wet digestible phase.

	% ^{14}C Liberated From the Soil After Wet Digestion
<u>Site Comparisons^x</u>	
Upper-Mid	11.8 a
Depression	16.5 a
<u>Treatment Comparison^t</u>	
Wet	13.7 a
Wet-Dry	13.1 a
Dry then Wet	15.6 a
<u>ANOVA</u>	
Site	ns
Treatment	ns
Site x Treatment	ns

^{x}Average of 12 replicates.
^{t}Average of 6 replicates.
Means followed by a common letter are not significantly different at the 5% level.
***-highly significant, **-significant, *-low significance, ns-not significant ($\alpha = 0.05$)

5.5.2.5   Mass Balance of the ^{14}C Phenanthrene Experiment   The summing of mineralization, volatilization, biomass, and wet digestible ^{14}C results in recoveries of 49.8 to 62.8% when excluding the continuously dry treatments (Table 5.17). The remaining ^{14}C (between 50 and 35%) was unrecoverable and could be attributed an unaccounted loss. The data indicates the majority of the ^{14}C phenanthrene in 5000 ppm diesel added to air dried, ground and stored soil was mineralized to CO_2 at 22°C.

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Table 5.17 Mass balance of the ^{14}C phenanthrene in 5000 ppm diesel fuel added to the soil under various moisture conditions.

Sample	% ^{14}C Volatilized	% ^{14}C Mineralized	% ^{14}C Incorporated Into Biomass	% ^{14}C Liberated From the Soil After Wet Digestion	Total ^{14}C Recovered
Upper-Mid Dry	0.1 ± 0.1	0.2 ± 0.2	*Not Determined	*Not Determined	0.4 ± 0.05
Upper-Mid Wet	0.1 ± 0.1	45.8 ± 9.3	1.7 ± 0.9	9.4 ± 4.8	56.5 ± 14.0
Upper-Mid Wet-Dry	0.3 ± 0.3	42.7 ± 4.4	1.2 ± 0.9	13.9 ± 14.0	57.6 ± 12.6
Upper-Mid Dry Then Wetted	0.1 ± 0.1	36.2 ± 24.7	1.4 ± 0.9	12.0 ± 3.3	49.8 ± 20.0
Depression Dry	0.1 ± 0.1	0.2 ± 0.2	*Not Determined	*Not Determined	0.3 ± 0.1
Depression Wet	0.2 ± 0.2	44.2 ± 8.4	0.8 ± 0.4	16.9 ± 0.8	62.3 ± 9.6
Depression Wet-Dry	0.1 ± 0.04	46.4 ± 8.5	1.9 ± 0.9	13.4 ± 7.4	61.8 ± 10.0
Depression Dry Then Wetted	0.1 ± 0.1	41.9 ± 7.4	1.0 ± 0.9	19.2 ± 11.2	62.8 ± 14.2

*Air dry soils were wetted during the experiment therefore no data could be analyzed because biomass and wet digestion were determined when the experiment was completed.

5.6 Conclusion

The mineralization of phenanthrene can be a significant fate under appropriate conditions. Phenanthrene mineralization in diesel fuel was rapid at both sites along the landscape. The process of aeration may have enhanced the mineralization of substrates (phenanthrene and glucose) in soil. The breakdown of glucose was greater under continuously wet conditions, while the incorporation into the biomass was higher under wet-dry conditions. The total glucose "degraded" (mineralization plus biomass) was essentially the same for both moisture treatments. The mineralization of phenanthrene, in 5000 ppm diesel, was also the same under both moisture conditions. The wetting and drying of continuously wet trials at the end of the experiment did increase the bioavailability of the ^{14}C phenanthrene remaining in the soil resulting in further mineralization. The mineralization of glucose in the depression was slightly less than in the upper-mid slope, but the incorporation into the biomass had the opposite effect. In the end there were no differences between landscape position and the total mineralization of glucose. The results indicate there will be no effect of landscape on the mineralization and incorporation into the biomass of phenanthrene and glucose in this catena.

It can be concluded aeration may be the optimum condition for mineralization of diesel fuel components *in situ*. The study indicated wetting and drying had no effect on the mineralization of phenanthrene in diesel, yet there was significant ^{14}C production compared to other studies (Chapter 3 and Chapter 4). The forced air through the

microcosm maintained aerobic conditions throughout the soil promoting microbial activity

and mineralization.

CHAPTER 6

General Discussion

The objective of the study was to determine the presence of phenanthrene degrading microorganisms in soil. Chapter 3 was a survey of the potential for mineralization of phenanthrene in diesel fuel in uncontaminated and previously contaminated soils. Chapter 4 looked at one soil located in a landscape and determined the effect of changing landscape on the mineralization of phenanthrene and the degradation of hydrocarbons in intact soil columns and disturbed soil microcosms. Chapter 5 investigated the effects of changing moisture potential (wetting and drying) on the mineralization of phenanthrene in diesel fuel.

6.1 Mineralization of Phenanthrene in Diesel Fuel

The literature has shown phenanthrene can be degraded by organisms native to the soil environment (Stringfellow and Aitken 1995; Møller and Ingvorsen 1993; Kästner et al. 1994; MacGillivray and Shiaris 1993; Atlas and Cerniglia 1995). Foght et al. (1990) found up to 4% (6 isolates out of 138) of organisms isolated from fresh water, marine and estuaries were able to mineralize phenanthrene. In this study the mineralization of

phenanthrene in freshly sampled soils previously uncontaminated with hydrocarbons was low but not zero. The mineralization of the 9th C in phenanthrene was usually less than 10% in all soils investigated with mineralization half lives between 132 and 15 days. When mineralization was higher than 10% the standard deviation was also high indicating the process was sporadic. Even though the total mineralization was low, the half lives indicated there was a potential for significant mineralization. It appears the phenanthrene was not bioavailable (sorption) or the soil lacked certain factors (nutrients, organic matter etc.) needed for degradation. The accumulation of degradation intermediates, unable to proceed to $^{14}CO_2$, may have also contributed to the decreased mineralization. The fact that all soils had organisms capable of phenanthrene mineralization is important because this isn't always the case. Kästner et al. (1994) demonstrated no zone forming units in freshly sampled uncontaminated soil using a method of soil extraction and plating on minimal medium plates with phenanthrene as the sole carbon source.

Texture, organic carbon and microbial metabolic diversity were poor indicators of the total mineralized phenanthrene in soil. The poor relationship may be due to bioavailability. It appears phenanthrene was sorbed readily in the soil because extractions at the end of the experiment indicated the majority of the ^{14}C was in a methanol extractable phase. When considering the mineralization rate, there was a correlation with organic carbon and metabolic diversity. This indicates there were more active microorganisms capable of mineralizing the phenanthrene before it was no longer bioavailable. Organic carbon and microbial metabolic diversity would be good indicators of the potential for phenanthrene mineralization in soil.

Chapter 4 investigated the effect phenanthrene and diesel fuel addition had on respiration. It was concluded the addition of the hydrocarbon substrate increased the rate of CO_2 released over soils without diesel addition. This indicates the diesel fuel provided a substrate to be mineralized increasing the activity of the microbial populations. Phelps et al. (1994) also concluded the same trend when diesel fuel was added to soil but neglected to indicate control respiration rates. The mineralization of ^{14}C phenanthrene was low (10%) in this study, yet degradation of the total hydrocarbons was between 16 and 48%. The low amounts of phenanthrene mineralization may then be due to catabolite repression or competitive metabolism by the other substrates in diesel fuel. Stringfellow and Aitken (1995) demonstrated the competitive metabolism of phenanthrene in polyaromatic hydrocarbon degrading organisms. They found an inhibition of the uptake of phenanthrene by *Pseudomonas stutzeri* and *P. saccharophilia* in the presence of other polyaromatic hydrocarbon compounds. This property of the microbial population would then be consistent with the finding in this study.

Prior sensitization of hydrocarbons on the soil decreased the half life and acclimation period of phenanthrene degradation in diesel fuel due to prior enzyme stimulation in one of the sites investigated. Keuth and Rehm (1991) were able to isolate from a contaminated site *Arthrobacter polychromogenes* capable of mineralizing 47.7% phenanthrene. *Alcaligenes* sp., isolated from an oil polluted site, was able to mineralize 91% of the added phenanthrene after 22 days of incubation (MØller and Ingvorsen 1993). The present study indicated enhanced mineralization of phenanthrene in both the surface and subsurface of Site #10 (44-56%). The second site (Site #8) had poor mineralization (2-4%) yet was a site of a diesel spill. The results tend to indicate other factors besides

prior exposure (texture, organic matter, water content etc.) affect the mineralization of phenanthrene in diesel fuel.

6.2 Effect of Wetting and Drying and Landscape on Mineralization of Phenanthrene and Glucose

Van Gestel et al. (1993), Sorensen (1974), Amato et al. (1983), and Bottner (1985) concluded wetting and drying increased the decomposition of added plant substrate compared to continuously moist or continuously dry. Taylor and Parkinson (1988) found wet-dry treatments (wet 4 days, dried and quickly rewetted) had more degradation of aspen leaves (6.0g starting weight to 4.77g final weight) than continuously wet leaves (4.89g) after 3 months. They attributed some of the degradation to hydrolysis attack of cellulose during the drying treatment. Widrig and Manning (1995) subjected soil columns containing diesel fuel to wet-dry conditions. They found enhanced degradation of diesel components (~70% reduction) over continuously flooded systems (~50% reduction). Haider and Martin (1981) found wetting and drying (59%) had the same as or less degradation of lignin-coniferyl alcohols as wet trials (60%) after 24 months of incubation. In the current study the wetting and drying of phenanthrene or glucose in soil was not significantly different from continuously wet soil treatments. There was, however, a decrease in the mineralization of glucose during wet-dry cycles compared to continuously wet treatments. The incorporation into the microbial biomass had greater ^{14}C associated with wet-dry cycles. In the end there was no difference in the total glucose degraded (mineralization + biomass). The wetting and drying of phenanthrene had no differences

between wetting and drying and continuously wet treatments during mineralization and incorporation into the microbial biomass. It appears the effects of moisture potentials may be soil specific and dependent on the natural conditions from which the microbial populations have evolved.

The literature contains few examples of the effect of landscape on the degradation of hydrocarbons. Hanna et al. (1982) demonstrated that a depression has more available water (~15.04cm) than upper slope positions (~12.31cm) indicating a greater potential for microbial activity. Available water is influenced by the amounts of organic matter and texture of the soil. In the depression the water table is closer to the surface and can have greater amounts of clay and organic matter due to runoff. Van Kessel et al. (1993) also demonstrated various slope positions have a controlling effect on the microbial process of denitrification. The denitrification was higher on June 4th, 1991 in the depression (157 to 556 g N ha^{-1} d^{-1}) than on the knoll (37 to 302 g N ha^{-1} d^{-1}) signaling the effect of texture, organic matter and location of the water table in a landscape on microbial processes. In this example the availability of oxygen was a controlling factor in the microbial denitrification. Diesel fuel degradation is mainly an aerobic pathway (Atlas and Bartha 1993), therefore the depression may have significantly less bioremediation yet have the majority of the diesel present due to runoff. In Chapter 4 and 5, landscape had a no effect on the mineralization of phenanthrene in diesel fuel or glucose in the surface sites. There may be equal potential for phenanthrene and glucose mineralization along the catena. The upper-mid slope was not significantly different from the depression.

6.3 Type of Mineralization Study and Handling of Soil Samples

The mineralization of phenanthrene has been documented extensively in the literature (Howard et al. 1991; Foght et al.1990; Møller and Ingvorsen 1993). For the most part the majority of the studies have looked at disturbed soil samples, soil slurries and culture conditions and not intact soil columns. Most of the studies also used static microcosms without flow through systems (Keuth and Rehm 1991; Møller and Ingvorsen 1993). Mineralization in the literature ranged from 0% (Kästner et al. 1994 (2000 mg phenanthrene/L PAH coated agar plates)) to 80% (Møller and Ingvorsen 1993 (1 mg phenanthrene/kg soil)). In Chapter 4 mineralization was significantly higher (15x) in the intact soil columns (~8% mineralized) compared to microcosms in the same chapter and in Chapter 3 (0.5-3% mineralized) (Table 6.1). If the concentration of diesel fuel is ignored, the main difference between the two studies is structure and aeration (the concentration of diesel fuel may, however, cause toxicity and catabolite repression in soil systems). The soil cores in Chapter 4 (Site #5) were significantly higher than the microcosms in both chapters. This may indicate structure had a role in mineralization, but it is probably not the case. Chapter 5 (Site #2) soil was air dried and ground and had significantly higher mineralization than the same soil in Chapter 3. This removes the role of structure in mineralization because both soils in Chapter 3 and Chapter 5 were air dried and ground. Chapter 5 soil was aerated in the same manner as Chapter 4. The results may suggest it was in fact aeration that increased the mineralization in the soils studied. This suggests static microcosms characterize mineralization differently from aerated systems.

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Table 6.1 Comparison of the mineralization of phenanthrene in the surface from sites in Chapters 3, 4, and 5.

Chapter	Site	Apparatus	Physical Stress	Diesel (μg g^{-1} soil)	% ^{14}C Mineralized
Chapter 3	Site #5	Static Microcosm	Drying and grinding	5000	2.9 ± 1.4
Chapter 4	Site #5 (3a)	Aerated Core	None	50 000	6.5 ± 0.8
Chapter 4	Site #5 (3a)	Static Microcosm	Loss of Structure from Sampling	50 000	0.5 ± 0.1
Chapter 3	Site #2	Static Microcosm	Drying and grinding	5000	1.6 ± 0.2
Chapter 5	Site #2	Aerated Microcosm	Drying and grinding	5000	52.7 ± 2.9

6.4 Surface vs. Subsurface

It is anticipated based on the distribution of microbial activity in soil that the biodegradation potential in most cases will be higher in the surface compared to the subsurface. Depth from surface can be a controlling factor in the mineralization of compounds in the environment (Rainwater et al. 1993). The presence of organic matter and microorganisms play a large role in the active degradation of hydrocarbons in soil, yet organic matter also increases the likelihood of sorption. Foght et al. (1990) isolated various organisms capable of aromatic degradation from fresh water in northern Alberta and found only 4% of the bacteria were able to mineralize phenanthrene and none of these strains were able to degrade hexadecane. The results indicate the importance of having appropriate organisms present for mineralization and their selectivity for substrates. When considering Chapter 3 and 4, almost all surface soils did not have significantly greater total

mineralization of phenanthrene compared to subsurface soils. Where there was a significant amount of mineralization in Chapter 3 the surface soil had greater total mineralization and a greater mineralization rate (i.e. Site #4, surface 16.4%, subsurface 4.2%). When organic matter increases, microorganism activity is also increased (Smith et al. 1993) and is generally associated with enhanced mineralization due to the presence of nutrients. Manilal and Alexander (1991) found increasing organic matter seemed to decrease the degradation of phenanthrene by reducing the bioavailability due to adsorption (36% OM had 42% mineralization, 5.9% OM had 46% mineralization in 20 days). The rates of mineralization of phenanthrene in Chapter 3 were higher in the surface. Even though there were no differences in the total mineralization, the microorganisms were more active in degrading the available phenanthrene compared to the subsurface. When comparing the total extractable hydrocarbons in Chapter 4, the subsurface was significantly different from the surface. The surface had greater unrecoverable hydrocarbon than the subsurface indicating either enhanced degradation or greater adsorption of hydrocarbons. The same trend was noticed when looking at the sequential extraction of ^{14}C in Chapter 3. Higher radioactivity was recovered in the surface compared to the subsurface in the soxhlet and wet digestible fractions indicating greater adsorption in organic carbon soils. The respiration of the microorganisms in the same experiment indicated the majority of the sites had greater respiration in the surface compared to the subsurface indicating greater degradation of hydrocarbons. It can be concluded not only was there greater adsorption in the surface but microbial respiration was higher and increased degradation of the hydrocarbons was also present at these positions in the soil profile. Organic matter has two roles affecting mineralization because

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not only does it supply nutrients and a hospitable environment for microorganisms, it also decreases bioavailability. It appears mineralization may be dependent on the ability of the microorganisms to degrade phenanthrene before it becomes no longer bioavailable. When considering the role organic matter plays in soil, the potential for *in situ* bioremediation will be greater where organic carbon is present.

6.5 Fate of Phenanthrene and *In Situ* Bioremediation

The volatilization of phenanthrene was low in the microcosms and cores studied. The data seemed to indicate very little tendency for the transfer into the gaseous phase. The low volatility of this compound is due to its low vapour pressure, high molecular weight and high K_{oc} of this compound (Green and Karickhoff 1990; Piatt et al. 1996). The combination of these and other properties of phenanthrene reduce the likelihood of volatilization from soil *in situ*. Mineralization of ^{14}C was found to be less than 10% in most cases where the soil had no previous hydrocarbon contamination. *In situ* other factors such as nutrient addition and aeration would need to be implemented to enhance this process. In the current study it was found that a steady supply of air may increase mineralization, though this principle was not fully tested or proven (it was merely an observation). Widrig and Manning (1995) and Phelps et al. (1994) both concluded the addition of water, nutrients and air markedly increased the amounts of hydrocarbons degraded in soil columns with up to 78% of the total extractable hydrocarbons being reduced in the second example. It would, therefore, be prudent to study and implement these techniques to increase the degradation process. Extraction of the residual ^{14}C

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remaining in the microcosms and cores indicate the major fate (up to 60%) of the radioactivity added was to remain in the soil. The majority of the radioactivity (up to 50%) was associated with a methanol, soxhlet and wet digestible fraction therefore it can be concluded the ^{14}C was probably not bioavailable. The movement of hydrocarbons into the subsurface can be a major problem in bioremediation (Norris et al. 1994; Dohse and Leonard 1994). The transport of phenanthrene, degradation intermediates, and diesel fuel through the intact soil column in Chapter 4 indicates this may be a major fate. The subsurface can be a harsh environment for microbial growth and activity due to the absence of oxygen, nutrients and growth factors. Without these factors hydrocarbons may be resistant to breakdown (Phelps et al. 1994). It was concluded in intact soil columns up to 30% of the radioactivity added may be found in a water extractable phase resulting in a potential for transport. This water extractable fraction may contain phenanthrene degradation intermediates that are more soluble in water (hydrophilic), therefore may move through the soil profile to a greater extent. The total extractable hydrocarbons had as much as 30% of the diesel "pooling" in the sand fraction. The radioactive phenanthrene and degradation intermediates had as much as 15% of the total added diffuse to the bottom of the core through ~40cm of soil. It is important to note none of the movement was due to preferential flow along the sides of the column because concentrations of phenanthrene were similar at the walls and center of the core.

CHAPTER 7

Summary and Conclusions

The objectives of this study was to determine the fate of phenanthrene, the presence of microorganisms in soils to degrade phenanthrene and the examination of certain physical properties that may influence mineralization (texture, organic carbon, landscape, water potential etc.). It was concluded there were native microbial populations capable of mineralizing phenanthrene, though the amounts of ^{14}C label mineralized was low. The mineralization half lives (15 to 132 days) indicate the added phenanthrene could be mineralized rapidly but other controlling factors (bioavailability, aeration, species selection, toxicity etc.) limited the degradation. Though phenanthrene mineralization was low, the addition of hydrocarbons increased the rate of CO_2 released over soils without. This indicates the diesel fuel provided a substrate to be mineralized increasing the activity of the microbial populations. The mineralization of phenanthrene can be a significant fate in the lab depending on the treatment of the soil. In Chapter 5, the soil was air dried, ground and stored at room temperature before use. This treatment probably did not cause a significant increase in the potential for phenanthrene mineralization, since in Chapter 3 the same contaminant and drying effect was used on the soil yet poor mineralization occurred. The real factor affecting mineralization may in fact be the flow through

apparatus used in Chapter 5 where the soil was aerated to a greater extent compared to a static microcosm (Chapter 3). Prior sensitization of hydrocarbons on the soil decreased the half life and acclimation period of phenanthrene mineralization in diesel fuel. This phenomenon was not consistent because Site #8 with prior hydrocarbon exposure had little mineralization. The results tend to indicate other factors besides prior exposure (texture, organic matter, water content etc.) affect the degradation of phenanthrene in diesel fuel. Organic carbon and microbial metabolic diversity were good indicators of the potential of microorganisms for the mineralization rate of phenanthrene.

The wetting and drying of glucose in soil was not significantly different from continuously wet soil treatments in Chapter 5. The same results was seen for phenanthrene in 5000ppm diesel in the same chapter. Chapter 3 also indicated the same results. The drying and grinding of soil during the experiment had little or no effect on the further mineralization of phenanthrene. Organisms present in the soil investigated do not appear to favor wet-dry conditions over continuously wet soils. It appears the effects of moisture potentials may be soil specific and dependent on the natural conditions from which the microbial populations have evolved.

The landscape studied appears to have little effect on the mineralization of labeled ^{14}C phenanthrene in diesel fuel. The soils had no significant differences between the surface sites investigated indicating there was equal potential for phenanthrene mineralization along the catena. The same result was seen for glucose mineralization. The upper-mid slope was not significantly different from the depression indicating equal potential for glucose mineralization at these two slope positions.

The mineralization potential in intact soil columns was much higher (15x) than microcosms. This indicates microcosms without flow through systems provide a poor estimate of mineralization of phenanthrene in the environment, therefore may underestimate the potential of mineralization in a particular soil. It would be important to note the intact soil column experiment was performed in flow through systems while the microcosms were static. The differences in mineralization may have been due to an aeration problem and not structure.

The volatilization of phenanthrene was low in the microcosms and cores studied even with added aeration. The data seemed to indicate very little tendency for the transfer into the gaseous phase. Extraction of the residual ^{14}C remaining in the microcosms and cores indicate the major component (>50%) of the radioactivity added was either phenanthrene/degradation intermediates (up to 30%) or adsorb components (up to 60%). The movement of phenanthrene and diesel fuel through the intact soil column in Chapter 4 indicates movement will be a factor in fate with up to 30% of the hydrocarbons and 15% of the phenanthrene transported below the 40cm depth.

Surface soils did not have greater mineralization of phenanthrene and other hydrocarbons than the subsurface in Chapters 3 and 4. The mineralization rates of phenanthrene in the surface in Chapter 3 were significantly higher than the subsurface. This indicates the microbial populations were more active in mineralizing the phenanthrene before it became no longer bioavailable. The respiration of the microorganisms in Chapter 4 indicated the majority of the surface sites had greater respiration than the subsurface which in turn results in greater hydrocarbon degradation. There will also be more

bioavailable ^{14}C and a greater potential for transport in subsurface soils due to the decreased adsorption of hydrocarbons in low organic carbon environments.

The majority of scientific studies result in more questions asked than problems answered. Further study in this field should include the determination of the radioactive products remaining in the soil at the end of the experiment. As for the radioactivity remaining in the soil, was it cometabolized to end products and not further degraded to CO_2 or was it simply not degraded at all? Why was there low mineralization of phenanthrene yet there was significant microbial activity? Why was there not more significant differences in phenanthrene mineralization between surface and subsurface soils in Chapter 3? When prior exposure to soil occurs why does one soil have extensive degradation while another does not? If texture and organic carbon have little effect on mineralization, what soil physical property(s), if any, determine mineralization? What factor involved in drying, grinding and storing of the soil in Chapter 5 caused a significant amount of mineralization of phenanthrene or was it simply an apparatus effect? What property of soil structure increases mineralization between intact soil columns and microcosms? Why were there no differences between landscape positions yet the physical, chemical and biological properties differ so much in a catena? These and many more questions need to be answered to determine the properties of phenanthrene mineralization in the environment.

CHAPTER 8

Contribution to Knowledge

The study demonstrated there was a mineralization potential of phenanthrene in freshly sampled uncontaminated soils. It also indicated there may be ways of enhancing this process through aeration. Prior hydrocarbon exposure will not guarantee the presence of microbial populations capable of mineralization of phenanthrene. Wetting of dry soil did not enhance or significantly decrease the potential for phenanthrene mineralization in all soils. When the soil dries naturally in the environment there will be the same potential for mineralization when rainfall occurs. Surface soil mineralization of phenanthrene was not always significantly different from subsurface soils investigated, though when significant mineralization occurred (>5%) it was associated with the surface. Mineralization rates of phenanthrene were higher in the surface even though total mineralization was the same. The mineralization of phenanthrene in the landscape indicated no significant differences between the sites investigated along the catena. Intact soil columns, as opposed to microcosms, may also be more accurate in determining the bioremediation potential of the environment. Aerated (flow through systems) as opposed to static mineralization determinations may increase the mineralization of phenanthrene and

be a better predictor of the mineralization in the environment. Static microcosms, on the other hand, may be a better predictor of the *in situ* non flow through soil conditions.

At the field scale, *in situ* bioremediation may be a feasible option in the remediation strategy. The study has indicated there are organisms native to the environment present to mineralize phenanthrene, therefore inoculation with industrial organisms may not be necessary. Though the mineralization of ^{14}C label in phenanthrene in diesel fuel in the environment would be less than 10% after about 1 year, certain factors such as aeration, nutrient addition, surfactant addition, and irrigation may enhance mineralization. Bioavailability would be a major factor in degradation due to the high rate of adsorption (which turned out to be the major fate). Transport was also a factor in the fate of hydrocarbons, especially in the subsurface, due to the movement through the soil profile, while volatilization was not a factor in the loss of phenanthrene. Major concerns to be considered before implementation of *in situ* bioremediation include: location of water table and aquifer, location of human populations, urgency of closure, and enhanced degradation (bioavailability, surfactant addition, nutrient and water addition etc.) would be essential in risk assessment.

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Wilson, J.M. and Griffin, D.M. 1975. Water potential and the respiration of microorganisms in the soil. Soil Biology and Biochemistry. 7: 199-204.

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APPENDIX

I a. Duncan New Multiple Range Test for comparing the surface soils for sequential extraction of ^{14}C from soil at the end of the soil microcosm survey experiment.

	% ^{14}C Water Extracted[t]	% ^{14}C Methanol Extracted[t]	% ^{14}C Soxhlet Extracted[t]	% ^{14}C Wet Digested[t]	%Total ^{14}C Extracted[t]
Site Comparisons					
Site #1	1.8 b	60.2 cd	8.5 a	6.3 ab	76.6 bc
Site #2	1.1 ab	51.3 bcd	12.0 a	9.6 ab	74.0 bc
Site #3	1.3 ab	45.9 abc	7.5 a	10.7 ab	65.4 ab
Site #4	1.0 ab	34.8 a	13.5 a	12.4 b	61.6 ab
Site #5	0.6 a	64.5 d	29.6 b	15.3 bc	110.0 d
Site #6	0.6 a	56.3 bcd	10.7 a	22.5 c	90.1 c
Site #7	1.4 ab	42.4 ab	8.1 a	1.9 a	53.8 a
Site #9	0.9 ab	43.1 ab	6.1 a	15.7 bc	65.8 ab
ANOVA					
Site	ns	**	***	**	***

[t]Average of 4 surface replicates. Means followed by a common letter are not significantly different at the 5% level.

***-highly significant, **-significant, *-low significance, ns-not significant ($\alpha = 0.05$)

I b. Biolog plate data for average intensity and substrate richness for the surface soils of 8 sites investigated without prior hydrocarbon exposure.

	Average Intensity (Time of Color Change)[t]	Substrate Richness (% Substrates Utilized)[t]
Site Comparisons		
Site #1	2.6 cd	65.1 ab
Site #2	2.2 a	79.7 bc
Site #3	2.4 bc	83.6 c
Site #4	2.7 d	70.8 abc
Site #5	2.6 cd	71.4 abc
Site #6	2.6 cd	57.6 a
Site #7	2.1 a	68.2 ab
Site #9	2.3 ab	74.5 bc
ANOVA		
Site	***	*
Correlation		
% Clay (r^2)	0.08	0.001
% Sand (r^2)	8×10^{-5}	0.009
% Organic Carbon (r^2)	0.3	0.007
% Mineralized (r^2)	0.16	0.007

[t]Average of 4 surface replicates. Means followed by a common letter are not significantly different at the 5% level.

***-highly significant, **-significant, *-low significance, ns-not significant ($\alpha = 0.05$)

I c. Duncan New Multiple Range Test for comparing the surface soils for ^{14}C Phenanthrene mass balance over the course of the microcosm experiment.

	% ^{14}C Volatilized[t]	% ^{14}C Phenanthrene Mineralized[t]	% ^{14}C Extracted[t]	Total ^{14}C Recovered[t]
Site Comparisons				
Site #1	6.2 c	4.6 a	76.6 bc	87.3 b
Site #2	2.9 a	1.6 a	74.0 bc	78.4 ab
Site #3	4.8 b	3.7 a	65.4 ab	73.9 ab
Site #4	2.6 a	16.4 b	61.6 ab	80.6 b
Site #5	3.1 a	2.9 a	110.0 d	116.0 c
Site #6	2.4 a	1.2 a	90.1 c	93.8 b
Site #7	2.9 a	2.3 a	53.8 a	59.0 a
Site #9	2.2 a	9.1 ab	65.8 ab	77.0 ab
ANOVA				
Site	***	***	*	***

[t]Average of 4 surface replicates. Means followed by a common letter are not significantly different at the 5% level.

***-highly significant, **-significant, *-low significance, ns-not significant ($\alpha = 0.05$)

II a. Model data for the mineralization of ^{14}C phenanthrene in the surface of Site 1 of the Survey Experiment (Chapter 3). Quadruplicate reps were each modeled then an average and standard deviation was calculated.
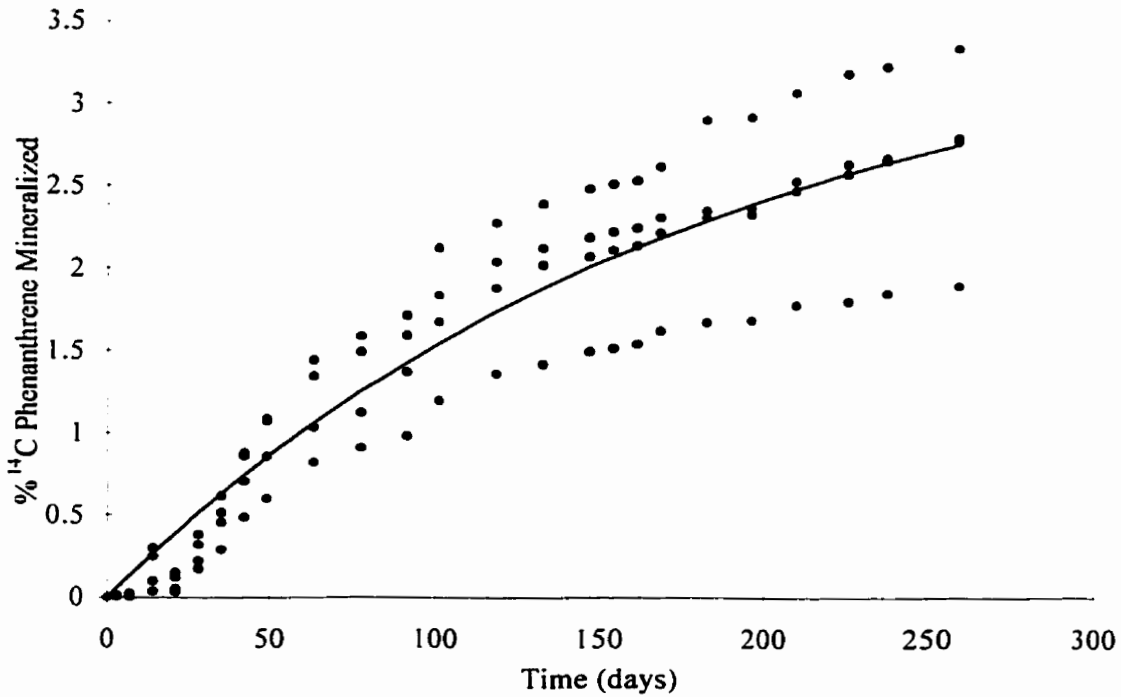

II b. Model data for the mineralization of ^{14}C phenanthrene in the subsurface of Site 1 of the Survey Experiment (Chapter 3). Quadruplicate reps were each modeled then an average and standard deviation was calculated.
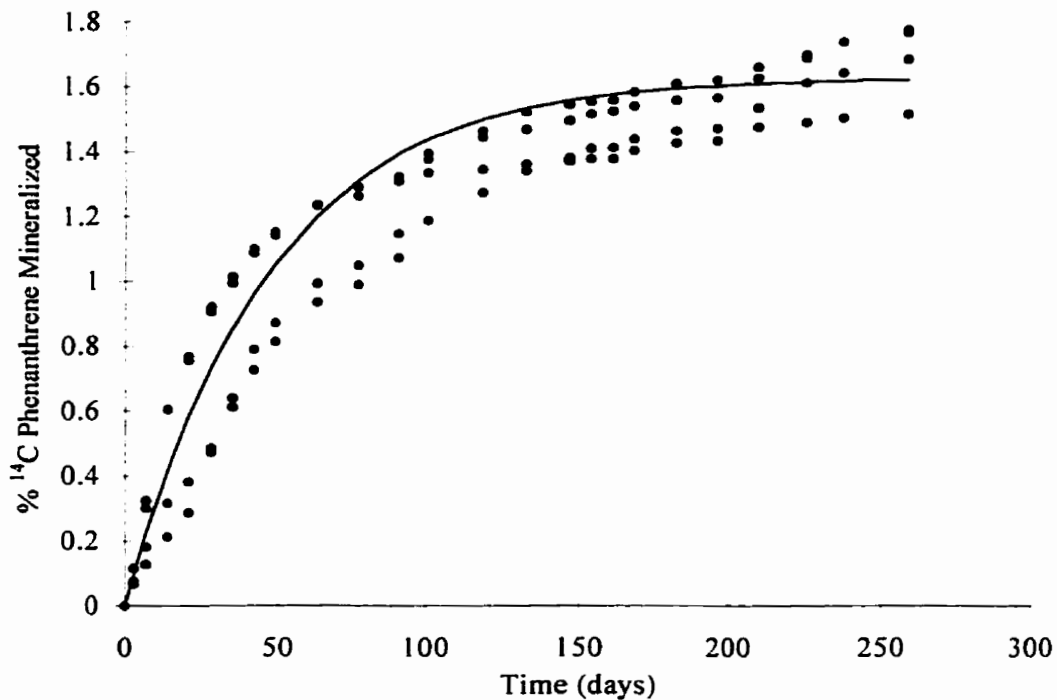
II c. Model data for the mineralization of ^{14}C phenanthrene in the surface of Site 2 of the Survey Experiment (Chapter 3). Quadruplicate reps were each modeled then an average and standard deviation was calculated.
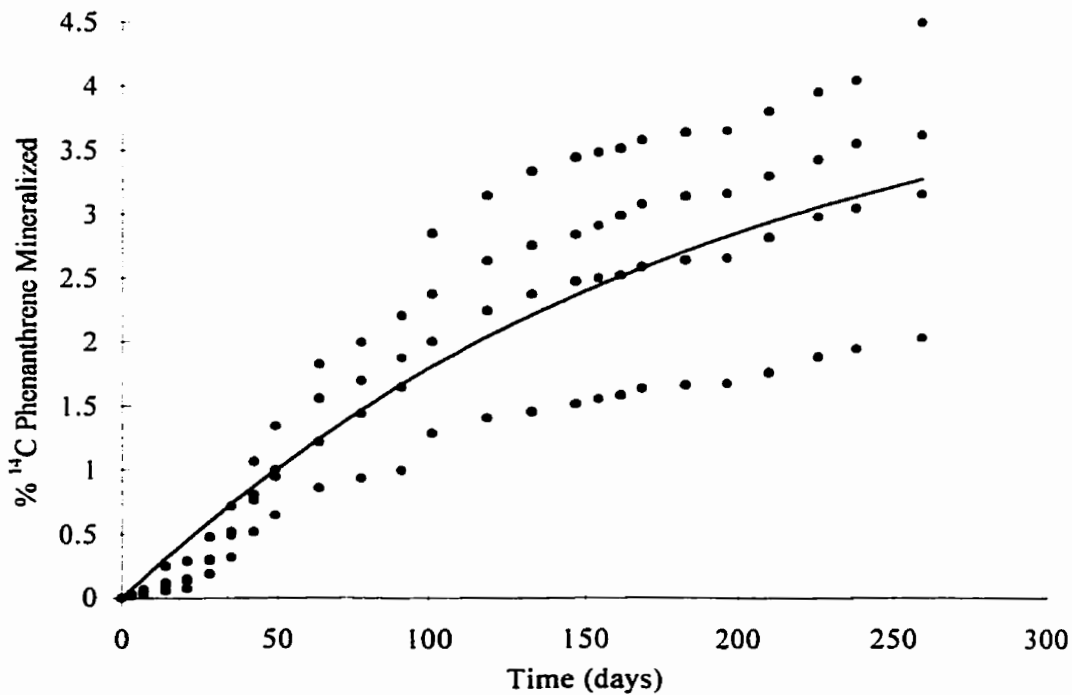
II d. Model data for the mineralization of ^{14}C phenanthrene in the subsurface of Site 2 of the Survey Experiment (Chapter 3). Quadruplicate reps were each modeled then an average and standard deviation was calculated.
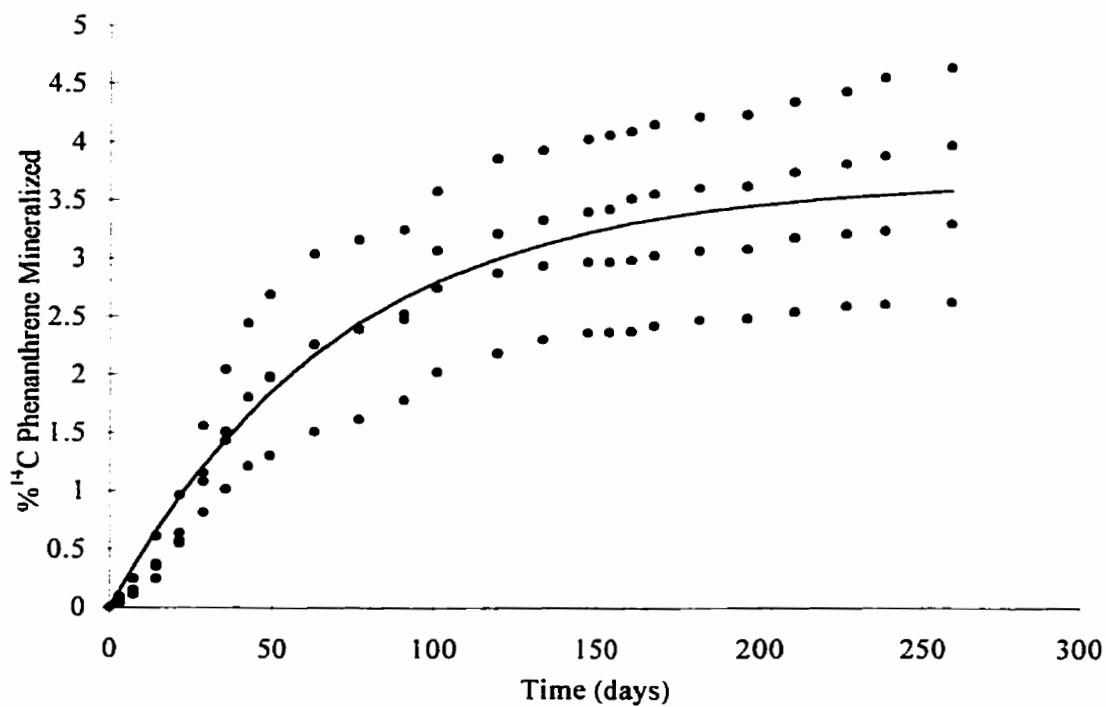
II e. Model data for the mineralization of ^{14}C phenanthrene in the surface of Site 3 of the Survey Experiment (Chapter 3). Quadruplicate reps were each modeled then an average and standard deviation was calculated.
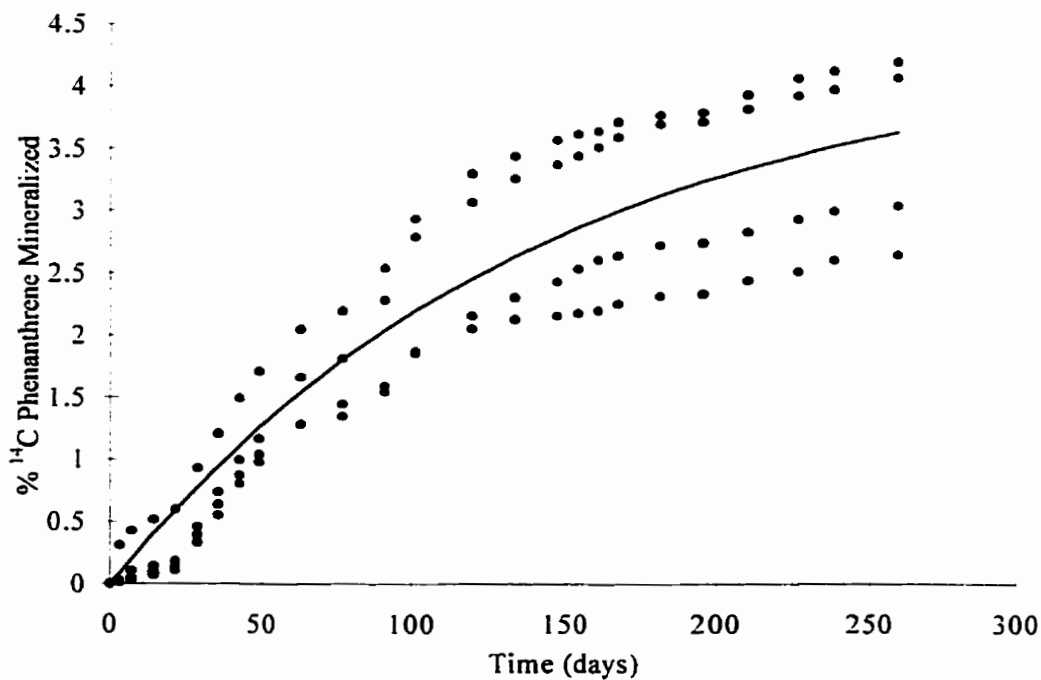
II f. Model data for the mineralization of ^{14}C phenanthrene in the subsurface of Site 3 of the Survey Experiment (Chapter 3). Quadruplicate reps were each modeled then an average and standard deviation was calculated.
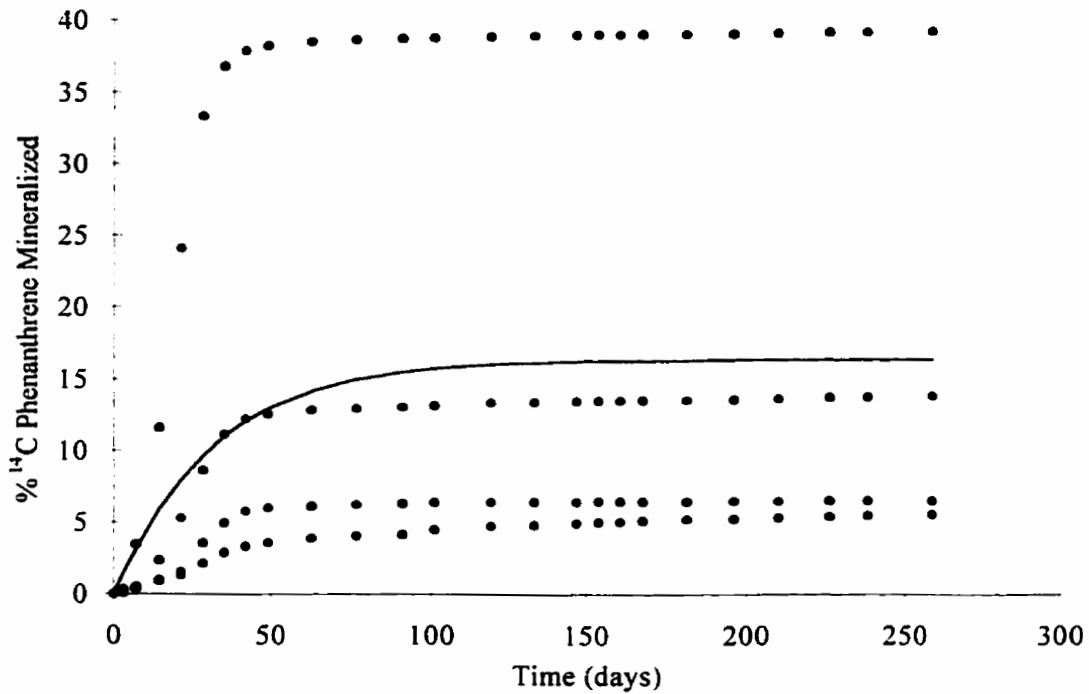
II g. Model data for the mineralization of ^{14}C phenanthrene in the surface of Site 4 of the Survey Experiment (Chapter 3). Quadruplicate reps were each modeled then an average and standard deviation was calculated.
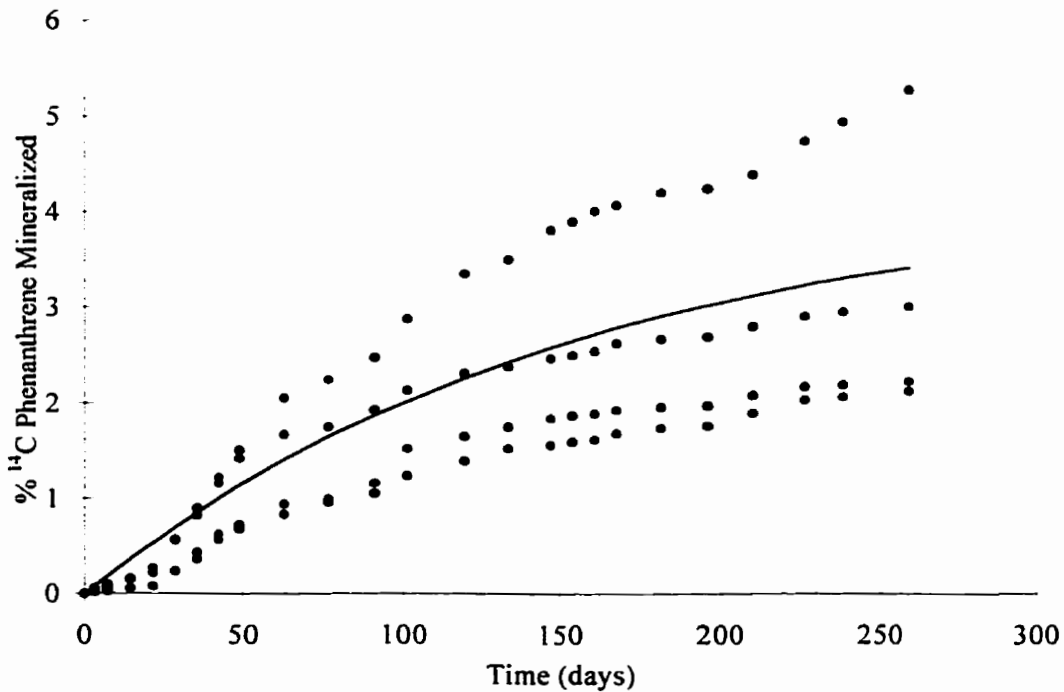
II h. Model data for the mineralization of ^{14}C phenanthrene in the subsurface of Site 4 of the Survey Experiment (Chapter 3). Quadruplicate reps were each modeled then an average and standard deviation was calculated.
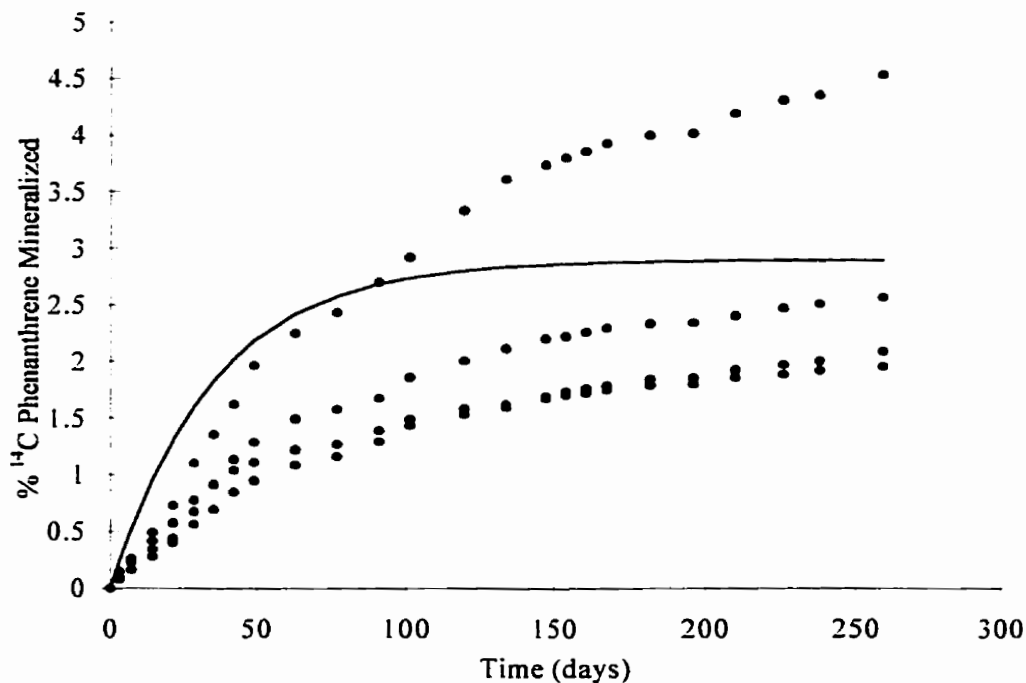
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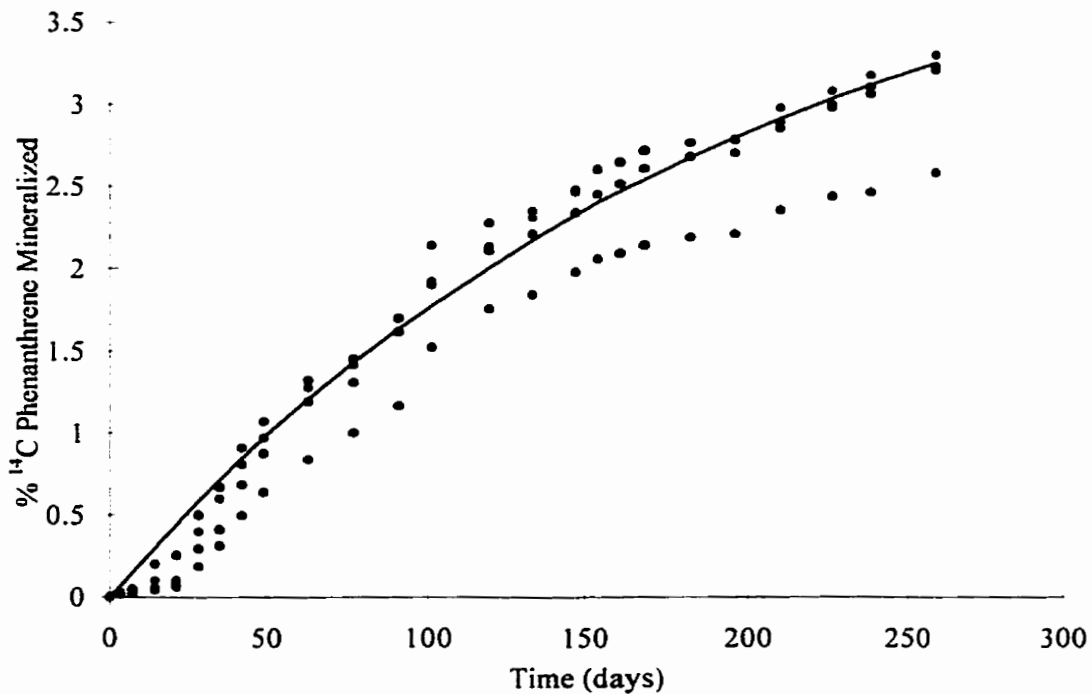
II j. Model data for the mineralization of ^{14}C phenanthrene in the subsurface of Site 5 of the Survey Experiment (Chapter 3). Quadruplicate reps were each modeled then an average and standard deviation was calculated.
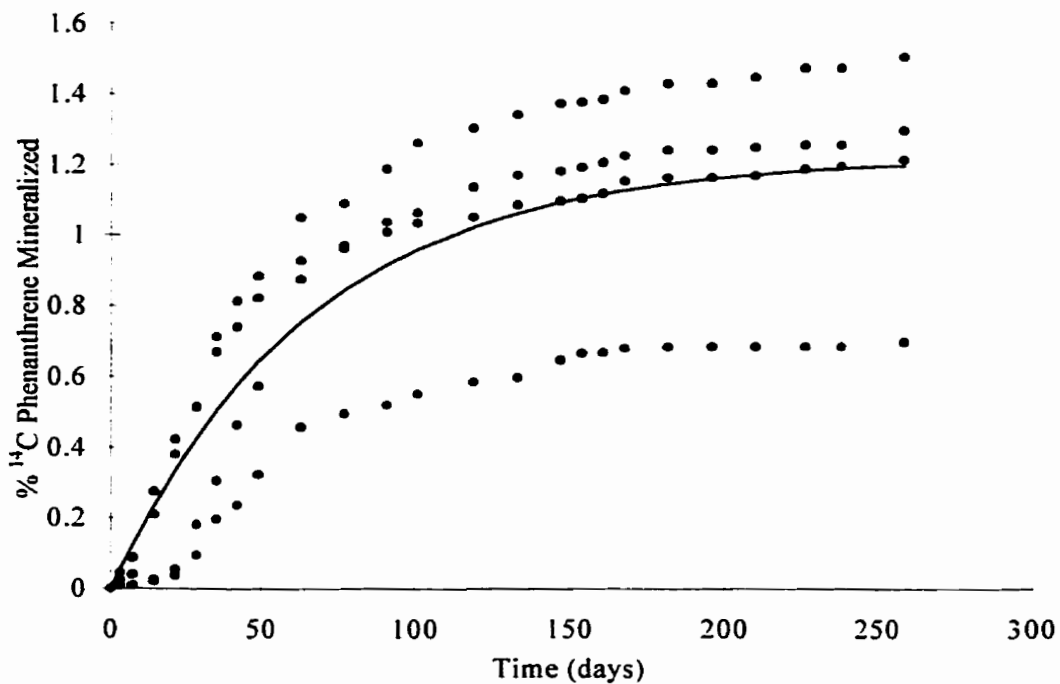
II k. Model data for the mineralization of ^{14}C phenanthrene in the surface of Site 6 of the Survey Experiment (Chapter 3). Quadruplicate reps were each modeled then an average and standard deviation was calculated.
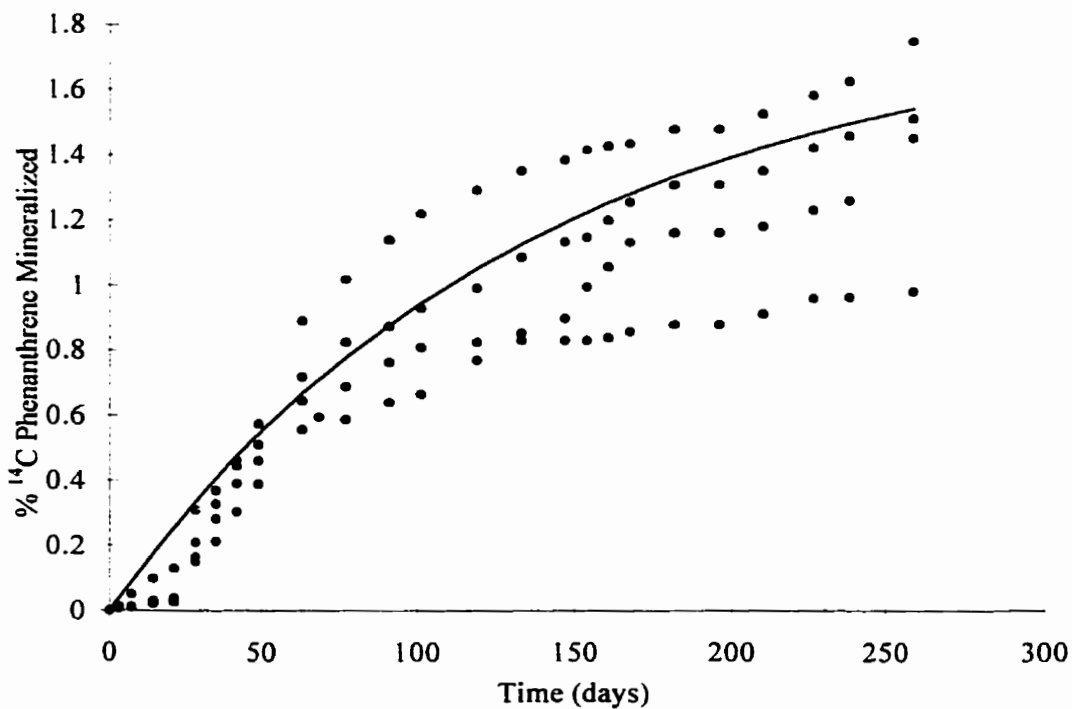
II l. Model data for the mineralization of ^{14}C phenanthrene in the subsurface of Site 6 of the Survey Experiment (Chapter 3). Quadruplicate reps were each modeled then an average and standard deviation was calculated.
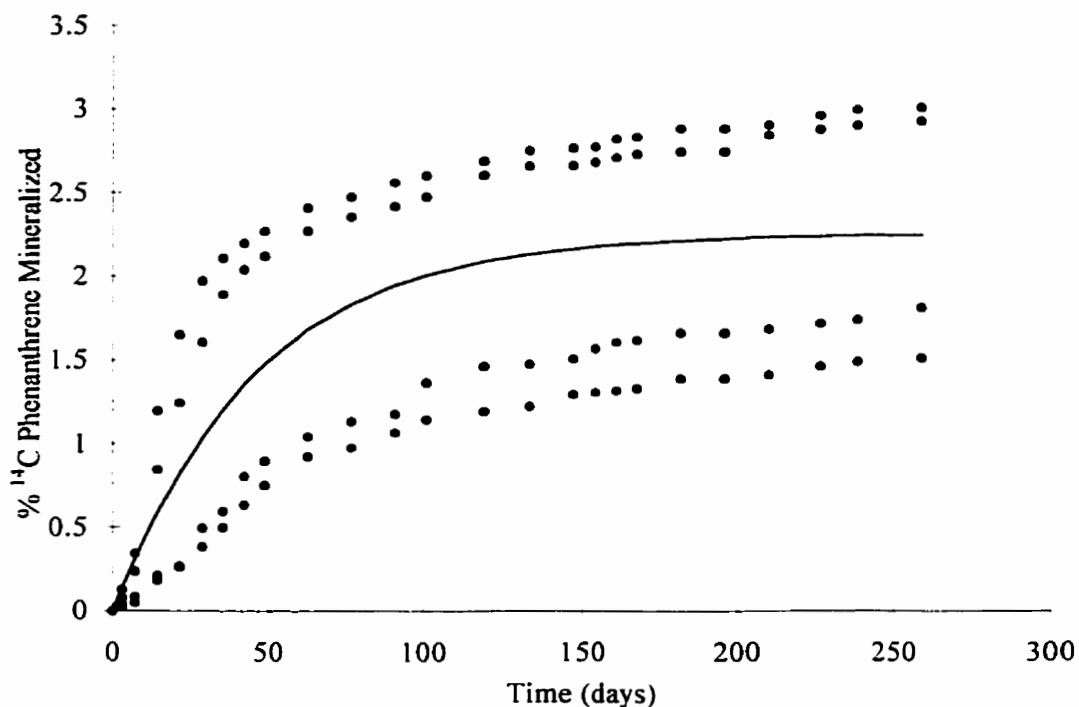
II m. Model data for the mineralization of ^{14}C phenanthrene in the surface of Site 7 of the Survey Experiment (Chapter 3). Quadruplicate reps were each modeled then an average and standard deviation was calculated.
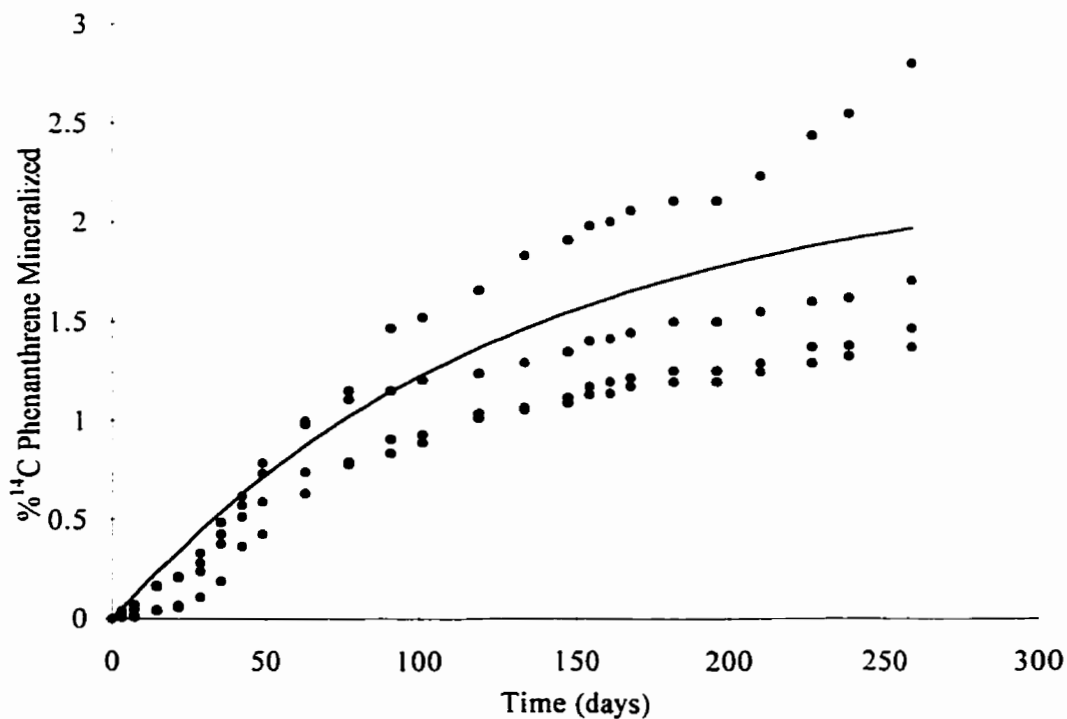
II n. Model data for the mineralization of ^{14}C phenanthrene in the subsurface of Site 7 of the Survey Experiment (Chapter 3). Quadruplicate reps were each modeled then an average and standard deviation was calculated.
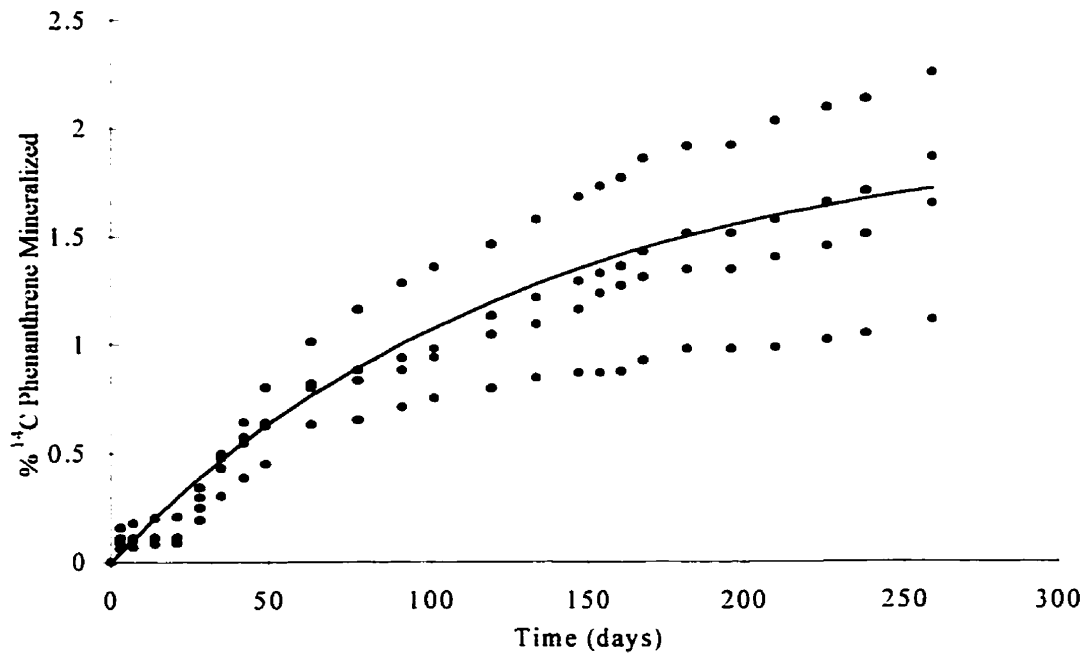
II o. Model data for the mineralization of ^{14}C phenanthrene in the surface of Site 8 of the Survey Experiment (Chapter 3). Quadruplicate reps were each modeled then an average and standard deviation was calculated.
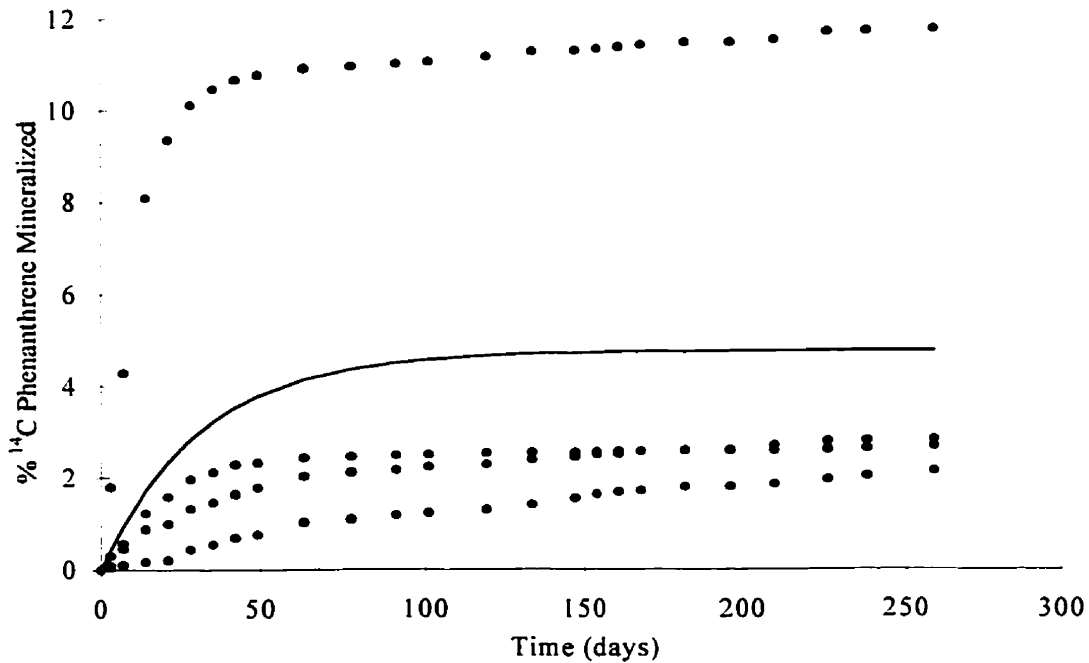
II p. Model data for the mineralization of ^{14}C phenanthrene in the subsurface of Site 8 of the Survey Experiment (Chapter 3). Quadruplicate reps were each modeled then an average and standard deviation was calculated.
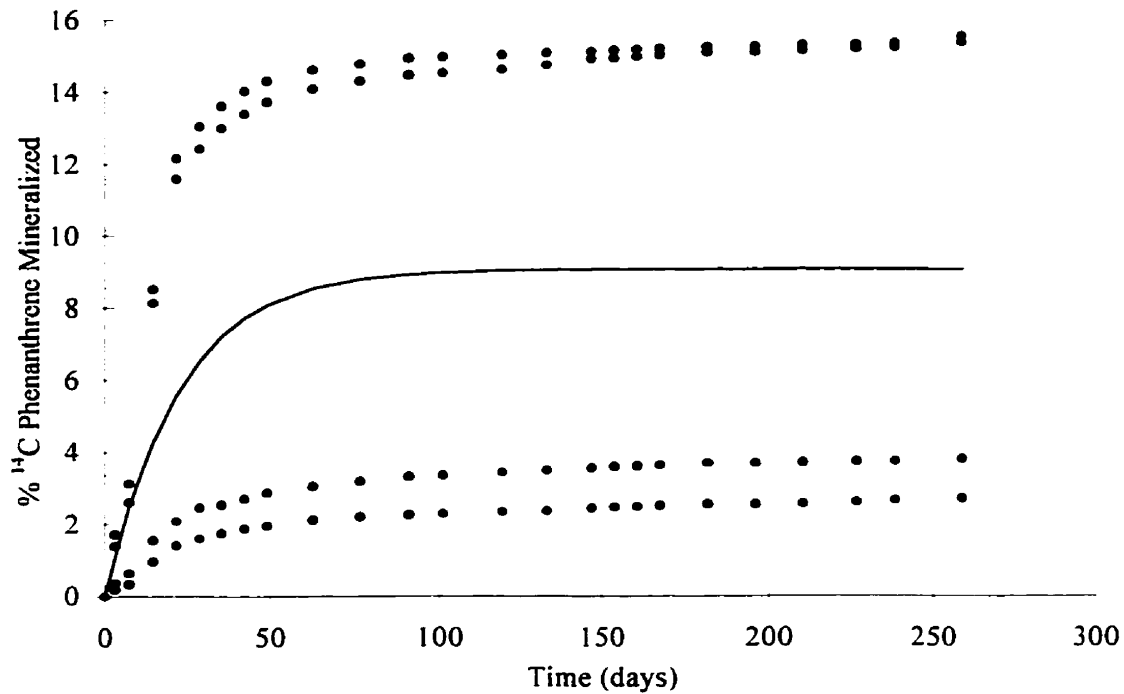
II q. Model data for the mineralization of ^{14}C phenanthrene in the surface of Site 9 of the Survey Experiment (Chapter 3). Quadruplicate reps were each modeled then an average and standard deviation was calculated.
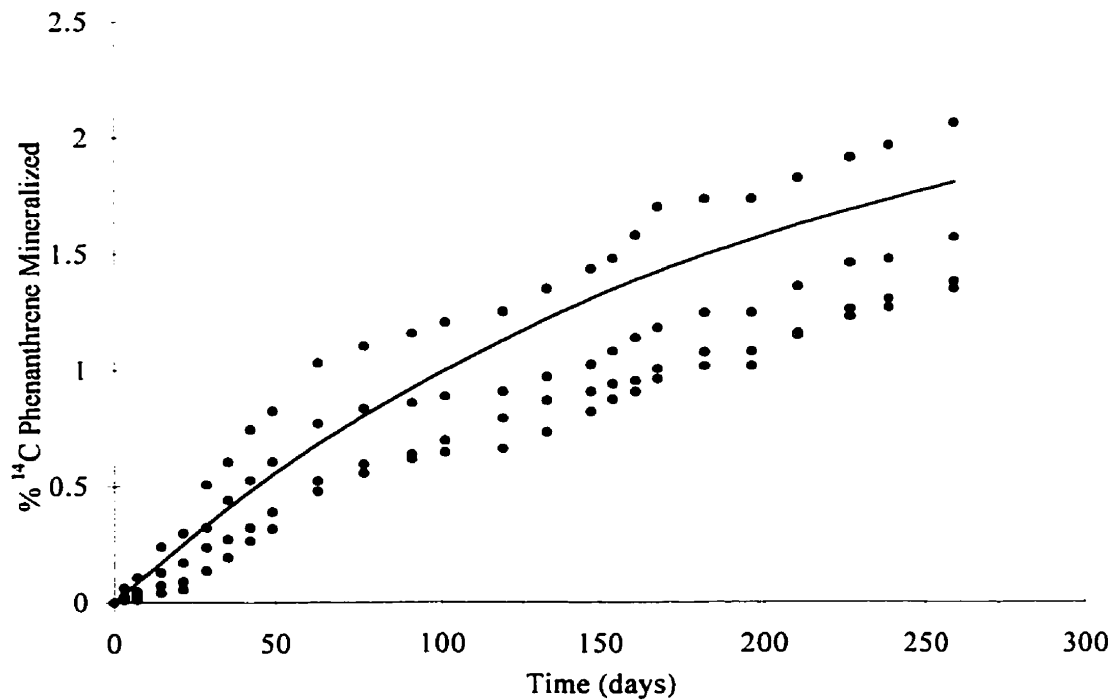
II r. Model data for the mineralization of ^{14}C phenanthrene in the subsurface of Site 9 of the Survey Experiment (Chapter 3). Quadruplicate reps were each modeled then an average and standard deviation was calculated.

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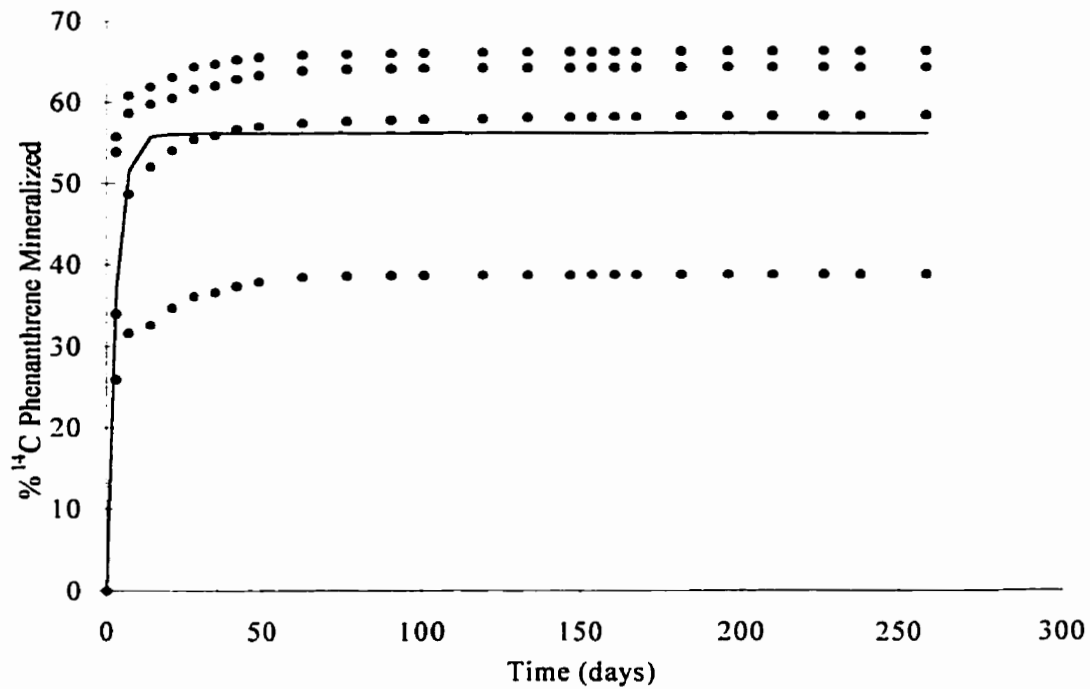
II s. Model data for the mineralization of ^{14}C phenanthrene in the surface of Site 10 of the Survey Experiment (Chapter 3). Quadruplicate reps were each modeled then an average and standard deviation was calculated.
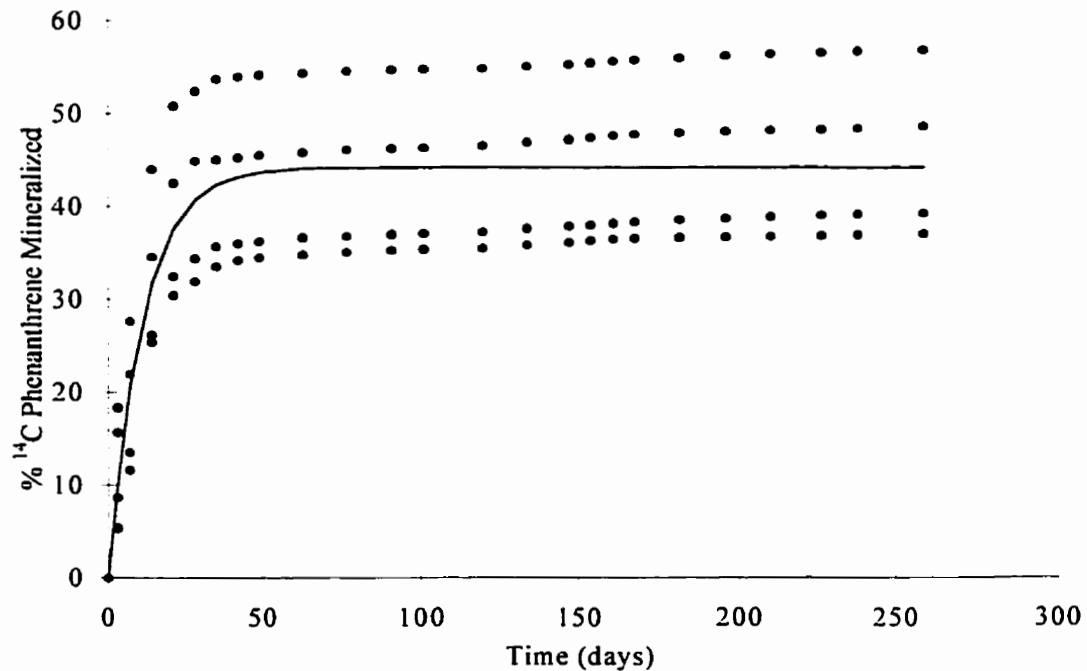
II t. Model data for the mineralization of ^{14}C phenanthrene in the subsurface of Site 10 of the Survey Experiment (Chapter 3). Quadruplicate reps were each modeled then an average and standard deviation was calculated.
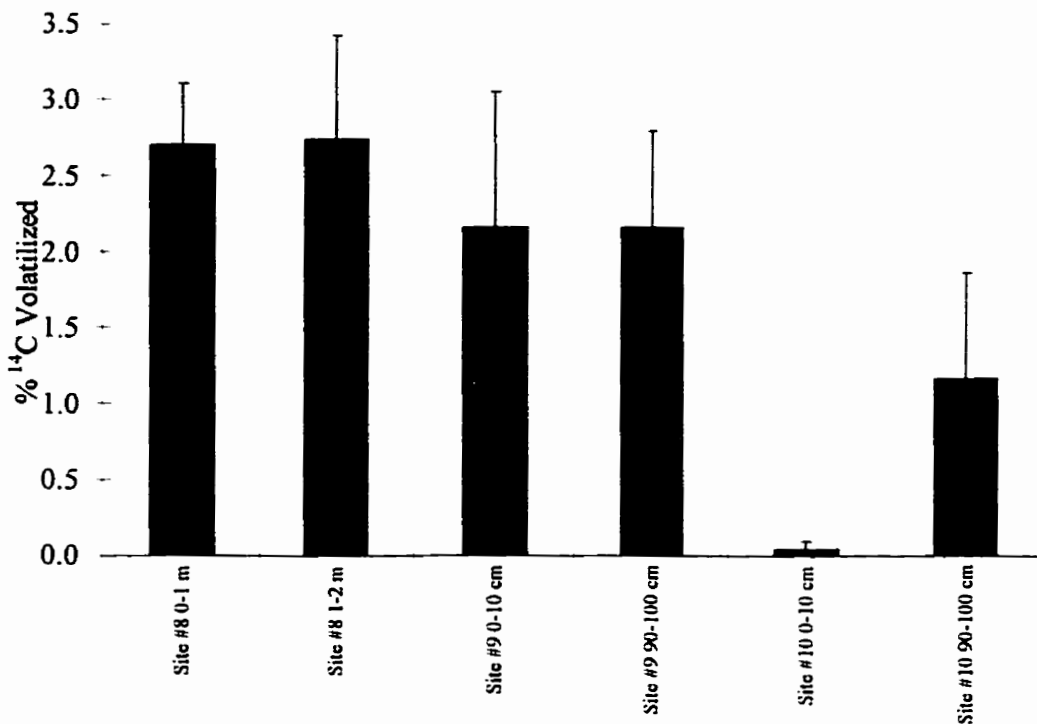
III a. ^{14}C Phenanthrene volatilized in Sites 8 (spill site), 9 (uncontaminated), and 10 (spill site) in the Soil Survey Experiment (Chapter 3).
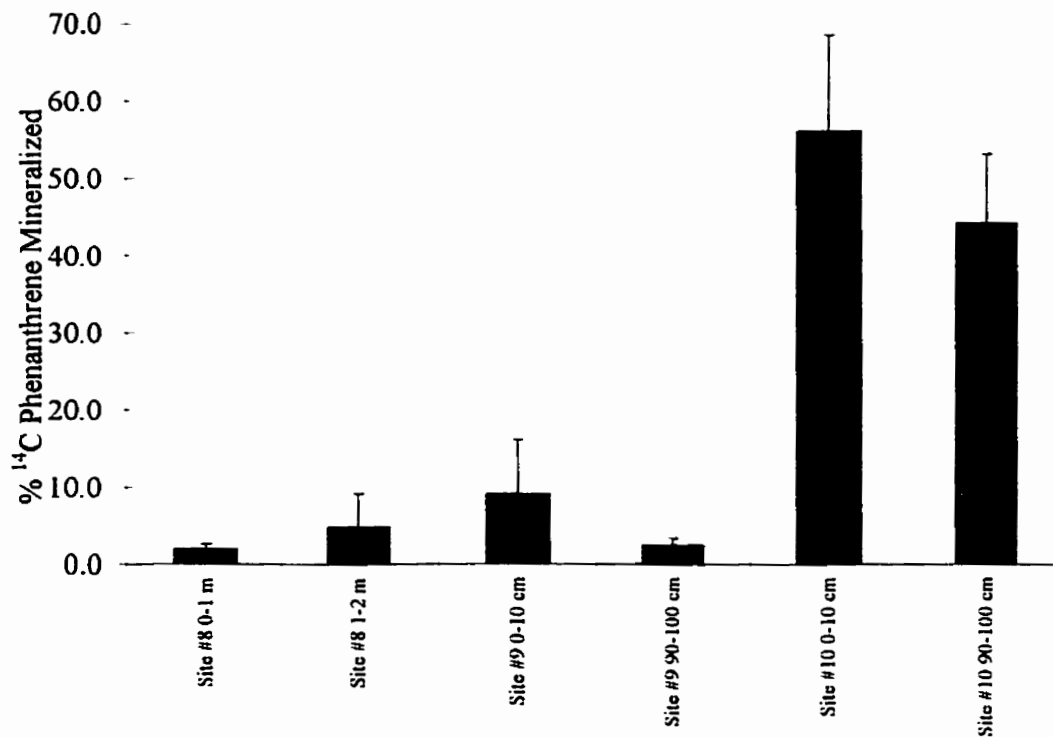
III b. ^{14}C Phenanthrene mineralization in Sites 8 (spill site), 9 (uncontaminated), and 10 (spill site) in the Soil Survey Experiment (Chapter 3).

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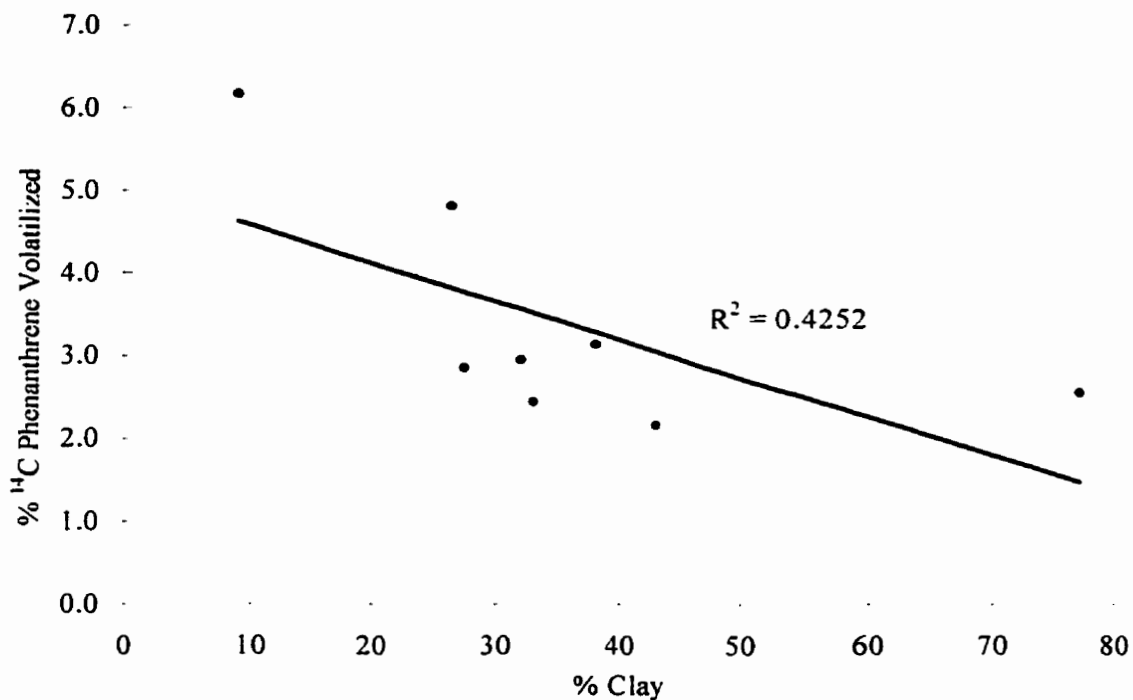
IV a. ^{14}C Phenanthrene volatilized versus clay content in the surface of the non hydrocarbon exposure sites in the Soil Survey Experiment (Chapter 3).
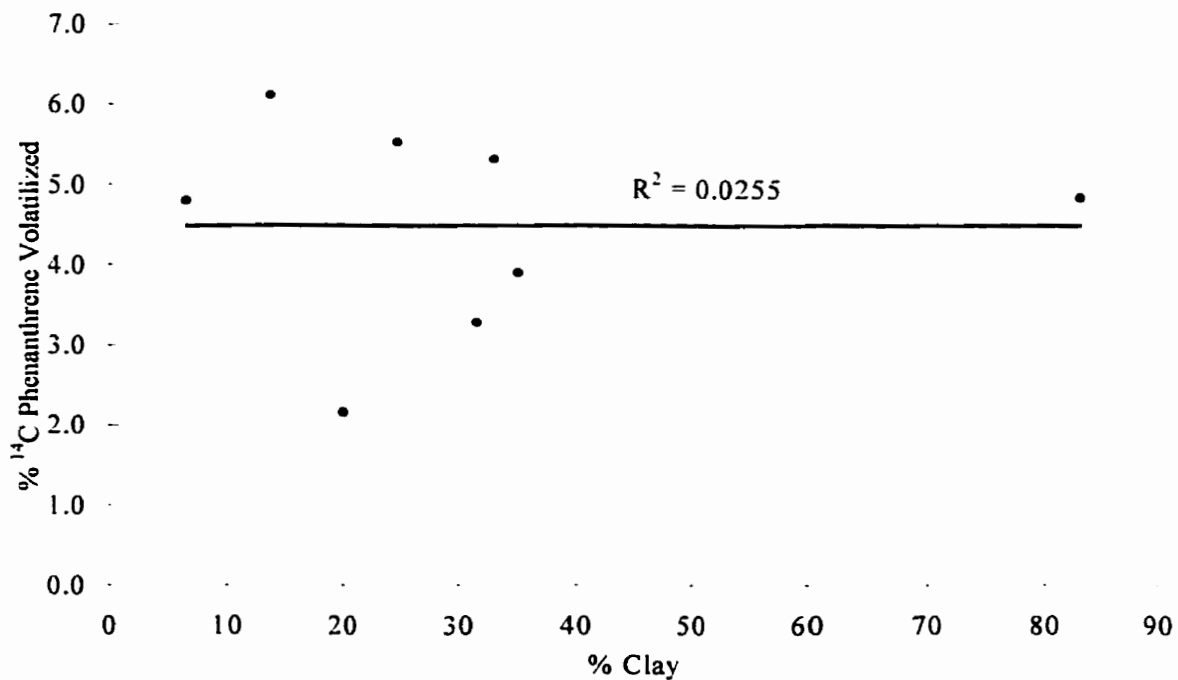
IV b. ^{14}C Phenanthrene volatilized versus clay content in the subsurface of the non hydrocarbon exposure sites in the Soil Survey Experiment (Chapter 3).
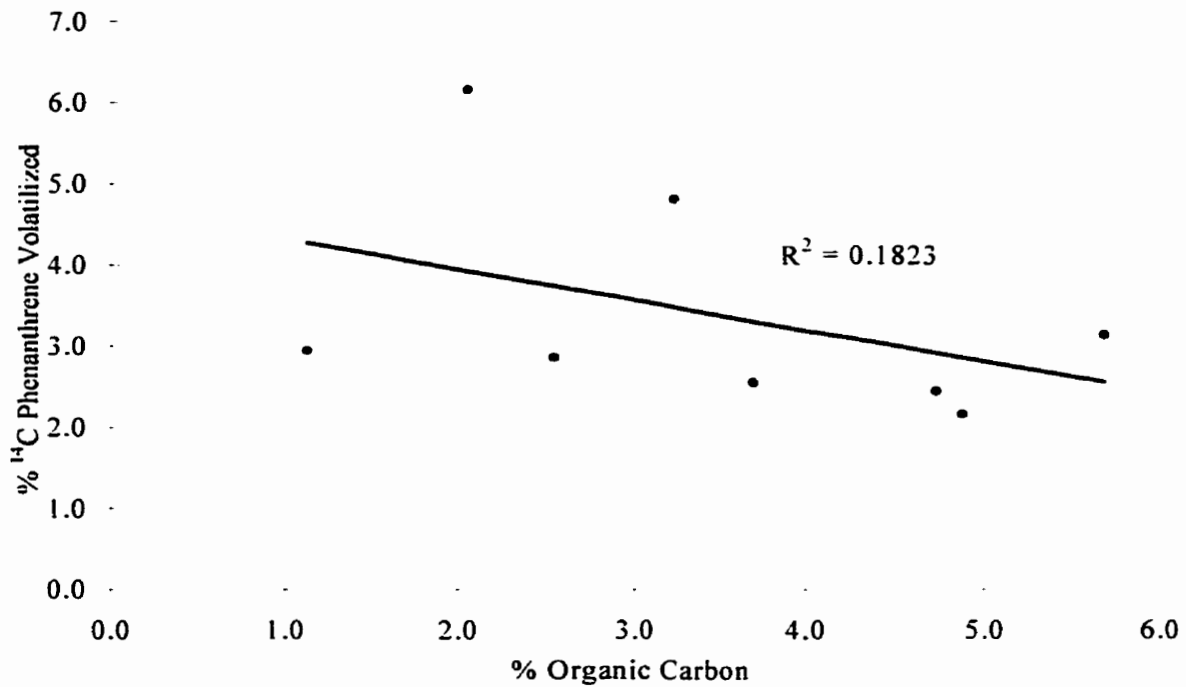
IV c. ^{14}C Phenanthrene volatilized versus organic carbon content in the surface of the non hydrocarbon exposure sites in the Soil Survey Experiment (Chapter 3).
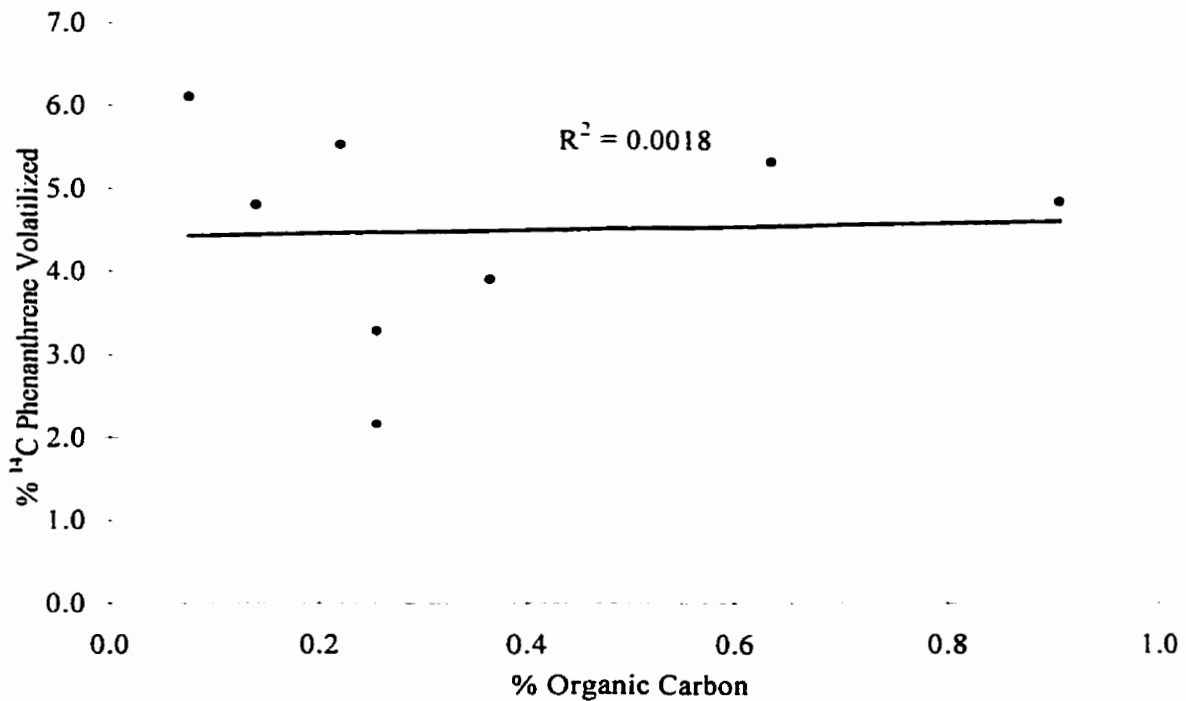
IV d. ^{14}C Phenanthrene volatilized versus organic carbon content in the subsurface of the non hydrocarbon exposure sites in the Soil Survey Experiment (Chapter 3).

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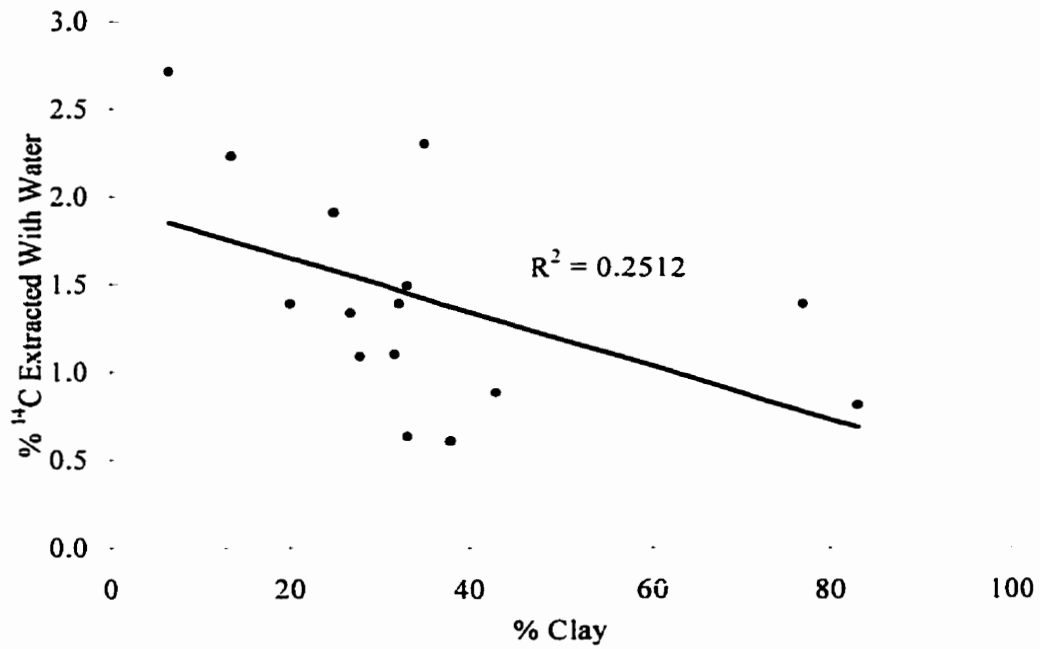
IV e. Correlation between percent clay and % ^{14}C extracted from water in sites not previously contaminated with hydrocarbons (Chapter 3).
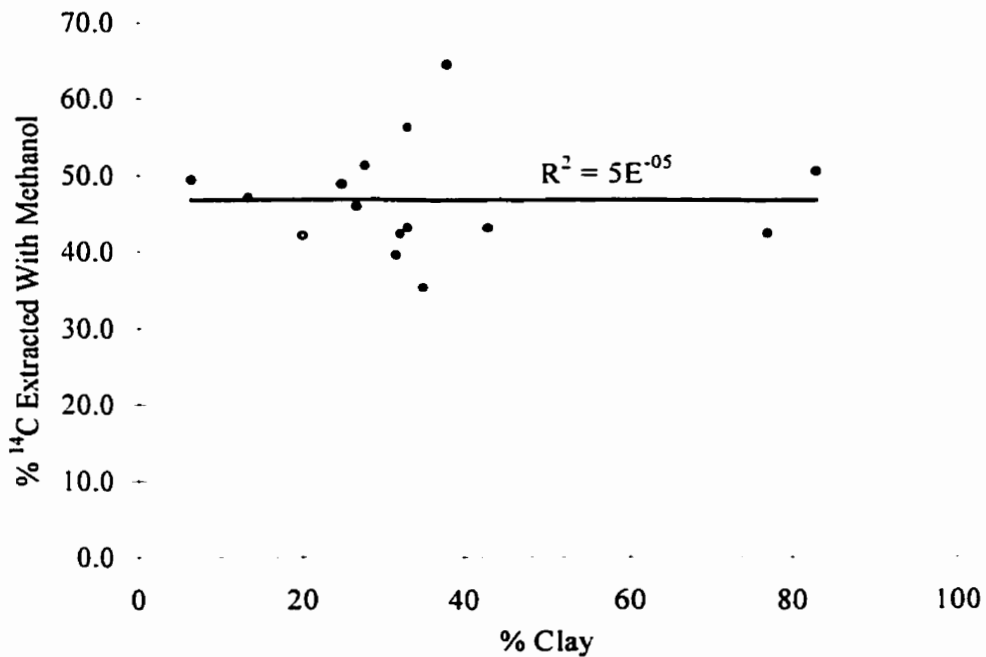
IV f. Correlation between percent clay and % ^{14}C extracted from methanol in sites not previously contaminated with hydrocarbons (Chapter 3).

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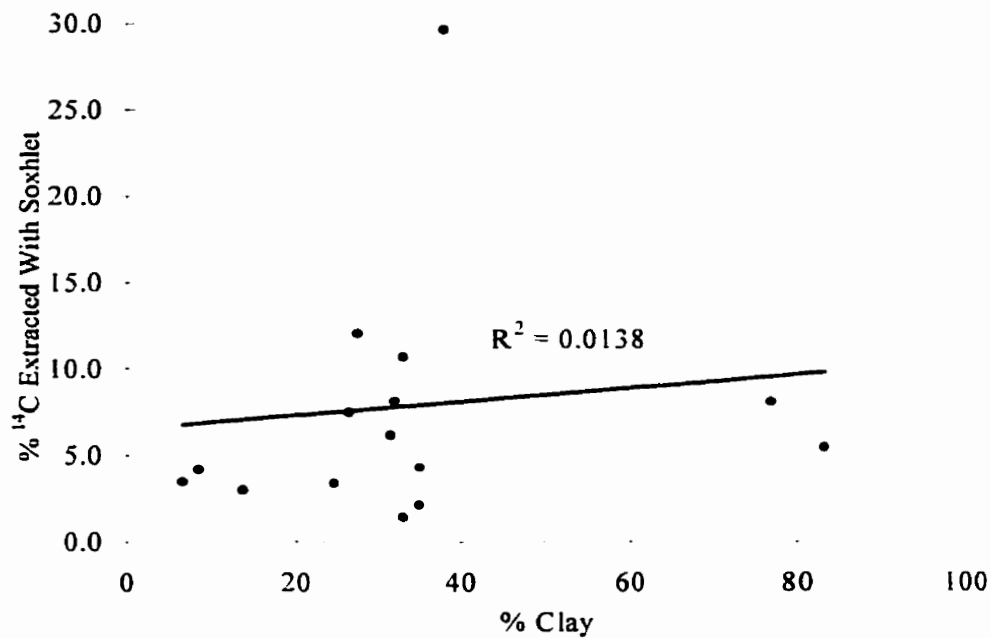
IV g. Correlation between percent clay and % ^{14}C extracted with DCM in a soxhlet apparatus in sites not previously contaminated with hydrocarbons (Chapter 3).
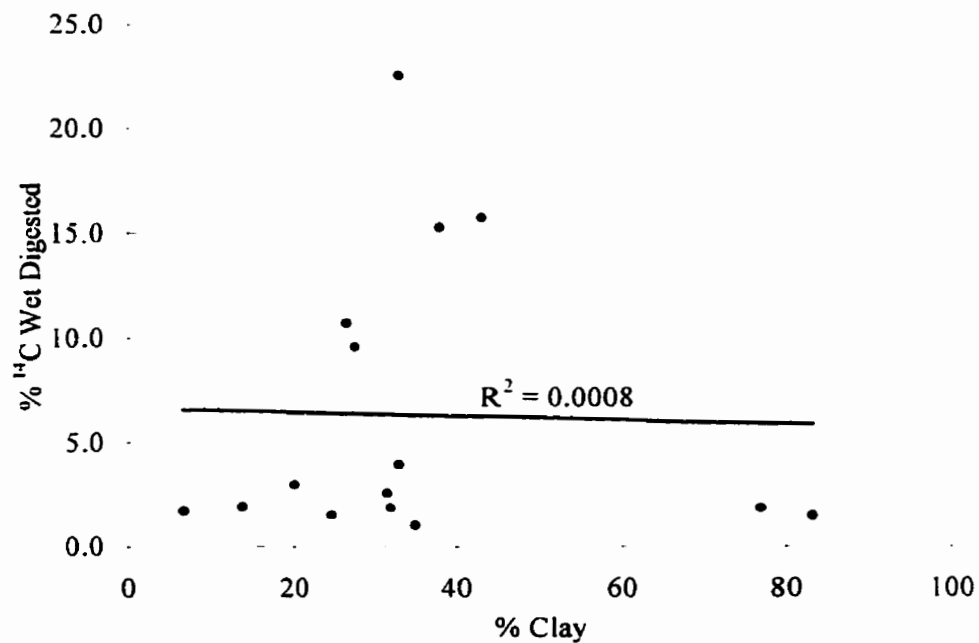
IV h. Correlation between percent clay and % ^{14}C extracted by wet digestion of the organic matter in sites not previously contaminated with hydrocarbons (Chapter 3).
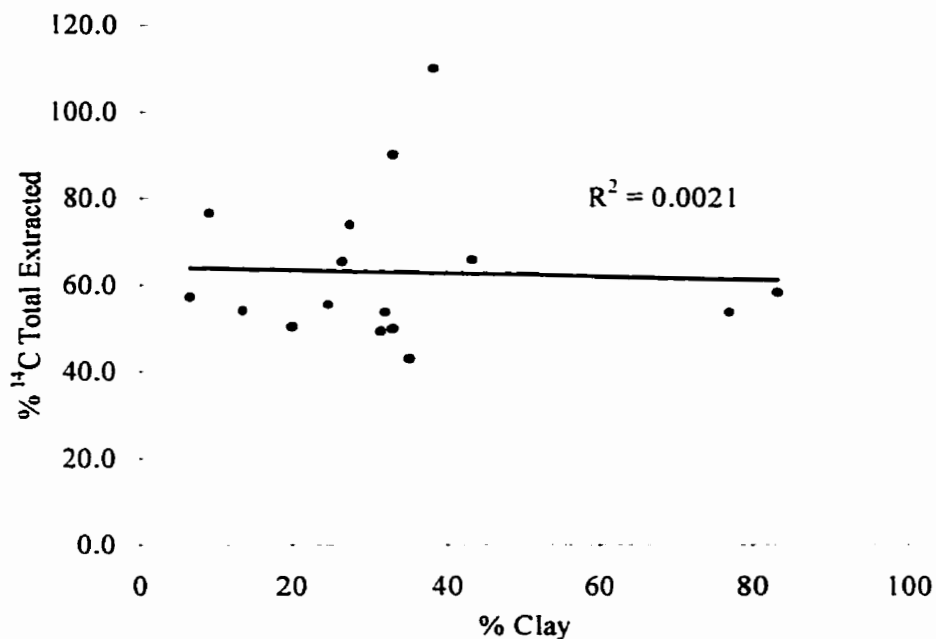
IV i. Correlation between percent clay and total % ^{14}C recovered in sites not previously contaminated with hydrocarbons (Chapter 3).
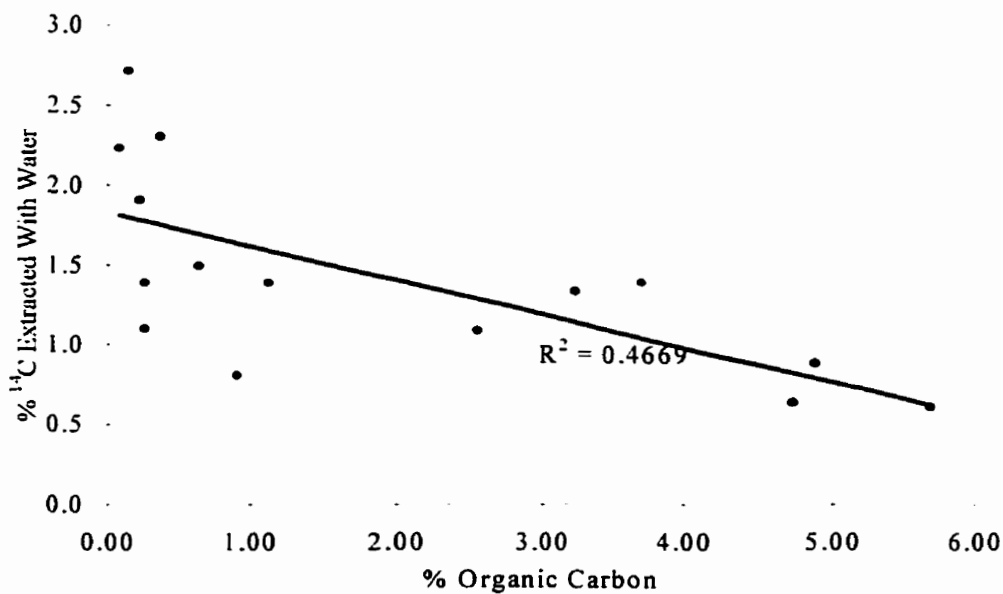
IV j. Correlation between percent organic carbon and % ^{14}C extracted with water in sites not previously contaminated with hydrocarbons (Chapter 3).
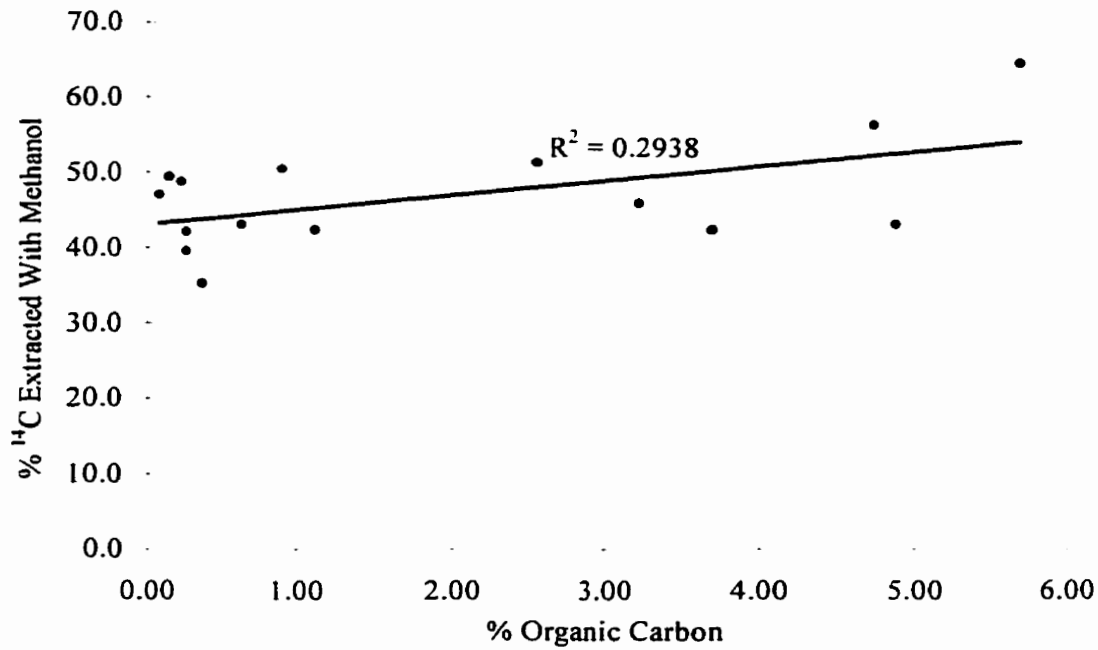
IV k. Correlation between percent organic carbon and % ^{14}C extracted with methanol in sites not previously contaminated with hydrocarbons (Chapter 3).
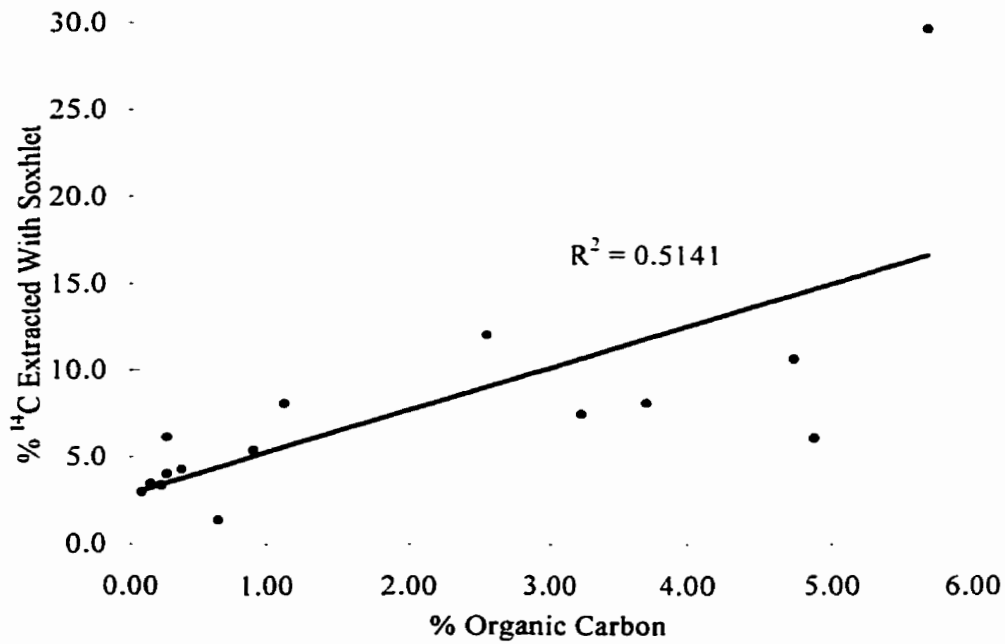
IV l. Correlation between percent organic carbon and % ^{14}C extracted with DCM in a soxhlet apparatus in sites not previously contaminated with hydrocarbons (Chapter 3).
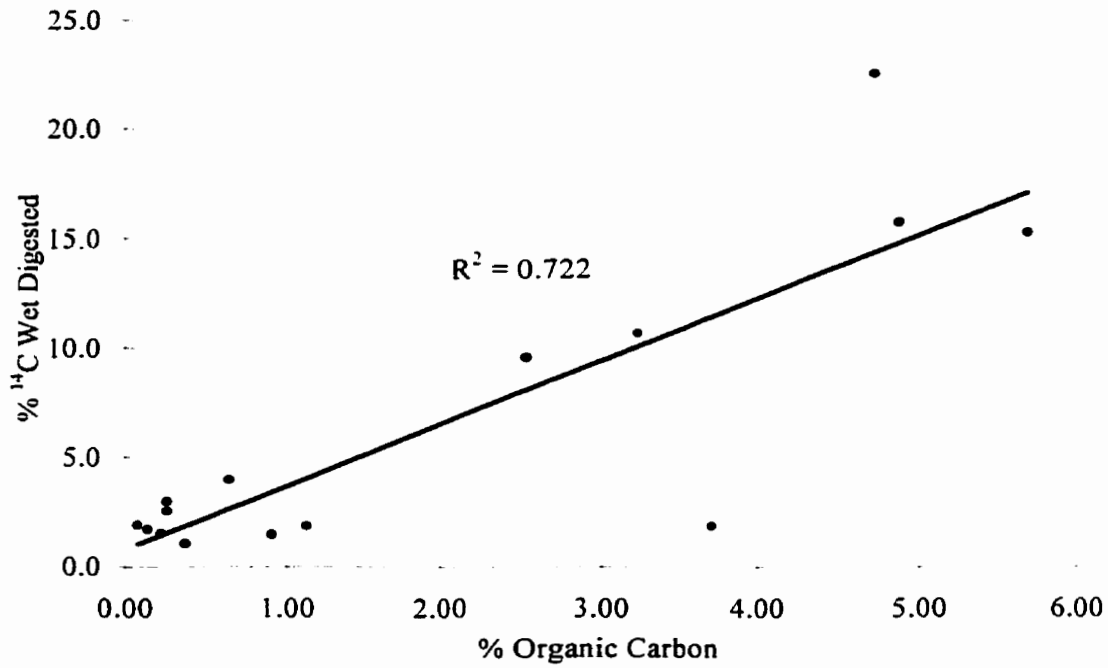
IV m. Correlation between percent organic carbon and % ^{14}C extracted by wet digestion of the organic matter in sites not previously contaminated with hydrocarbons (Chapter 3).
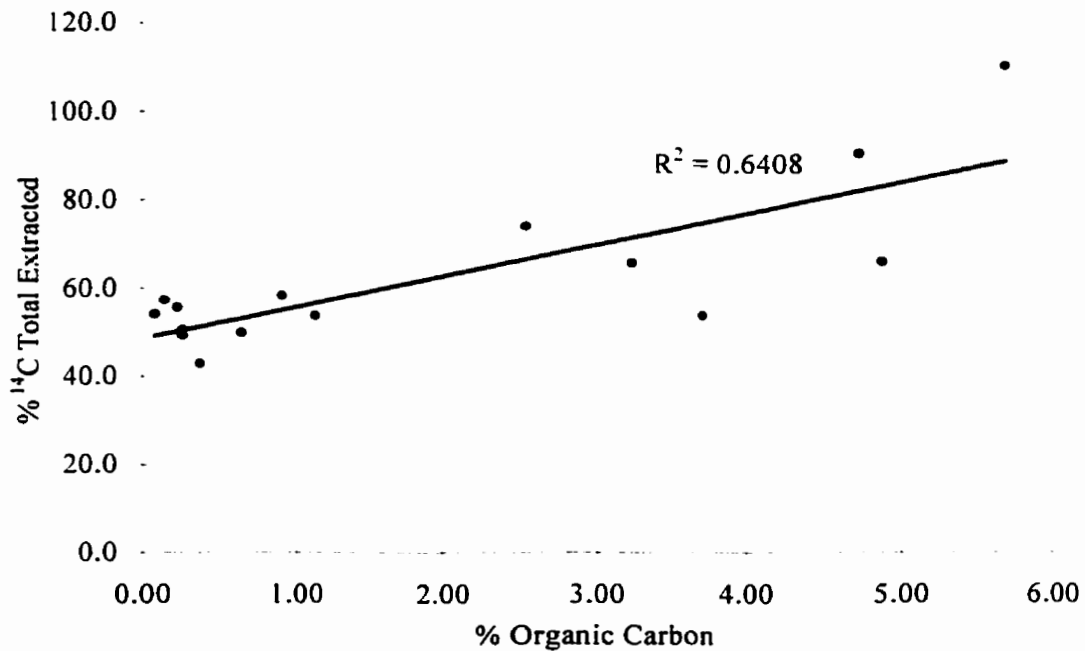
IV n. Correlation between percent organic carbon and total % ^{14}C recovered in sites not previously contaminated with hydrocarbons (Chapter 3).

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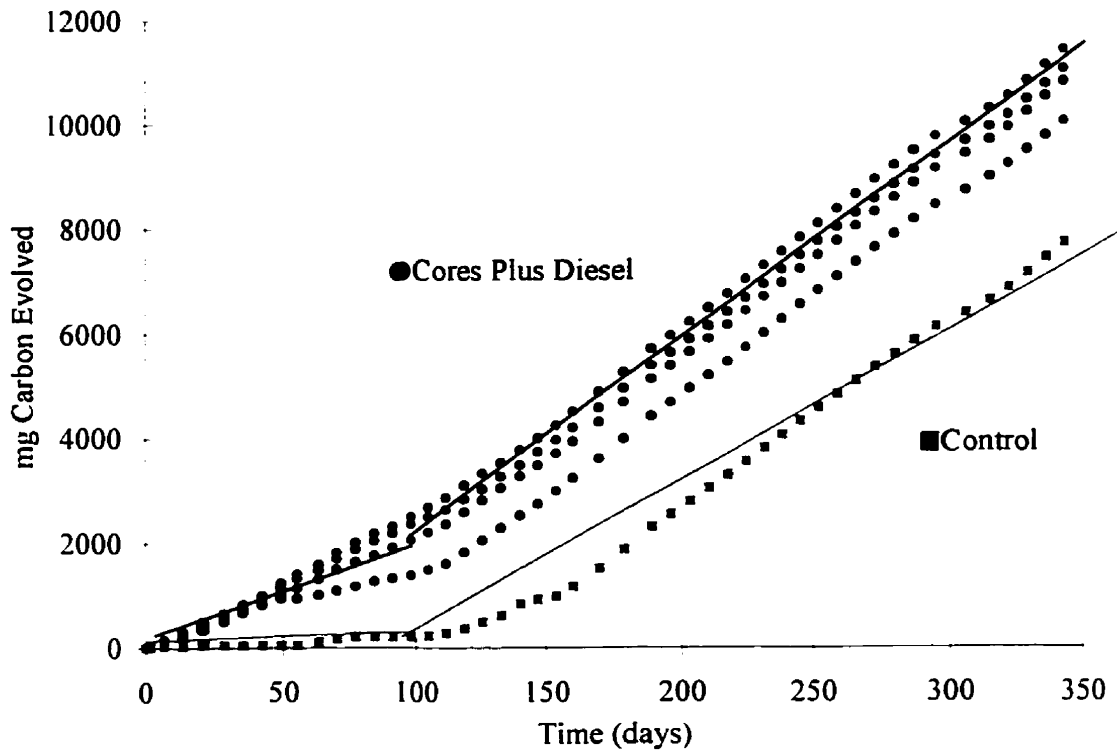
V a. Microbial respiration in the surface (0-50cm) at Site 5-1 in an intact soil column sampled along a landscape (Chapter 4).
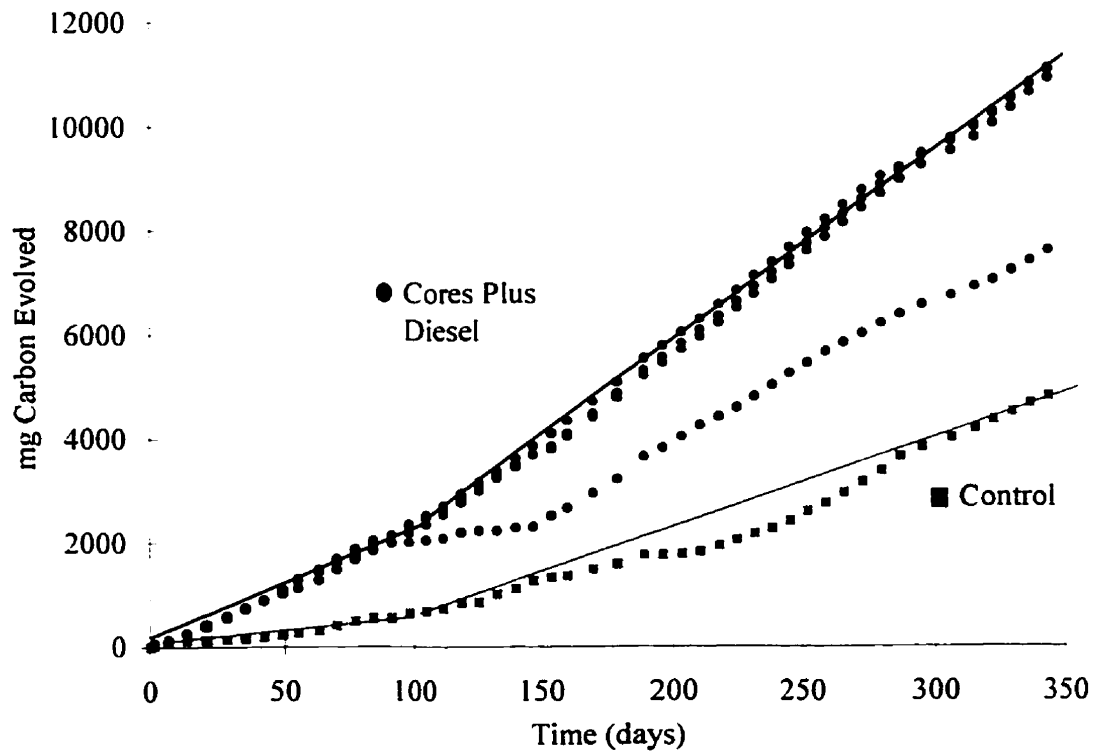
V b. Microbial respiration in the surface (0-50cm) at Site 5-2 in an intact soil column sampled along a landscape (Chapter 4).
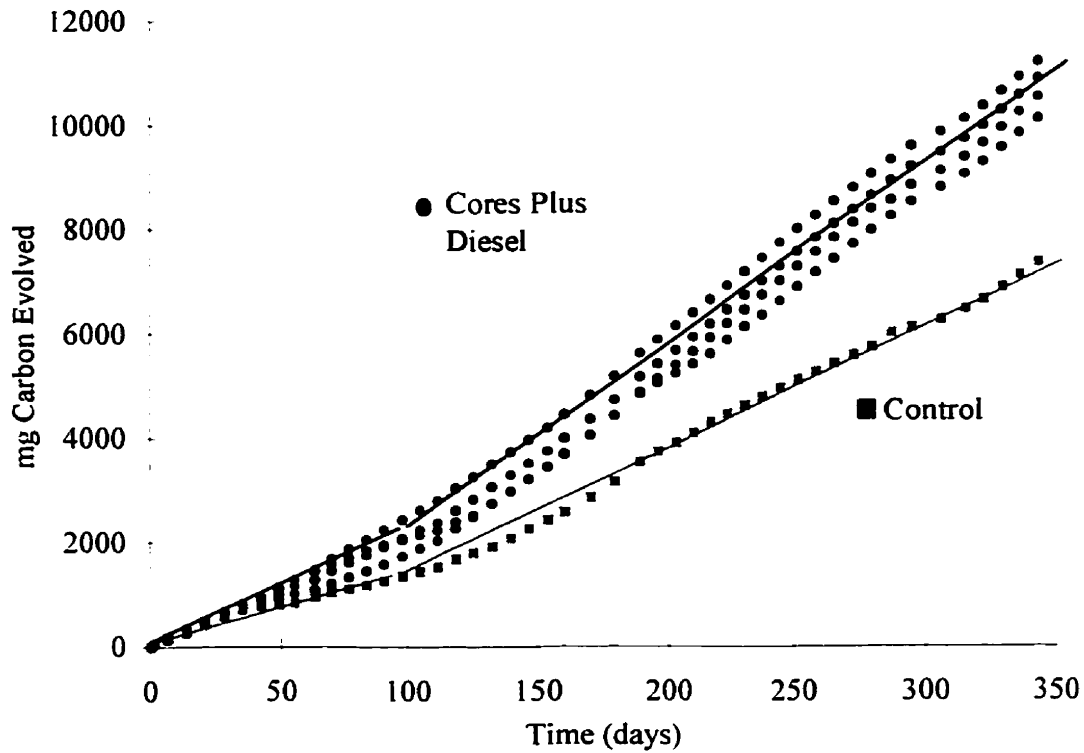
V c. Microbial respiration in the surface (0-50cm) at Site 5-3a in an intact soil column sampled along a landscape (Chapter 4).
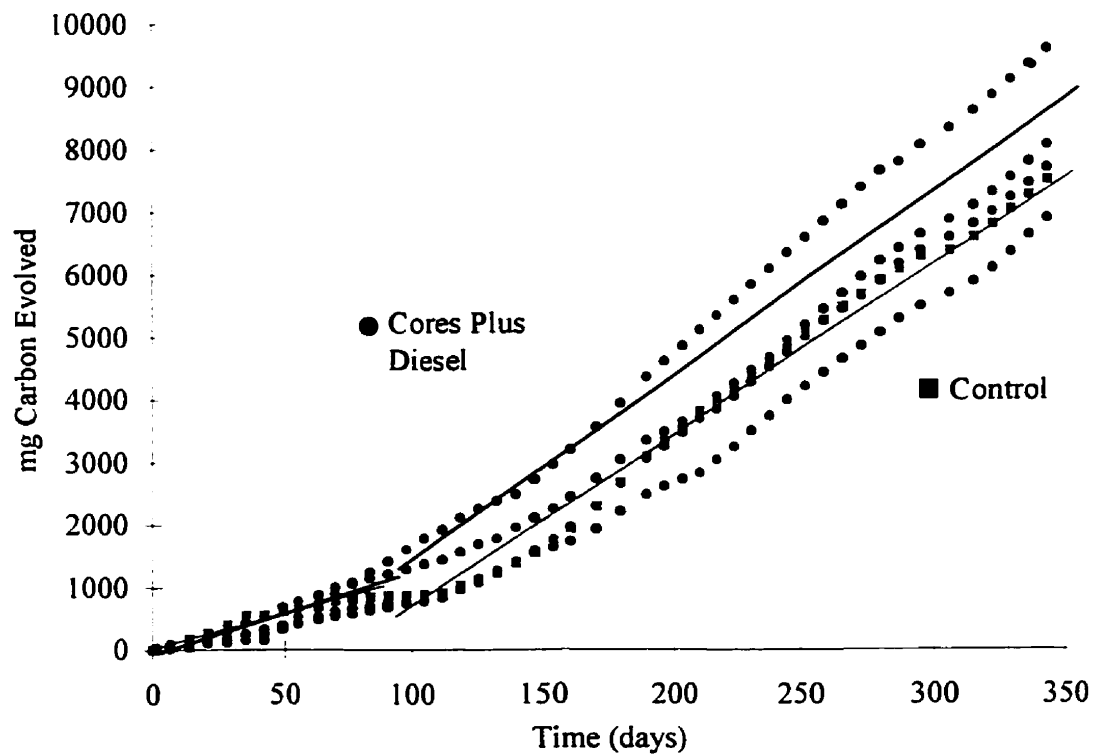
V d. Microbial respiration in the subsurface (50-100cm) at Site 5-3b in an intact soil column sampled along a landscape (Chapter 4).

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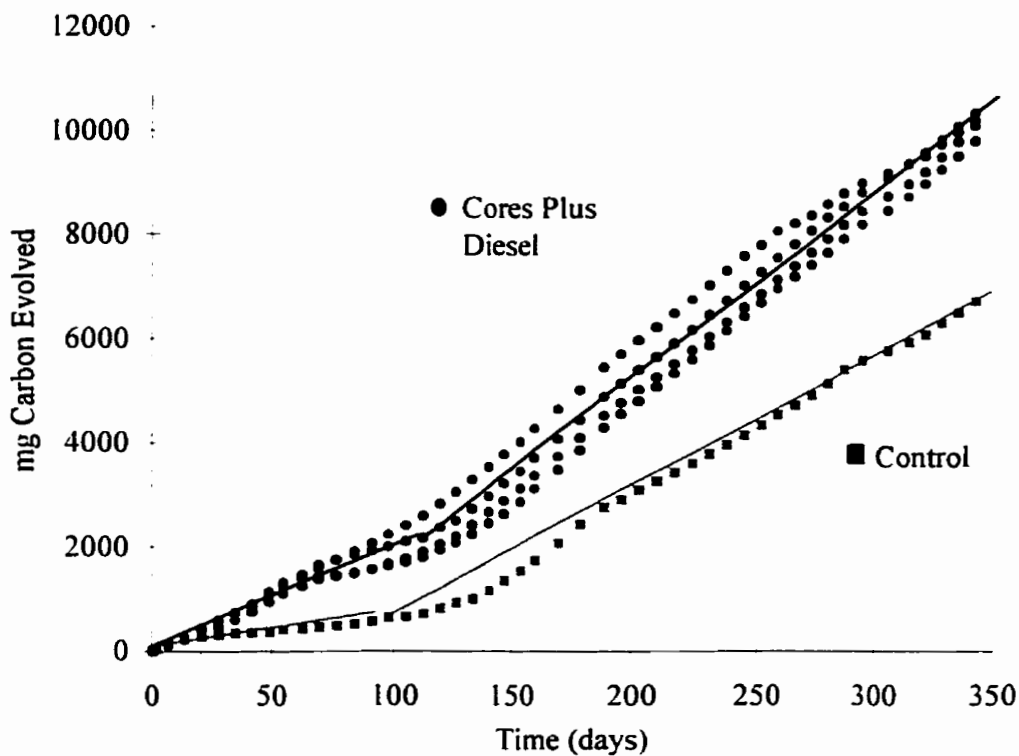
V e. Microbial respiration in the surface (0-50cm) at Site 5-4 in an intact soil column
      sampled along a landscape (Chapter 4).
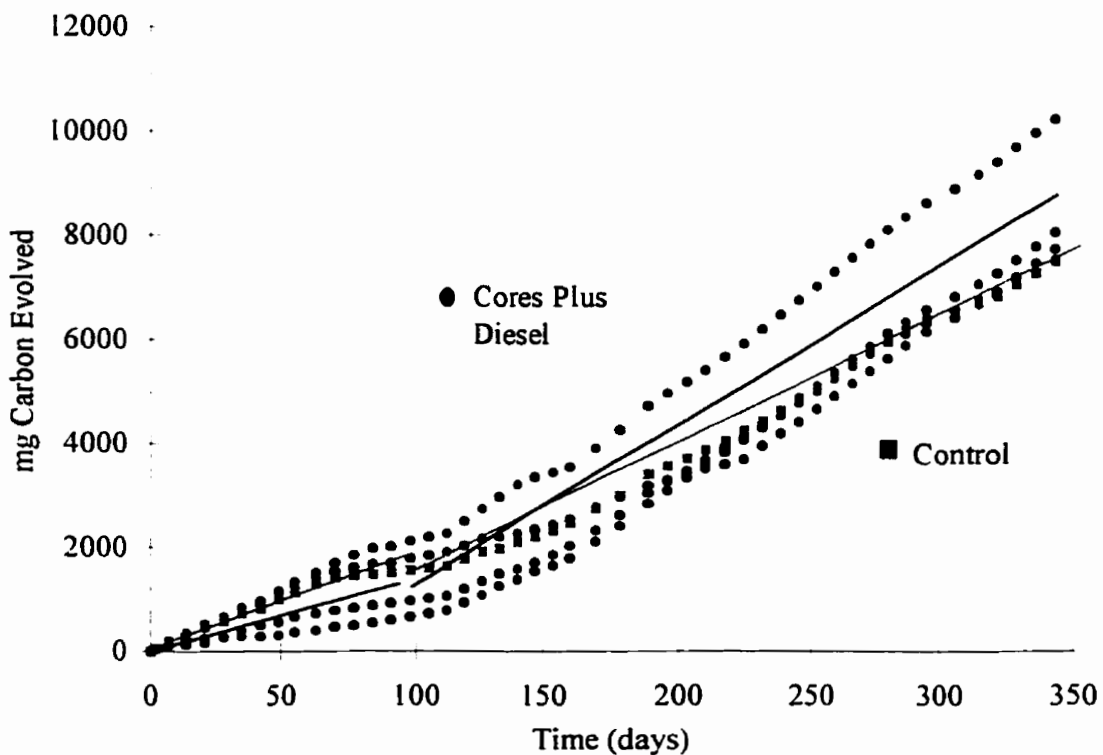
V f. Microbial respiration in the surface (0-50cm) at Site 5-5a in an intact soil column
      sampled along a landscape (Chapter 4).
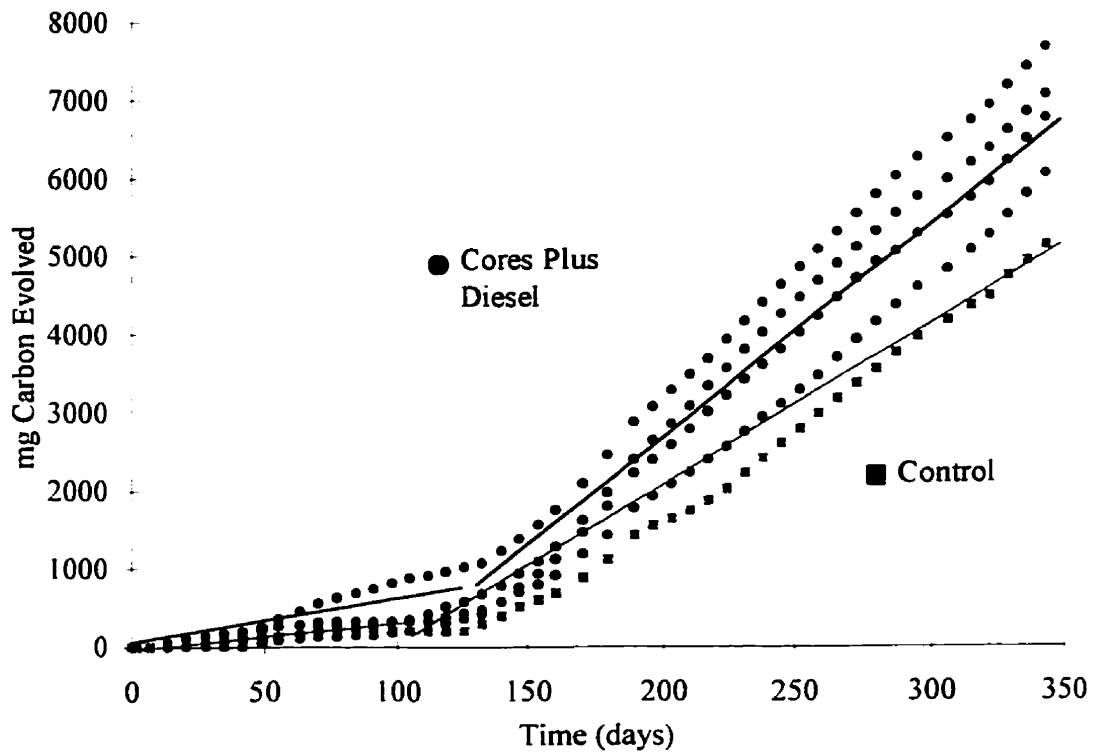
V g. Microbial respiration in the subsurface (50-100cm) at Site 5-5b in an intact soil column sampled along a landscape (Chapter 4).
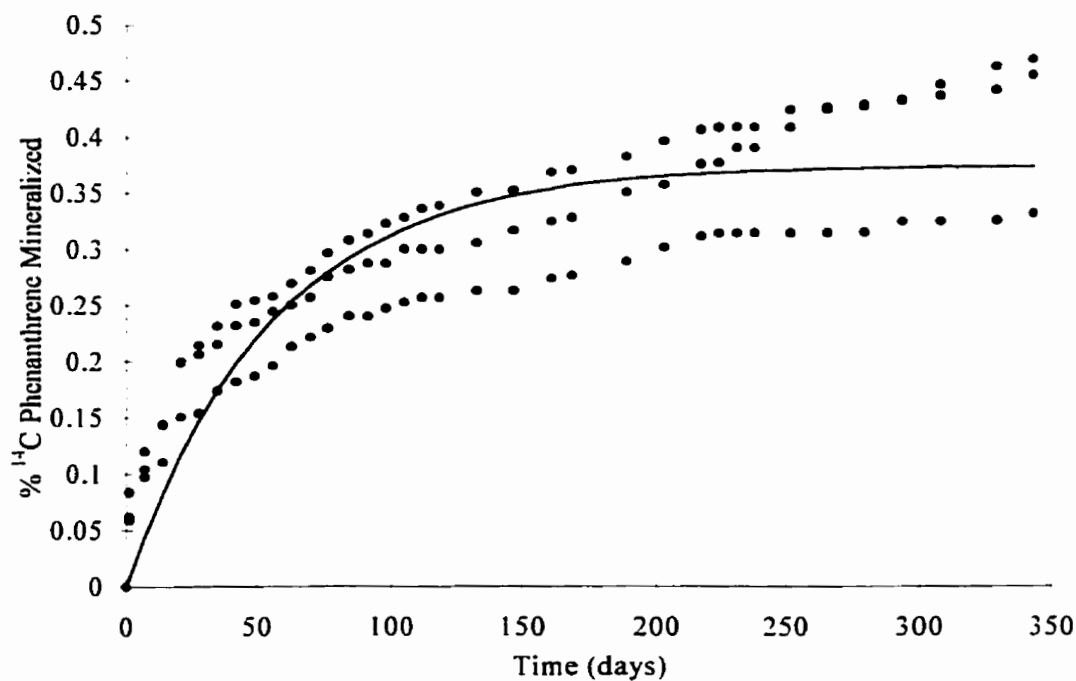
VI a. Model data for the mineralization of ^{14}C phenanthrene in the surface of Site 5-1 (0-10cm) sampled along a landscape. The mineralization was monitored in microcosms (Chapter 4). Triplicate reps were each modeled then an average and standard deviation was calculated.
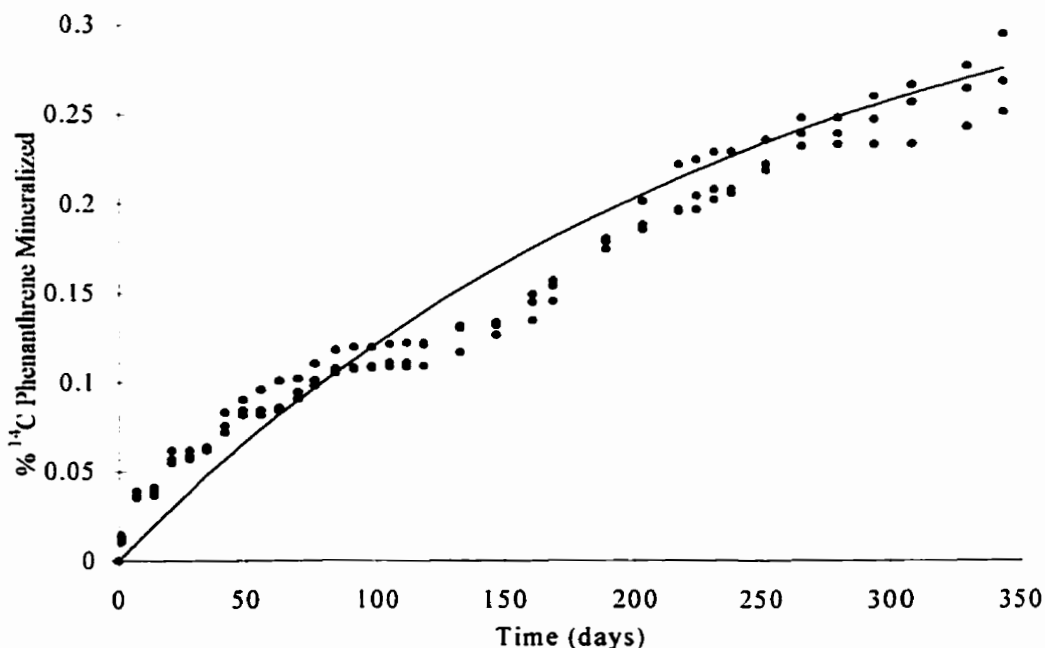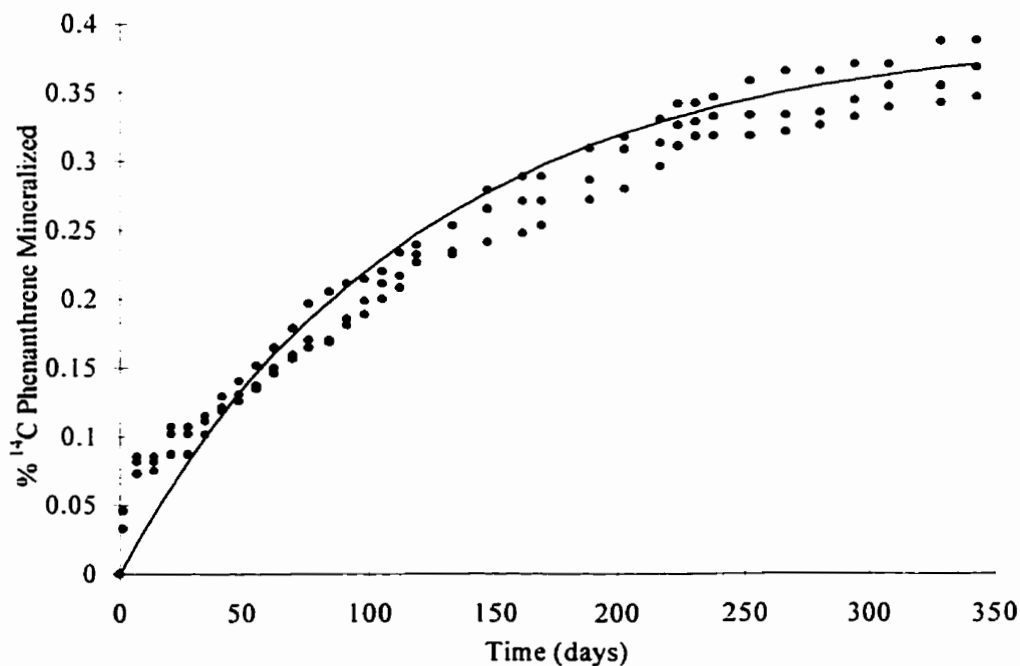

VI b. Model data for the mineralization of ^{14}C phenanthrene in the subsurface of Site 5-1 (40-50cm) sampled along a landscape. The mineralization was monitored in microcosms (Chapter 4). Triplicate reps were each modeled then an average and standard deviation was calculated.
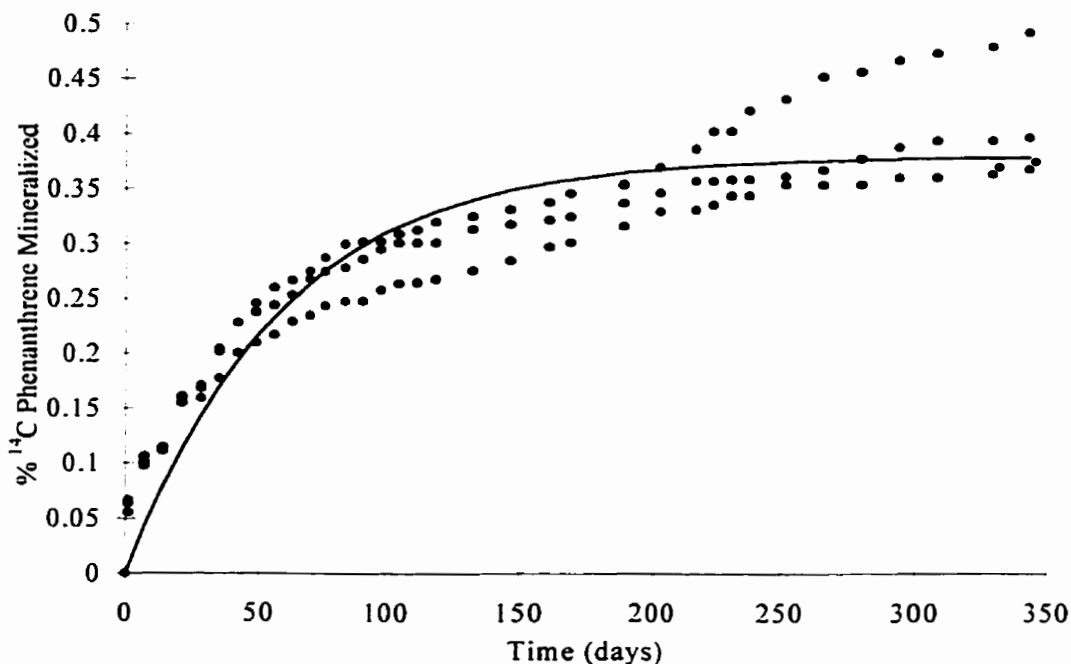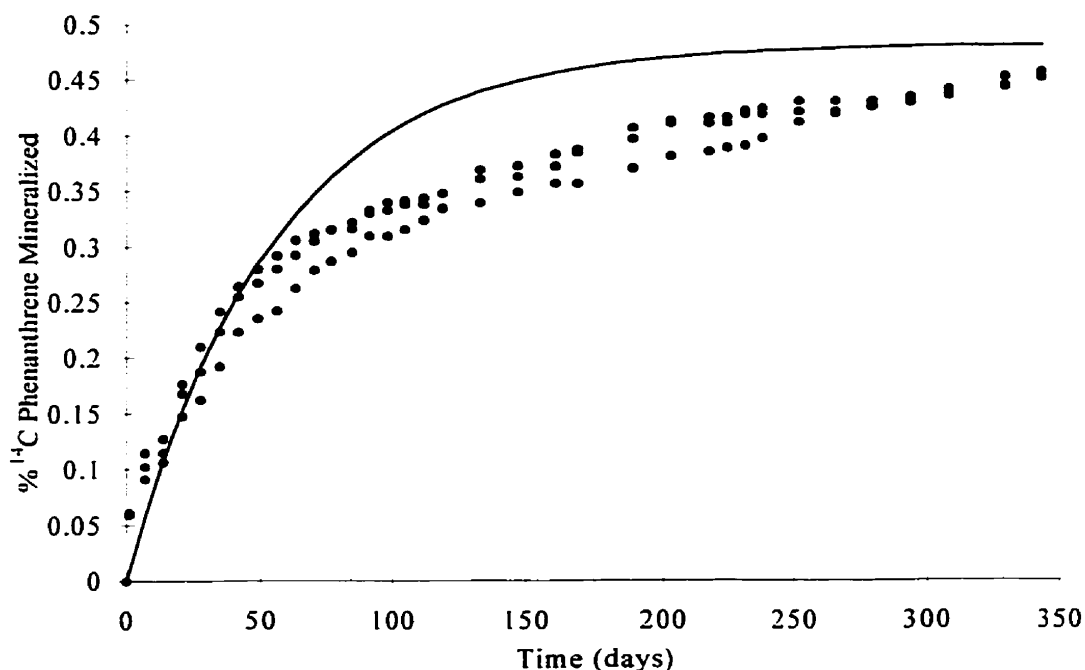
VI c. Model data for the mineralization of ^{14}C phenanthrene in the surface of Site 5-2 (0-10cm) sampled along a landscape. The mineralization was monitored in microcosms (Chapter 4). Triplicate reps were each modeled then an average and standard deviation was calculated.
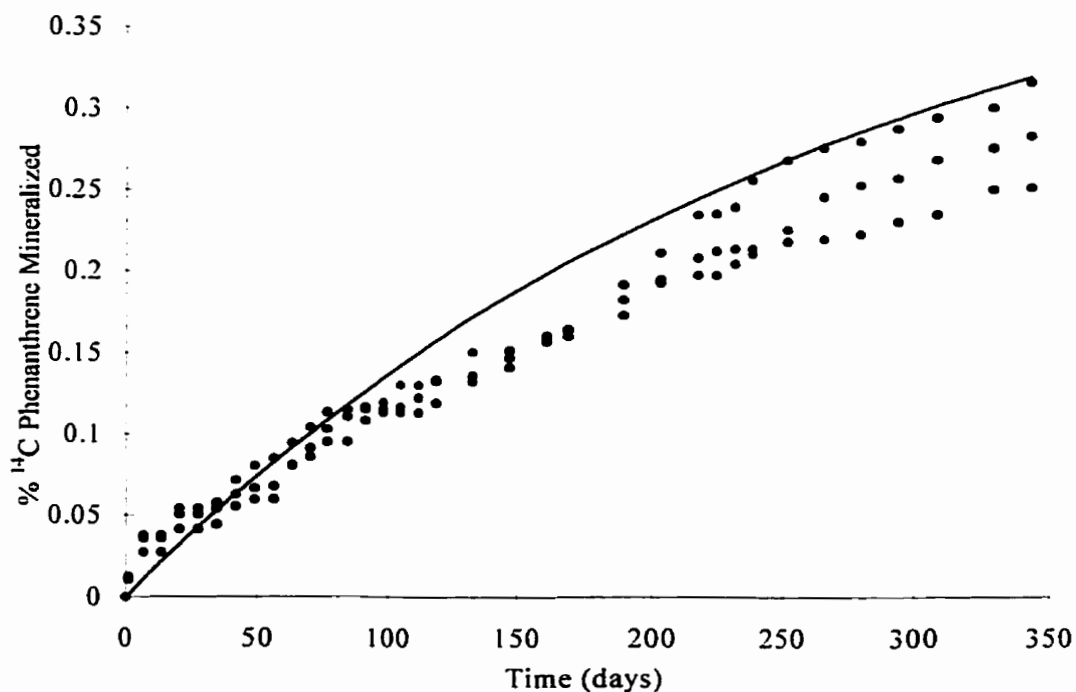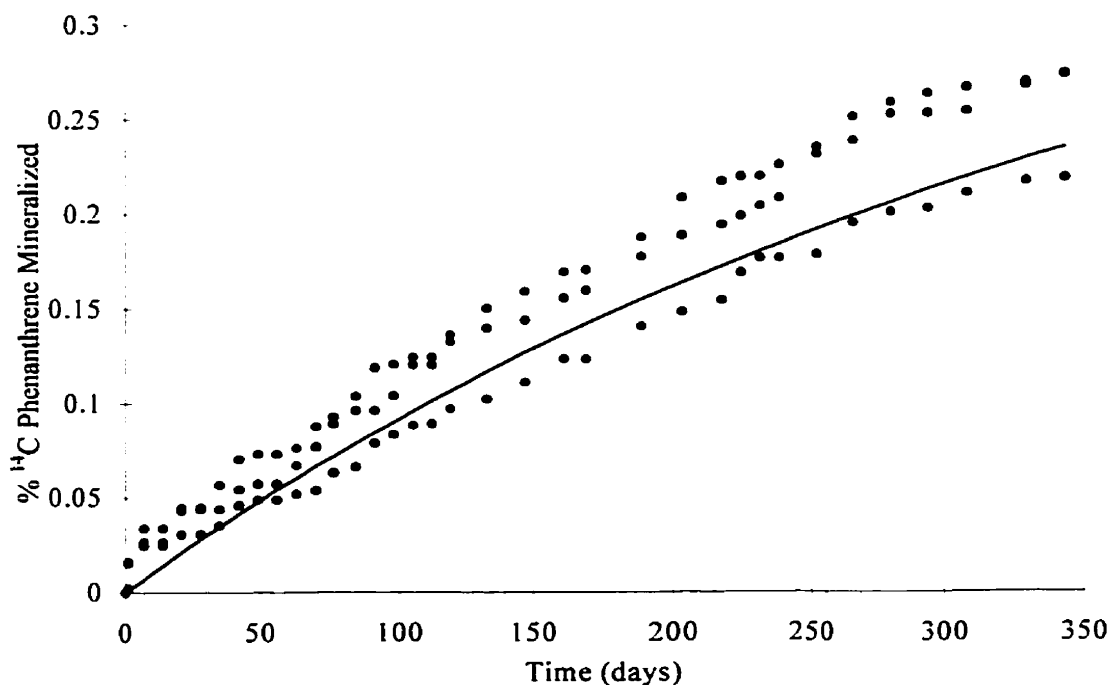

VI d. Model data for the mineralization of ^{14}C phenanthrene in the subsurface of Site 5-2 (40-50cm) sampled along a landscape. The mineralization was monitored in microcosms (Chapter 4). Triplicate reps were each modeled then an average and standard deviation was calculated.
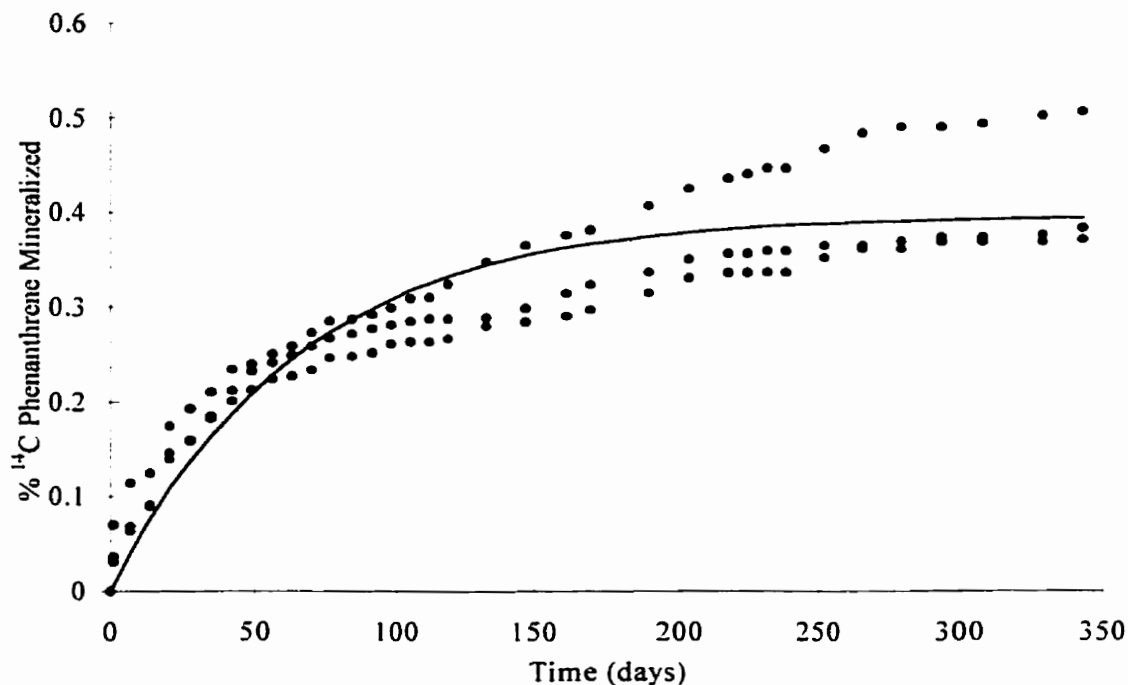
VI e. Model data for the mineralization of ^{14}C phenanthrene in the surface of Site 5-3a (0-10cm) sampled along a landscape. The mineralization was monitored in microcosms (Chapter 4). Triplicate reps were each modeled then an average and standard deviation was calculated.
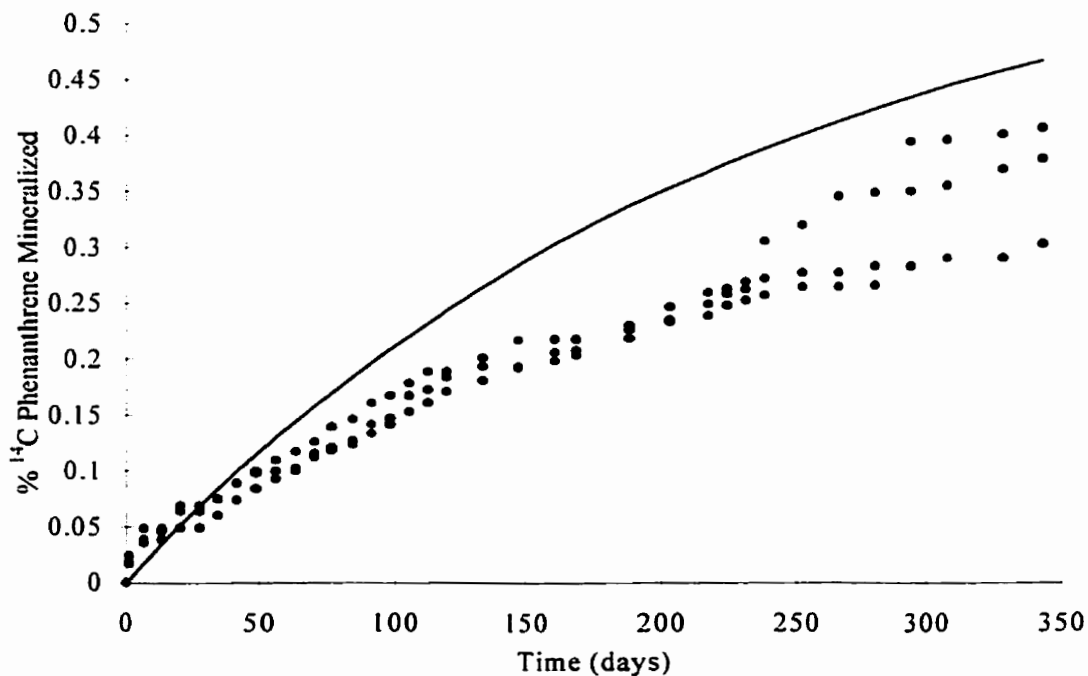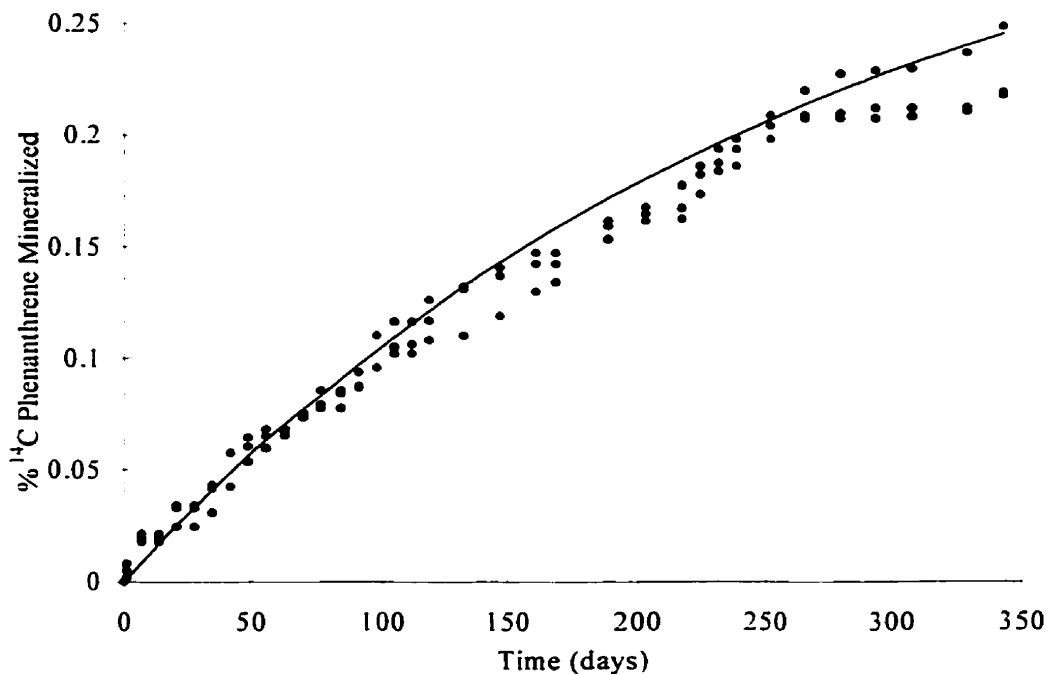

VI f. Model data for the mineralization of ^{14}C phenanthrene in the subsurface of Site 5-3a (40-50cm) sampled along a landscape. The mineralization was monitored in microcosms (Chapter 4). Triplicate reps were each modeled then an average and standard deviation was calculated.
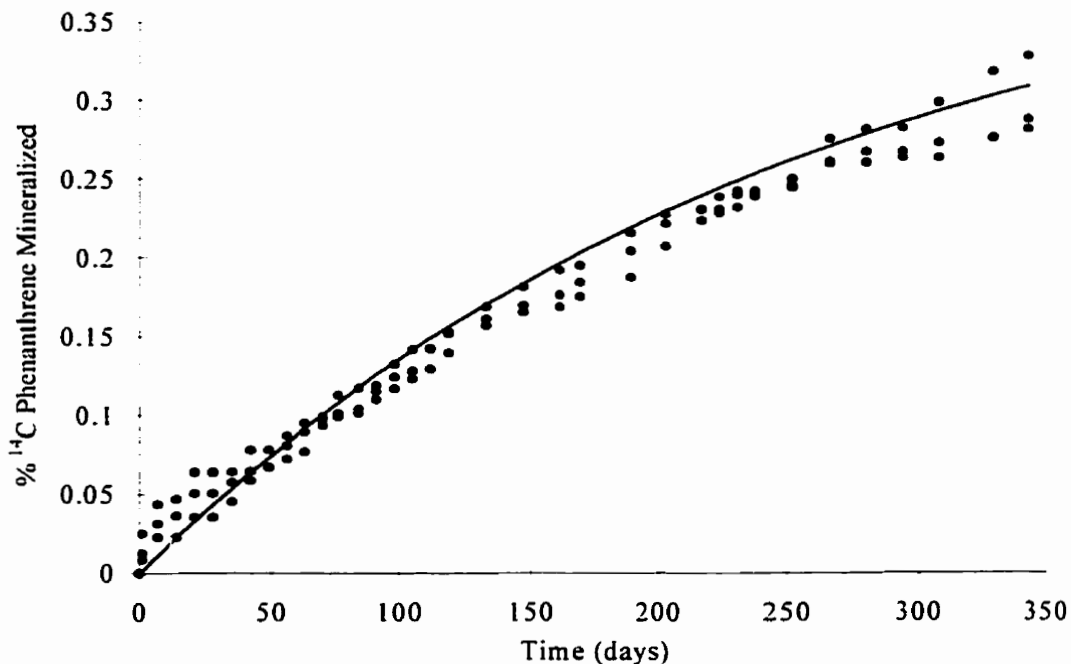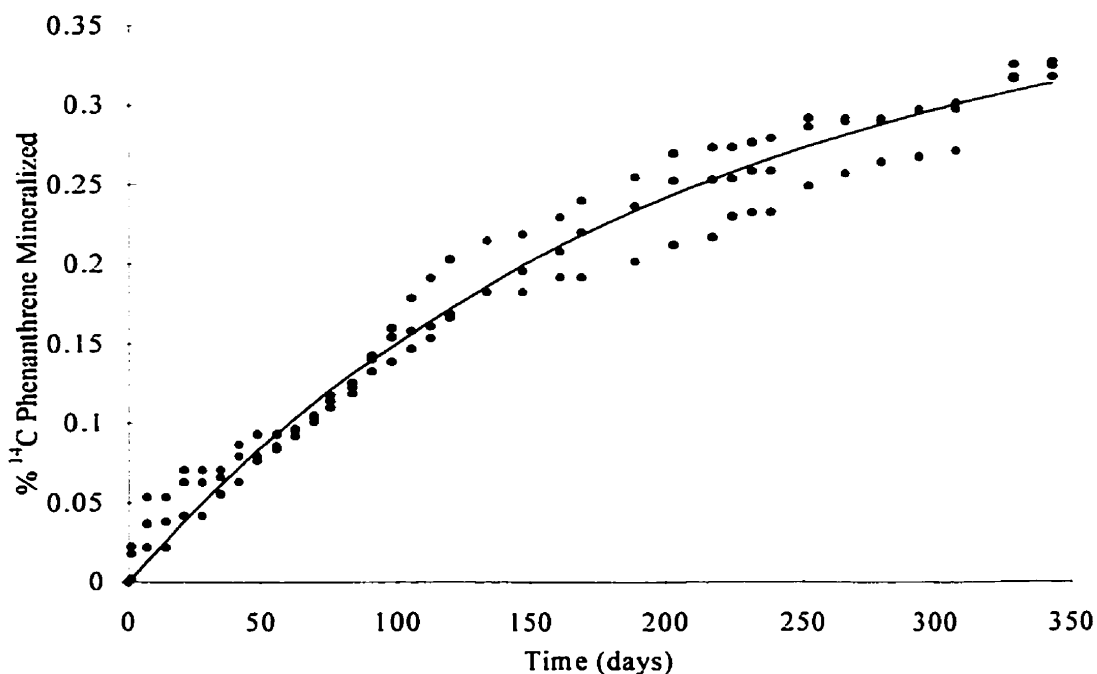
VI g. Model data for the mineralization of ^{14}C phenanthrene in the subsurface of Site 5-3b (40-50 cm) sampled along a landscape. The mineralization was monitored in microcosms (Chapter 4). Triplicate reps were each modeled then an average and standard deviation was calculated.
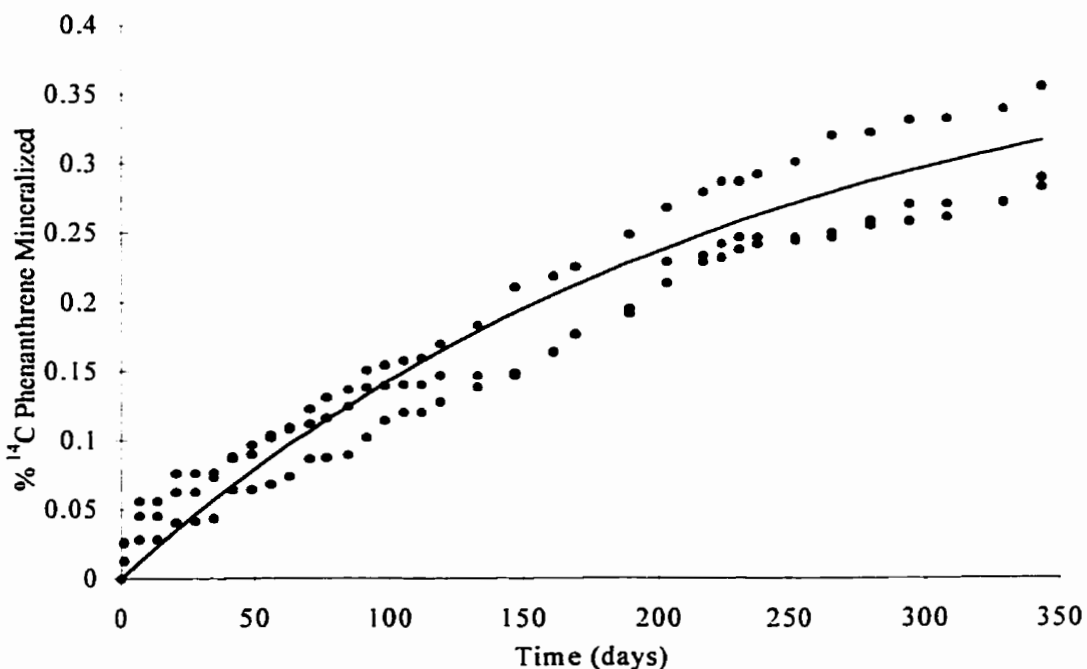
VI h. Model data for the mineralization of ^{14}C phenanthrene in the subsurface of Site 5-3b (90-100 cm) sampled along a landscape. The mineralization was monitored in microcosms (Chapter 4). Triplicate reps were each modeled then an average and standard deviation was calculated.
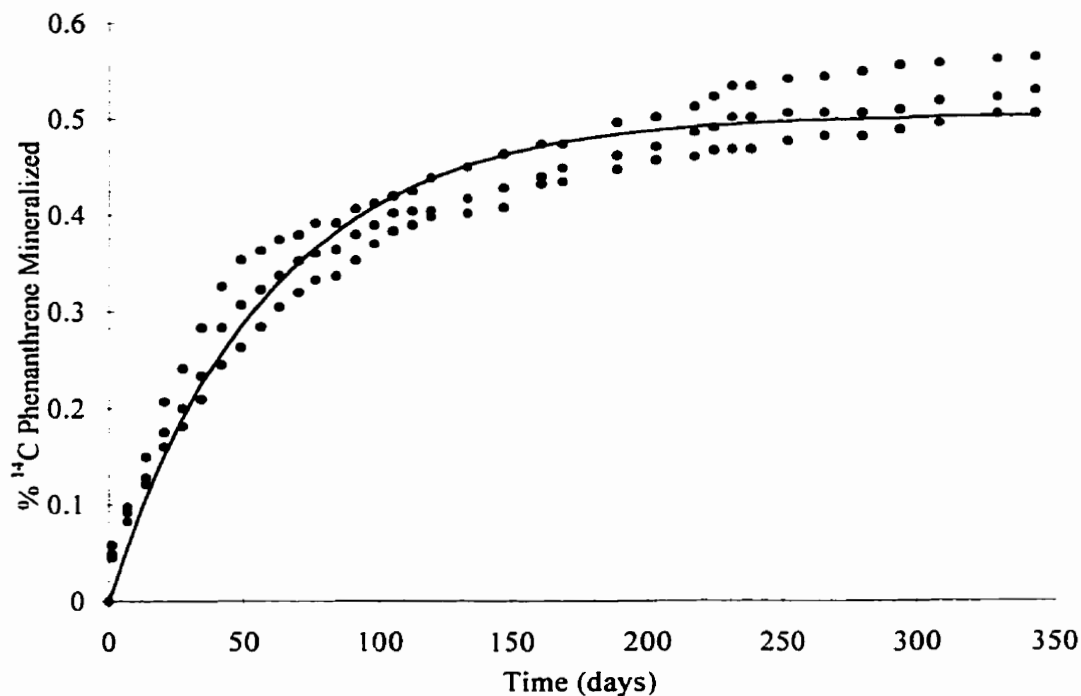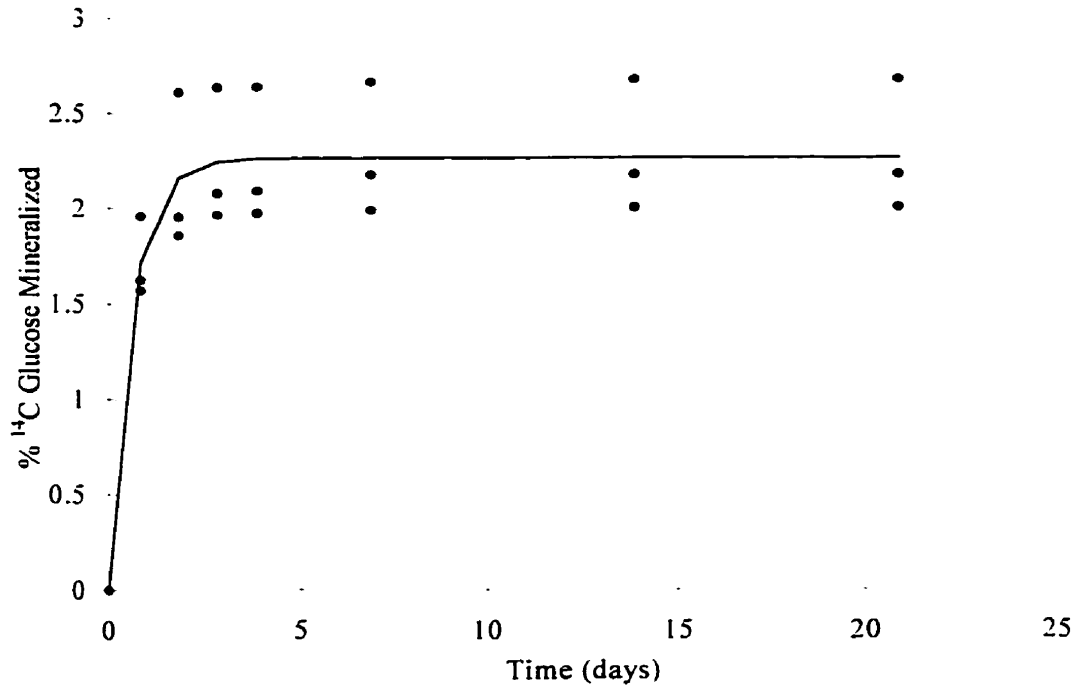
VI i. Model data for the mineralization of ^{14}C phenanthrene in the surface of Site 5-4 (0-10 cm) sampled along a landscape. The mineralization was monitored in microcosms (Chapter 4). Triplicate reps were each modeled then an average and standard deviation was calculated.
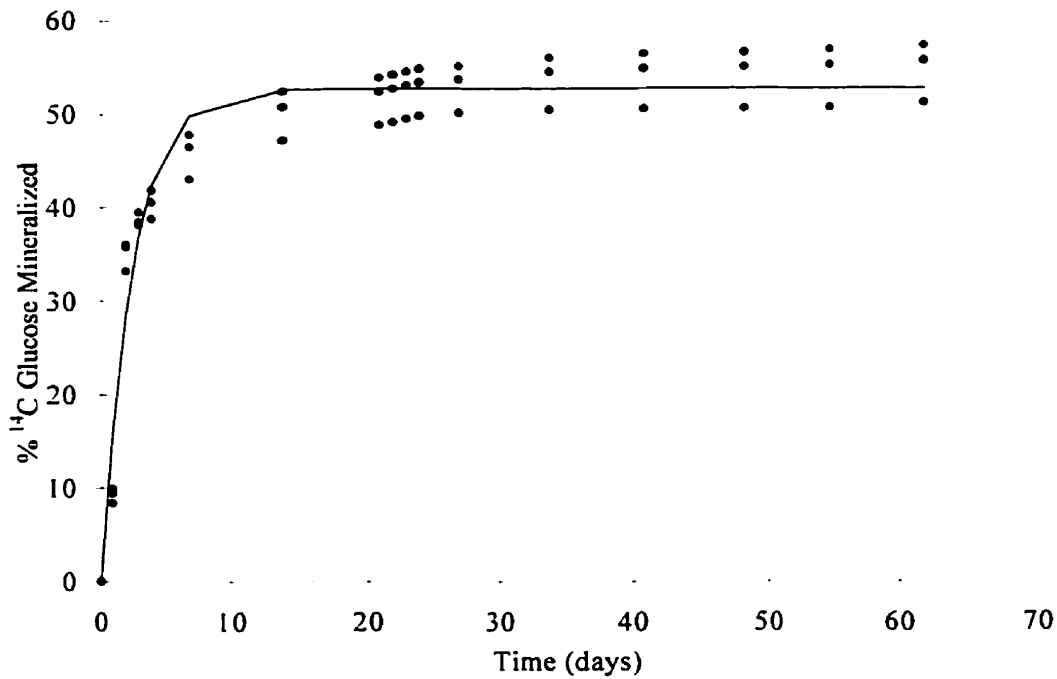

VI j. Model data for the mineralization of ^{14}C phenanthrene in the subsurface of Site 5-4 (40-50 cm) sampled along a landscape. The mineralization was monitored in microcosms (Chapter 4). Triplicate reps were each modeled then an average and standard deviation was calculated.

VI k. Model data for the mineralization of ^{14}C phenanthrene in the surface of Site 5-5a (0-10 cm) sampled along a landscape. The mineralization was monitored in microcosms (Chapter 4). Triplicate reps were each modeled then an average and standard deviation was calculated.
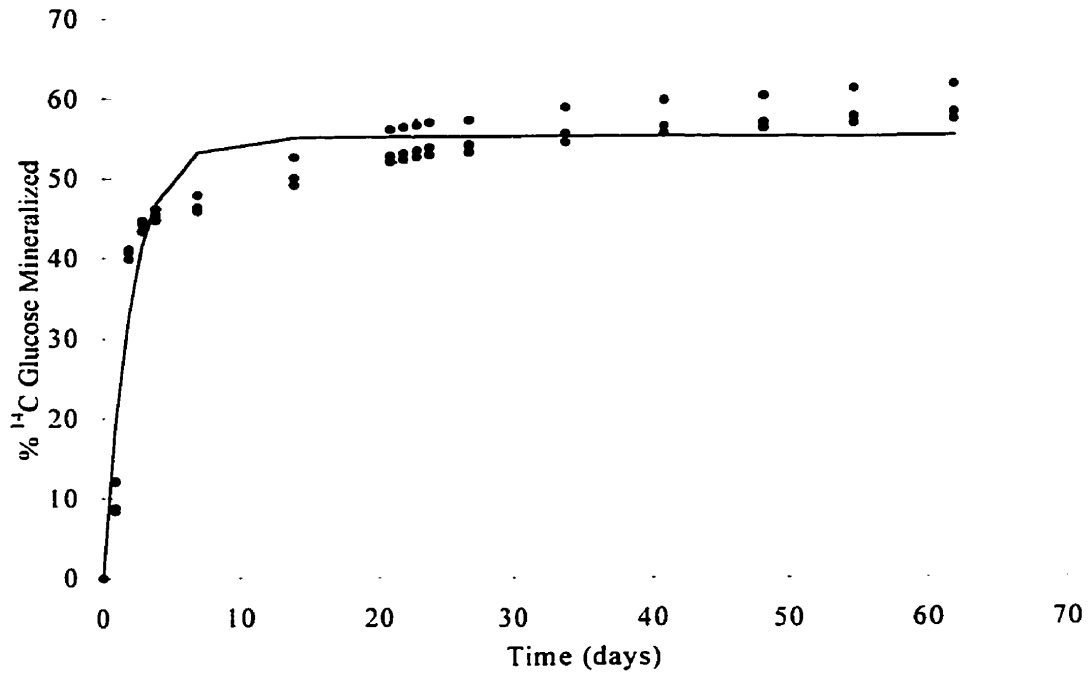

VI l. Model data for the mineralization of ^{14}C phenanthrene in the subsurface of Site 5-5a (40-50 cm) sampled along a landscape. The mineralization was monitored in microcosms (Chapter 4). Triplicate reps were each modeled then an average and standard deviation was calculated.
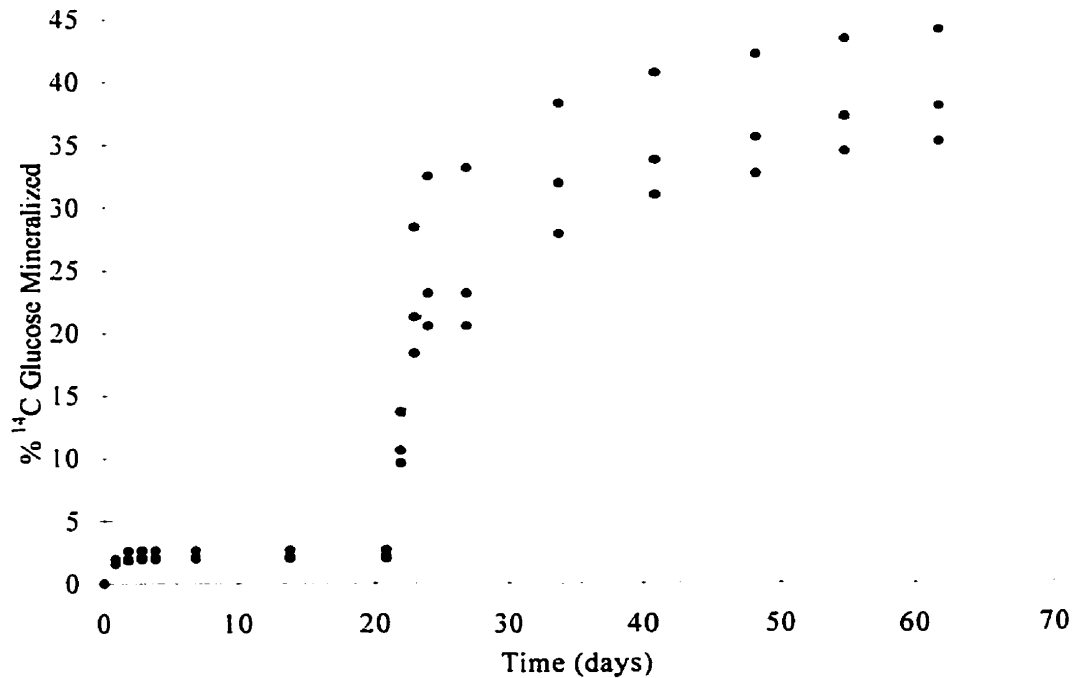
VI m. Model data for the mineralization of ^{14}C phenanthrene in the subsurface of Site 5-5b (50-60 cm) sampled along a landscape. The mineralization was monitored in microcosms (Chapter 4). Triplicate reps were each modeled then an average and standard deviation was calculated.

VI n. Model data for the mineralization of ^{14}C phenanthrene in the subsurface of Site 5-5b (90-100 cm) sampled along a landscape. The mineralization was monitored in microcosms (Chapter 4). Triplicate reps were each modeled then an average and standard deviation was calculated.

VII a. Model data for the mineralization of ^{14}C glucose in the upper-mid slope sampled along a landscape (Site 6). The mineralization was monitored in microcosms maintained at air dry conditions (Chapter 5). Triplicate reps were each modeled then an average and standard deviation was calculated.
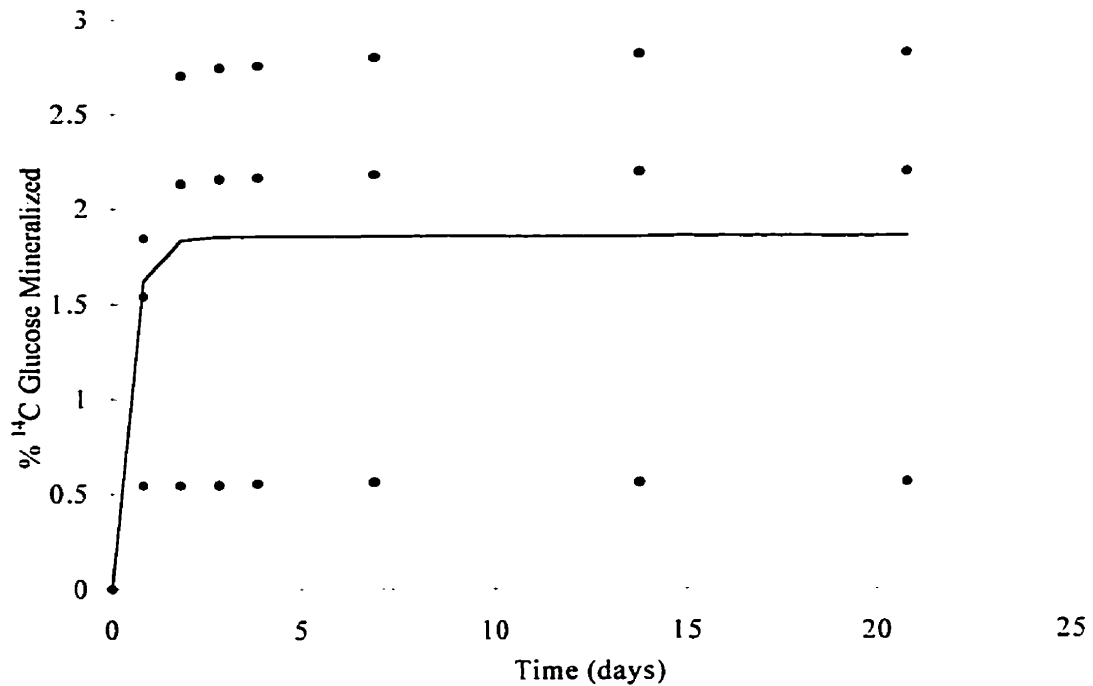

VII b. Model data for the mineralization of ^{14}C glucose in the upper-mid slope (Site 6). The mineralization was monitored in microcosms maintained at field capacity (Chapter 5). Triplicate reps were each modeled then an average and standard deviation was calculated.
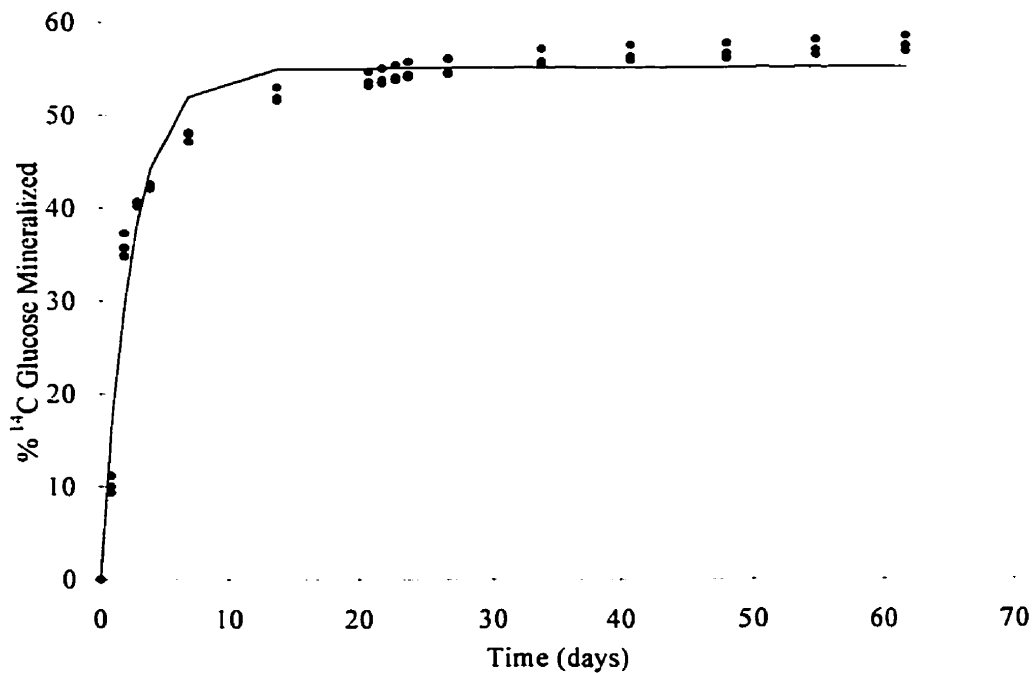
VII c. Model data for the mineralization of ^{14}C glucose in the upper-mid slope sampled along a landscape (Site 6). The mineralization was monitored in microcosms subjected to wet-dry conditions (Chapter 5). Triplicate reps were each modeled then an average and standard deviation was calculated.
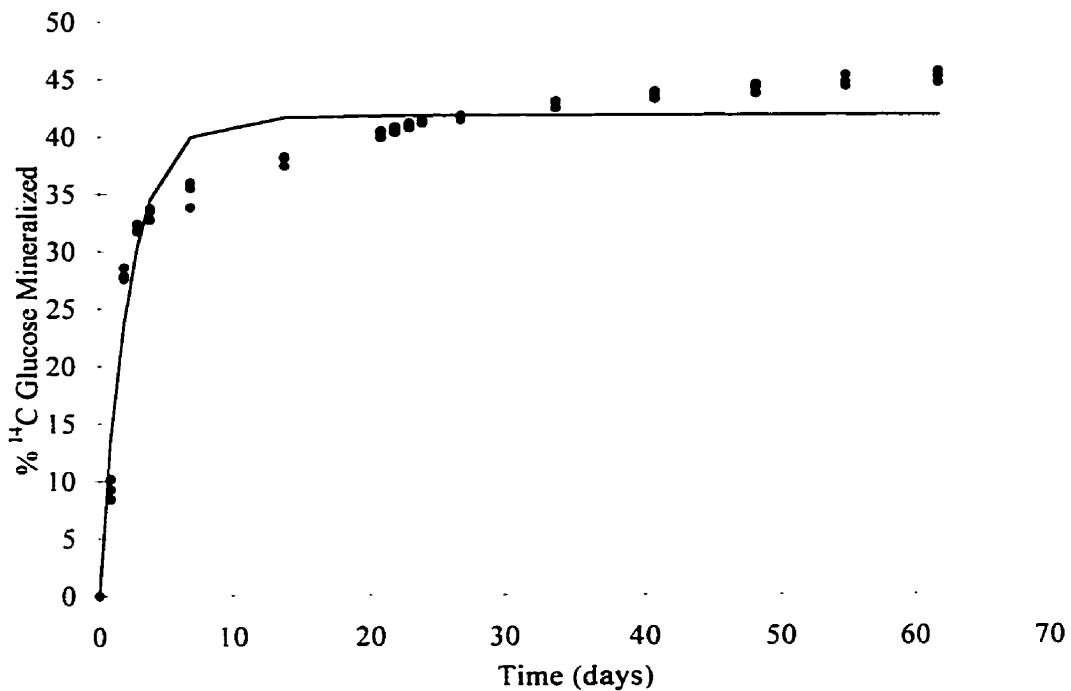

VII d. Data for the mineralization of ^{14}C glucose in the upper-mid slope (Site 6). The mineralization was monitored in microcosms maintained air dry for 21 days then subjected to wet-dry conditions (Chapter 5).
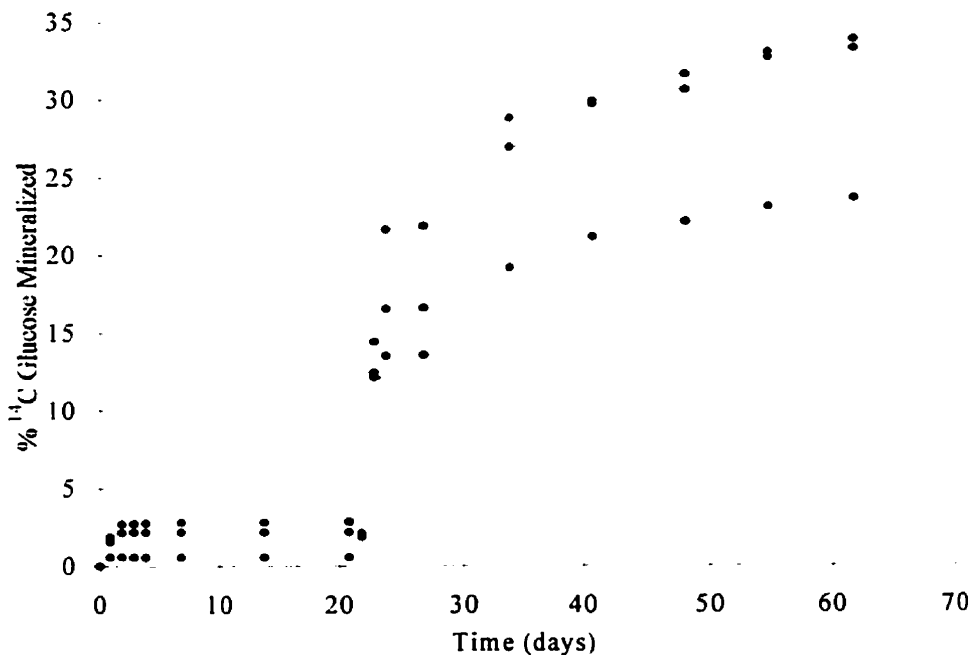
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VII e. Model data for the mineralization of ^{14}C glucose in the depression sampled along a landscape (Site 6). The mineralization was monitored in microcosms maintained at air dry conditions (Chapter 5). Triplicate reps were each modeled then an average and standard deviation was calculated.
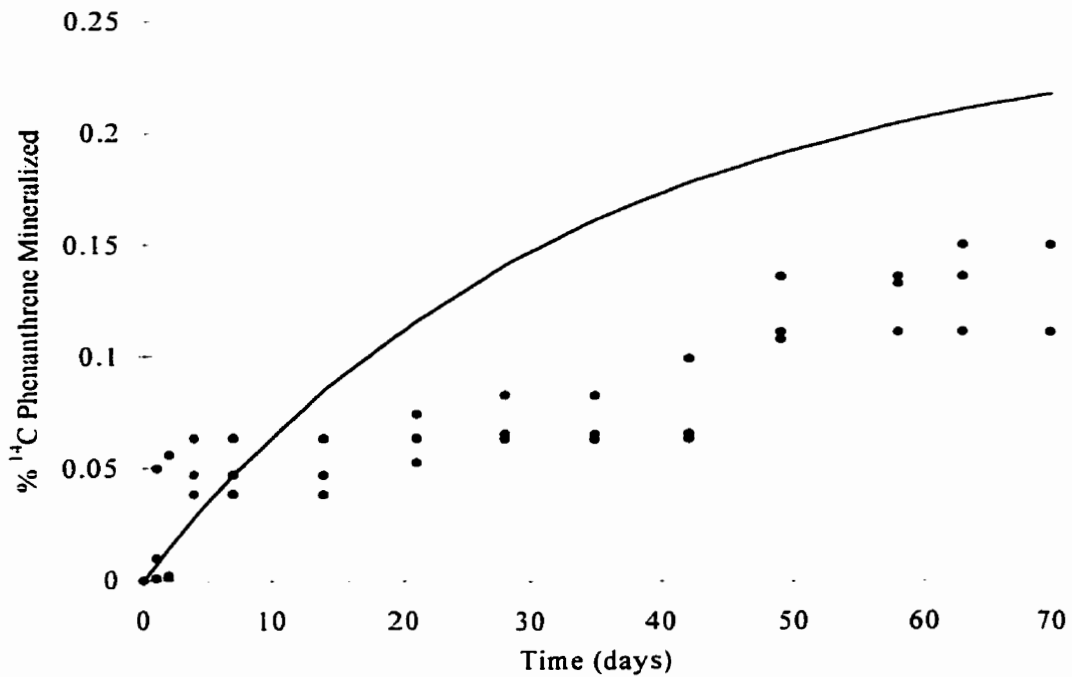

VII f. Model data for the mineralization of ^{14}C glucose in the depression (Site 6). The mineralization was monitored in microcosms maintained at field capacity (Chapter 5). Triplicate reps were each modeled then an average and standard deviation was calculated.
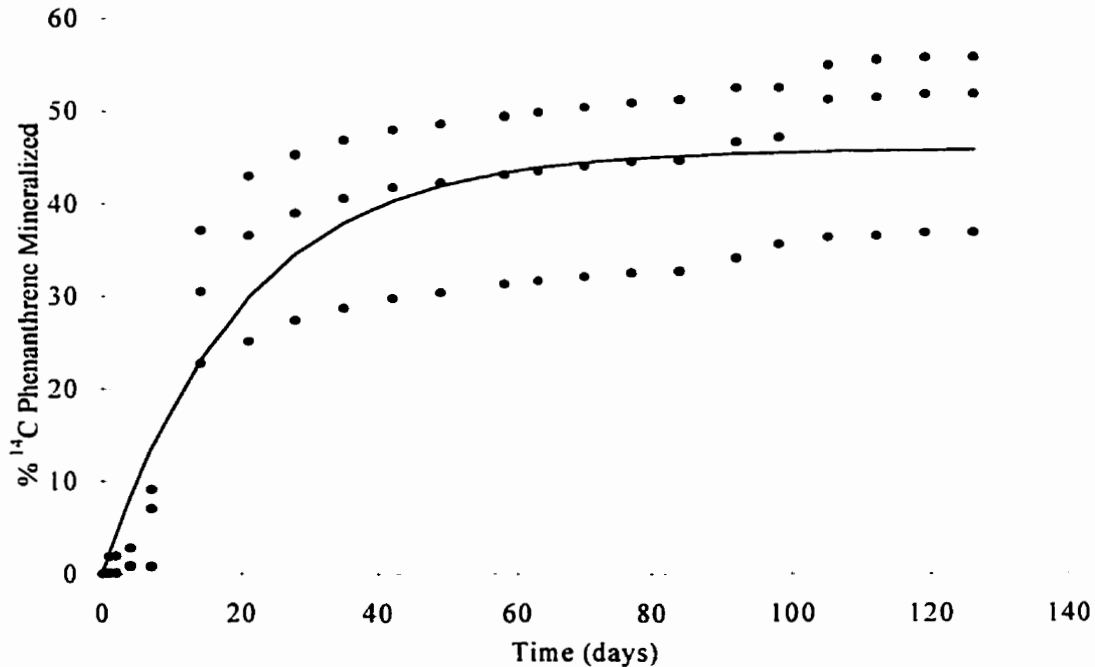
VII g. Model data for the mineralization of ^{14}C glucose in the depression sampled along a landscape (Site 6). The mineralization was monitored in microcosms subjected to wet-dry conditions (Chapter 5). Triplicate reps were each modeled then an average and standard deviation was calculated.
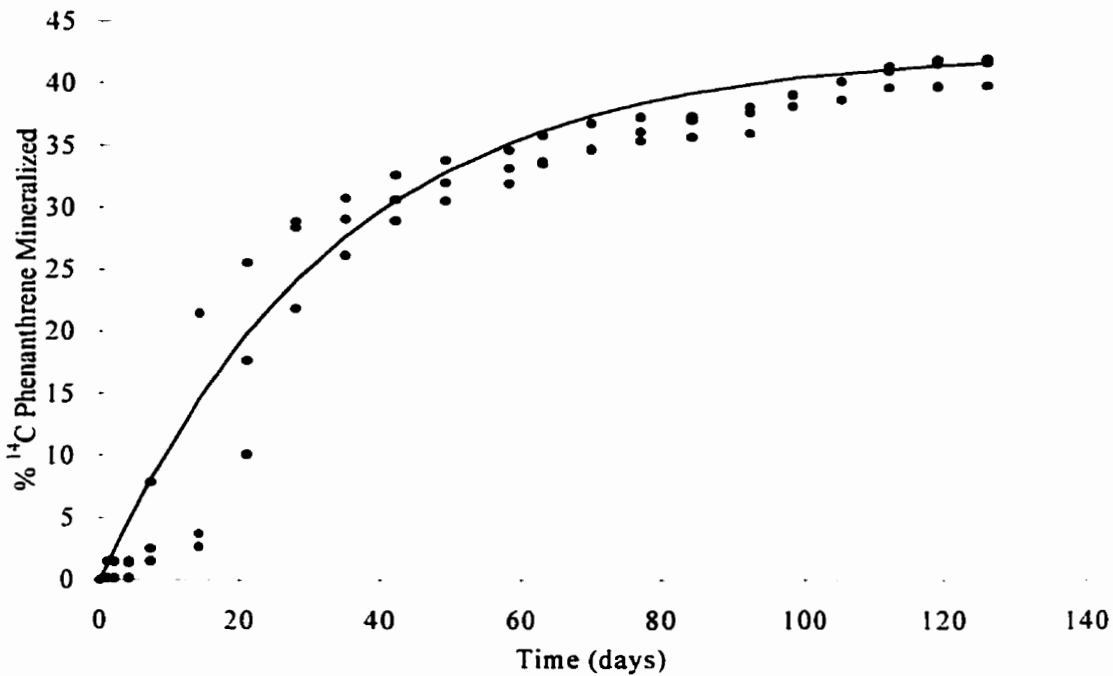

VII h. Data for the mineralization of ^{14}C glucose in the depression (Site 6). The mineralization was monitored in microcosms maintained air dry for 21 days then subjected to wet-dry conditions (Chapter 5).
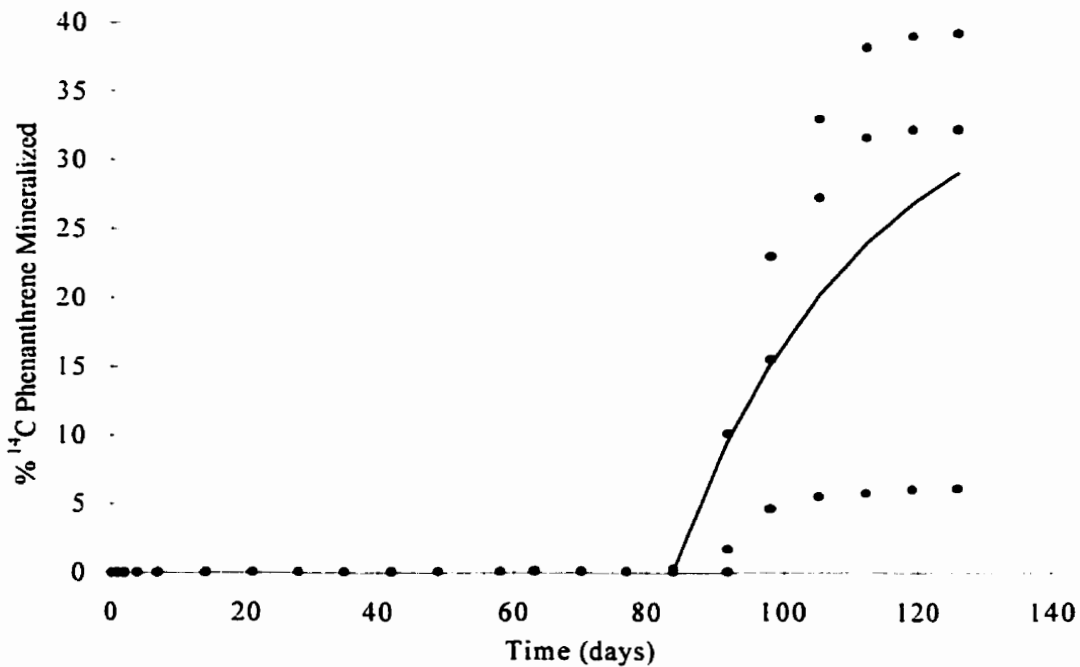
VIII a. Model data for the mineralization of ^{14}C phenanthrene in the upper-mid slope sampled along a landscape (Site 6). The mineralization was monitored in microcosms maintained at air dry conditions (Chapter 5). Triplicate reps were each modeled then an average and standard deviation was calculated.

VIII b. Model data for the mineralization of ^{14}C phenanthrene in the upper-mid slope (Site 6). The mineralization was monitored in microcosms maintained at field capacity (Chapter 5). Triplicate reps were each modeled then an average and standard deviation was calculated.

VIII c. Model data for the mineralization of ^{14}C phenanthrene in the upper-mid slope
sampled along a landscape (Site 6). The mineralization was monitored in
microcosms subjected to wet-dry conditions (Chapter 5). Triplicate reps were each
modeled then an average and standard deviation was calculated.

VIII d. Model data for the mineralization of ^{14}C phenanthrene in the upper-mid slope
(Site 6). The mineralization was monitored in microcosms maintained air dry for 77
days then subjected to wet-dry conditions (Chapter 5). Triplicate reps were each
modeled then an average and standard deviation was calculated.

VIII e. Model data for the mineralization of ^{14}C phenanthrene in the depression sampled along a landscape (Site 6). The mineralization was monitored in microcosms maintained at air dry conditions (Chapter 5). Triplicate reps were each modeled then an average and standard deviation was calculated.
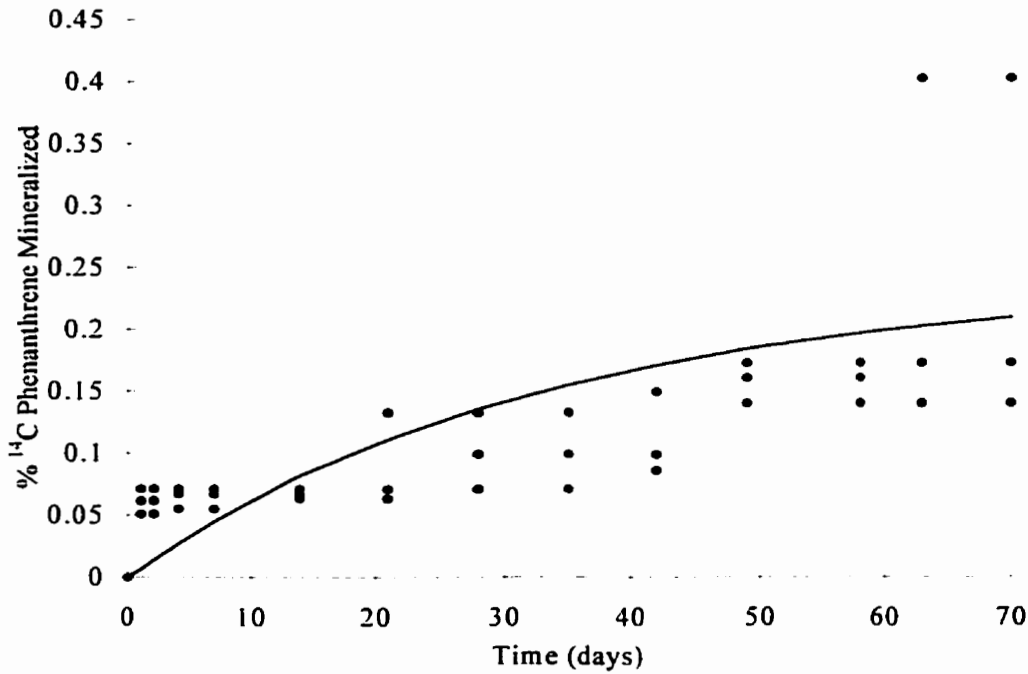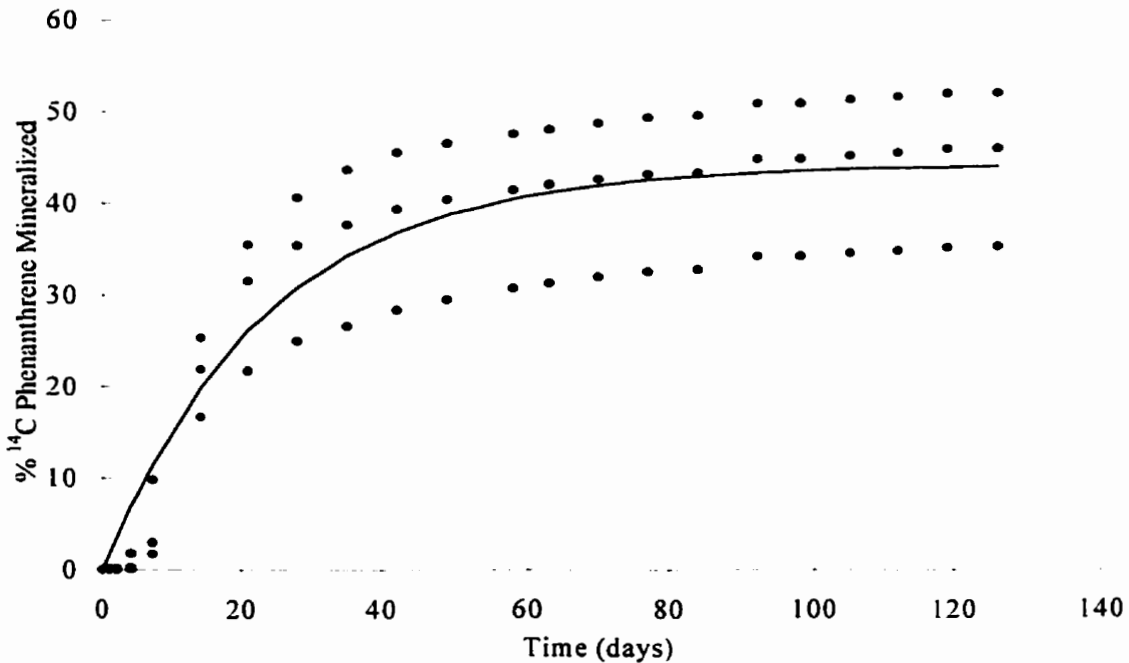

VIII f. Model data for the mineralization of ^{14}C phenanthrene in the depression (Site 6). The mineralization was monitored in microcosms maintained at field capacity (Chapter 5). Triplicate reps were each modeled then an average and standard deviation was calculated.

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VIII g. Model data for the mineralization of ^{14}C phenanthrene in the depression (Site 6). The mineralization was monitored in microcosms subjected to wet-dry conditions (Chapter 5). Triplicate reps were each modeled then an average and standard deviation was calculated.
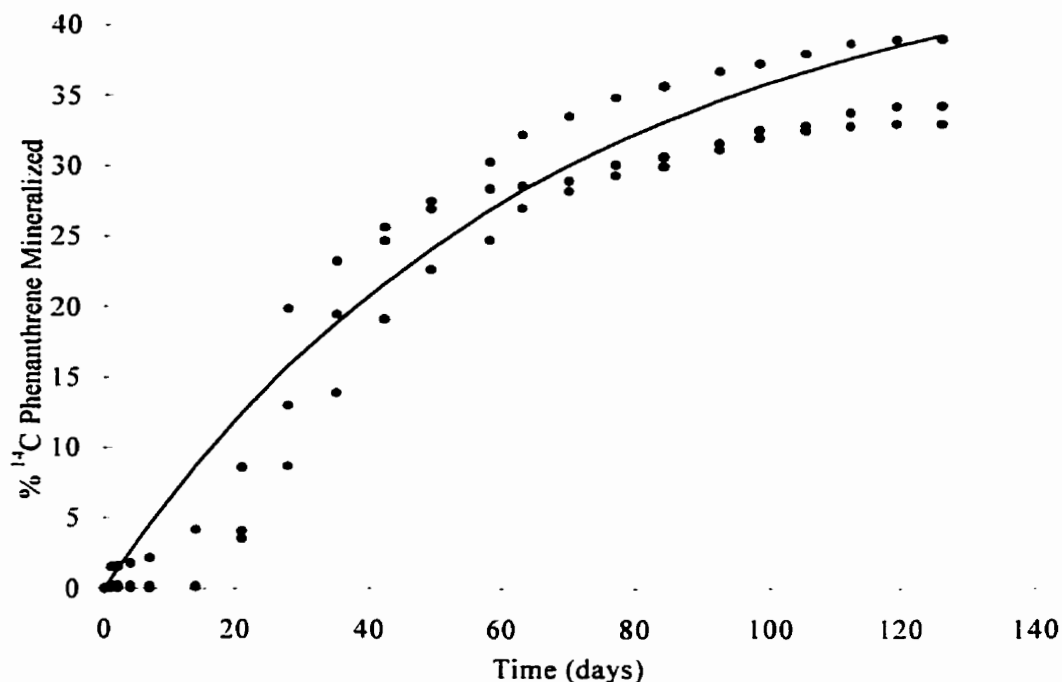

VIII h. Model data for the mineralization of ^{14}C phenanthrene in the depression (Site 6). The mineralization was monitored in microcosms maintained air dry for 77 days then subjected to wet-dry conditions (Chapter 5). Triplicate reps were each modeled then an average and standard deviation was calculated.
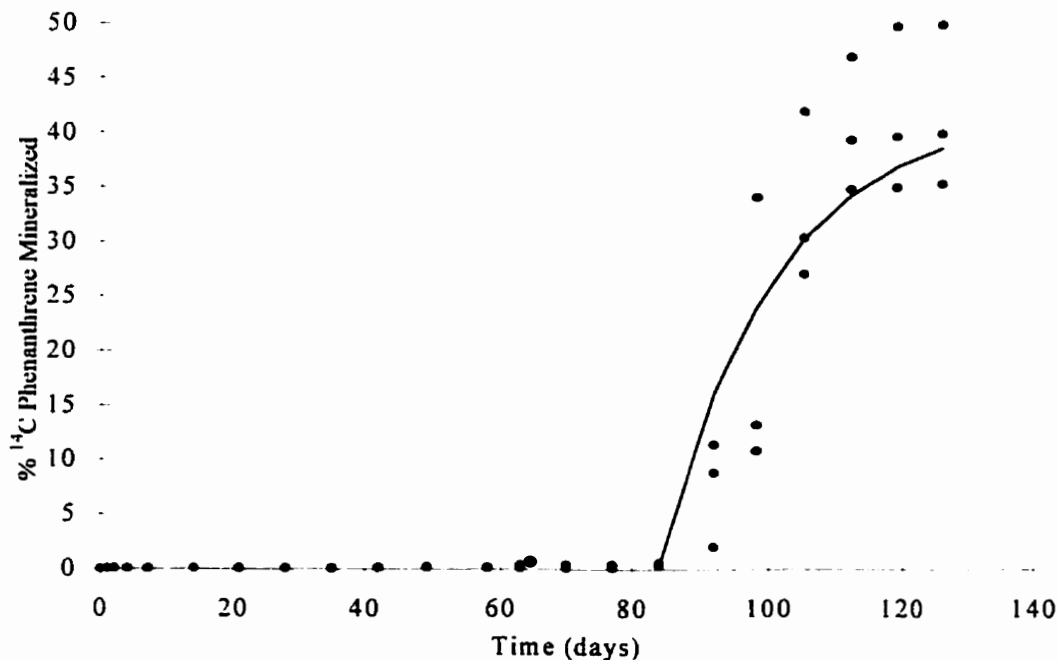
IX a. Duncan New Multiple Range Test for comparing the sites for ^{14}C Phenanthrene mass balance over the course of the survey microcosm experiment.

	% ^{14}C Volatilized	% ^{14}C Phenanthrene Mineralized	% ^{14}C Extracted	Total ^{14}C Recovered
Site Comparisons[t]				
Site #1	5.5 e	4.1 a	67.0 b	76.5 b
Site #2	4.5 cd	3.0 a	64.1 b	71.6 b
Site #3	5.1 de	4.0 a	60.5 ab	69.7 b
Site #4	3.7 bc	10.3 b	51.6 a	73.9 b
Site #5	4.2 c	3.7 a	80.0 c	87.9 c
Site #6	3.2 ab	1.5 a	66.6 b	71.2 b
Site #7	3.1 ab	2.3 a	51.6 a	56.9 a
Site #9	2.6 a	5.7 a	58.1 ab	66.5 ab
Depth Comparison[x]				
0-10 cm	3.4 a	5.2 a	74.7 b	83.3 b
90 -100 cm	4.6 b	3.4 a	52.3 a	60.3 a
ANOVA				
Site	***	*	***	***
Depth	***	ns	***	***
Site x Depth	***	*	***	***

[t]Average of 8 replicates.
[x]Average of 32 replicates.
Means followed by a common letter are not significantly different at the 5% level.
***-highly significant, **-significant, *-low significance, ns-not significant ($\alpha = 0.05$)

IX b. Site and depth means for ^{14}C Phenanthrene volatilized over the course of the intact soil column experiment.

	% ^{14}C Volatilized
Site Comparisons[t]	
Site 5-3a and 5-3b	0.007 a
Site -55a and 5-5b	0.006 a
Depth Comparison	
0-50 cm	0.006 a
50-100 cm	0.007 a
ANOVA	
Site	ns
Depth	ns
Site x Depth	ns

[t]Average of 8 replicates. Means followed by a common letter are not significantly different at the 5% level using Duncan New Multiple Range Test.
***-highly significant, **-significant, *-low significance, ns-not significant ($\alpha = 0.05$)

IX c. **Duncan New Multiple Range Test for comparing all sites for mass balance of ^{14}C recovered from the addition of phenanthrene. Values were take at the end of the soil survey experiment (259 days).**

Sample	% ^{14}C Volatilized	% ^{14}C Phenanthrene Mineralized	% ^{14}C Extracted	Total ^{14}C Recovered
Site Comparison[t]				
Site #8	2.7 b	3.4 a	55.5 b	61.6 a
Site #9[P]	2.6 b	5.7 a	58.1 b	66.5 a
Site #10	0.6 a	50.2 b	33.6 a	84.4 b
Depth Comparison[x]				
0-10 cm	1.6 a	22.4 a	52.0 a	76.0 b
90-100 cm	2.3 b	17.1 a	46.2 a	65.7 a
ANOVA				
Site	***	***	***	**
Depth	*	ns	ns	*
Site x Depth	ns	ns	ns	ns

[P]Site #9 (no previous hydrocarbon exposure) was a control for Site #10 (previous hydrocarbon exposure)

[t]Average of 8 replicates.

[x]Average of 12 replicates.

Means followed by a common letter are not significantly different at the 5% level.

***-highly significant, **-significant, *-low significance, ns-not significant ($\alpha = 0.05$)

IX d. **Site and depth means for total ^{14}C recovered for the intact soil column experiment.**

	Total ^{14}C Recovered
Site Comparisons[t]	
Site 5-3a and 5-3b	61.0 a
Site 5-5a and 5-5b	58.9 a
Depth Comparison	
0-50 cm	53.8 a
50-100 cm	66.2 b
ANOVA	
Site	ns
Depth	**
Site x Depth	ns

[t]Average of 8 replicates. Means followed by a common letter are not significantly different at the 5% level using Duncan New Multiple Range Test.

***-highly significant, **-significant, *-low significance, ns-not significant ($\alpha = 0.05$)

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IX e. **Duncan New Multiple Range Test for comparing the sites for the total ^{14}C recovered for the wet-dry experiment.**

	Total ^{14}C Recovered
<u>Site Comparisons</u>[x]	
Upper-Mid	38.7 a
Depression	44.1 a
<u>Treatment Comparison</u>[t]	
Wet	60.7 a
Wet-Dry	53.5 a
Dry then Wet	51.1 a
<u>ANOVA</u>	
Site	ns
Treatment	ns
Site x Treatment	ns

[x]Average of 12 replicates.

[t]Average of 6 replicates.

Means followed by a common letter are not significantly different at the 5% level.

***-highly significant, **-significant, *-low significance, ns-not significant ($\alpha = 0.05$)
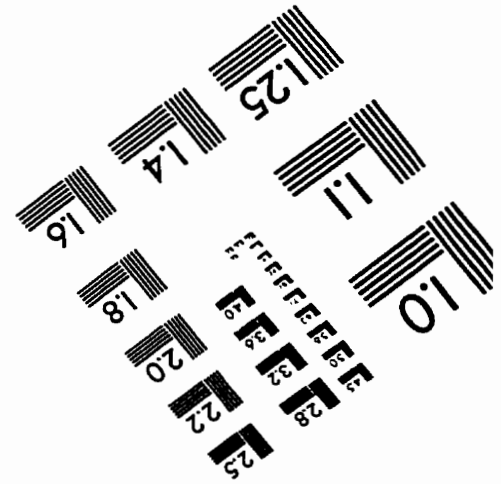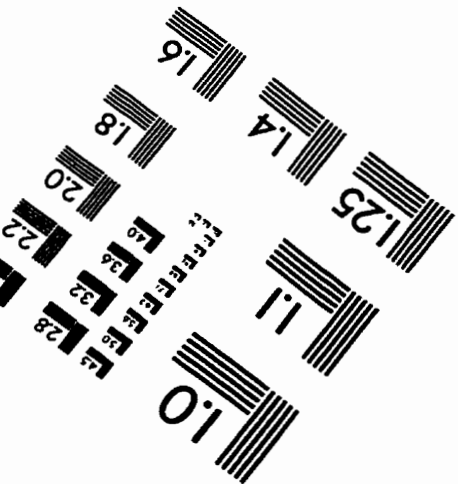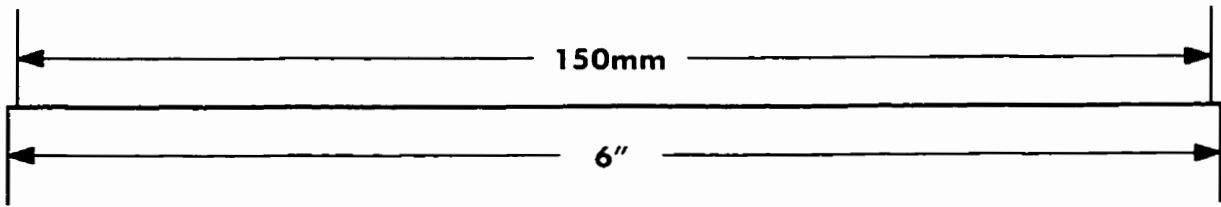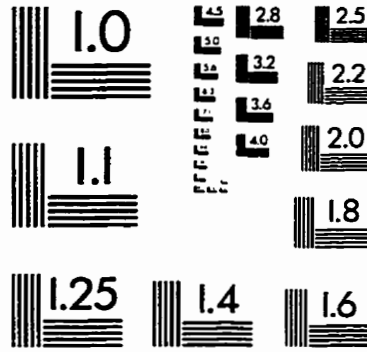
IX f. Location of ^{14}C in each soil profile sampled in the landscape.

| | % ^{14}C Recovered at Each Depth in the Soil Profile | | | | | | | | | | | |
	0-10 cm	10-20 cm	20-30 cm	30-40 cm	40-50 cm	Sand	50-60 cm	60-70 cm	70-80 cm	80-90 cm	Sand	Total % ^{14}C Extracted
Site 5-1	11.7 ± 3.5	16.0 ± 4.8	23.0 ± 7.7	9.5 ± 8.2	----	6.3 ± 2.1	----	----	----	----	----	66.2 ± 9.2
Site 5-2	9.2 ± 1.1	18.4 ± 9.1	11.6 ± 3.3	5.3 ± 2.2	----	4.9 ± 1.1	----	----	----	----	----	49.3 ± 9.0
Site 5-3a	8.0 ± 2.3	15.3 ± 6.5	9.2 ± 2.2	4.8 ± 2.3	----	9.3 ± 2.0	----	----	----	----	----	46.6 ± 9.8
Site 5-3b	----	----	----	----	----	----	14.0 ± 5.0	21.8 ± 6.9	10.5 ± 2.6	6.9 ± 3.8	13.1 ± 3.3	66.2 ± 7.9
Site 5-4	16.7 ± 4.7	13.9 ± 0.9	6.1 ± 1.9	2.6 ± 0.6		4.5 ± 1.8	----	----	----	----	----	43.8 ± 7.8
Site 5-5a	11.8 ± 6.3	11.0 ± 3.5	9.0 ± 4.9	9.4 ± 2.8	5.1 ± 1.2	7.3 ± 0.9	----	----	----	----	----	53.6 ± 5.3
Site 5-5b	----	----	----	----	----	----	25.0 ± 5.2	11.2 ± 5.3	10.6 ± 2.4	8.4 ± 4.2	8.7 ± 5.9	64.1 ± 8.8

IX g. The total extractable hydrocarbons recovered from the soil at various depths in the sites sampled in the landscape. Initial diesel fuel added was 171.7g.

	% Hydrocarbons Recovered at Each Depth in the Soil Profile											
	0-10 cm	10-20 cm	20-30 cm	30-40 cm	40-50 cm	Sand	50-60 cm	60-70 cm	70-80 cm	80-90 cm	Sand	% of Total Hydrocarbons Remaining in Soil
Site 5-1	20.2 ± 8.5	13.8 ± 13.0	28.0.0 ± 9.6	14.2 ± 9.6	----	2.9 ± 2.7	----	----	----	----	----	79.1 ± 16.5
Site 5-2	15.8 ± 14.1	23.6 ± 12.0	12.2 ± 11.4	1.4 ± 1.1	----	5.2 ± 7.2	----	----	----	----	----	58.3 ± 11.7
Site 5-3a	7.8 ± 7.9	9.4 ± 7.4	9.6 ± 6.2	2.4 ± 3.2	----	26.4 ± 17.6	----	----	----	----	----	55.5 ± 17.6
Site 5-3b	----	----	----	----	----	----	32.5 ± 10.0	20.5 ± 14.7	4.5 ± 2.1	2.8 ± 1.8	23.9 ± 15.8	84.2 ± 6.9
Site 5-4	36.0 ± 21.8	9.4 ± 16.4	0.7 ± 0.8	0.0 ± 0.0	----	14.9 ± 9.4	----	----	----	----	----	61.0 ± 16.9
Site 5-5a	18.8 ± 32.1	0.0 ± 0.0	0.7 ± 0.8	13.1 ± 24.8	0.7 ± 0.1	18.3 ± 21.2	----	----	----	----	----	51.2 ± 13.0
Site 5-5b	----	----	----	----	----	----	10.0 ± 5.9	18.0 ± 29.0	3.5 ± 3.0	2.2 ± 1.1	31.0 ± 24.5	64.6 ± 18.6

IMAGE EVALUATION
TEST TARGET (QA-3)

150mm

6"