National Library of Canada
Acquisitions and Bibliographic Services Branch
395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale du Canada
Direction des acquisitions et des services bibliographiques
395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file Votre référence

Our file Notre référence

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

Canada

UNIVERSITY OF ALBERTA

INTERACTIONS OF POLYNUCLEAR AROMATIC HYDROCARBONS WITH SOIL

BY

SOHEILA KARIMI LOTFABAD ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE.

DEPARTMENT OF CHEMICAL ENGINEERING

EDMONTON, ALBERTA
FALL 1994

National Library
of Canada

Acquisitions and
Bibliographic Services Branch

395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale
du Canada

Direction des acquisitions et
des services bibliographiques

395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file Votre référence

Our file Notre référence

The author has granted an irrevocable non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of his/her thesis by any means and in any form or format, making this thesis available to interested persons.

The author retains ownership of the copyright in his/her thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without his/her permission.

L'auteur a accordé une licence irrévocable et non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de sa thèse de quelque manière et sous quelque forme que ce soit pour mettre des exemplaires de cette thèse à la disposition des personnes intéressées.

L'auteur conserve la propriété du droit d'auteur qui protège sa thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

ISBN 0-315-95056-0

Canada

UNIVERSITY OF ALBERTA

RELEASE FORM

NAME OF AUTHOR: Soheila Karimi Lotfabad

TITLE OF THESIS: Interactions of Polynuclear Aromatic Hydrocarbons with Soil

DEGREE Master of Science

YEAR THIS DEGREE GRANTED: 1994

Signed: Soheila Karimi

Permanent Address:
#7, 33Reza, Reza St.
Mashhad, Iran

Date: Aug. 22, 1994

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis untitled **INTERACTIONS OF POLYNUCLEAR AROMATIC HYDROCARBONS WITH SOIL** submitted by **SOHEILA KARIMI LOTFABAD** in partial fulfillment of the requirement for the degree of **MASTER OF SCIENCE**.

Dr. M.R.Gray, supervisor

M. Dudas
Dr. M. Dudas

Dr. M.A. Pickard

DATE: Aug 22, 1994

ABSTRACT

Interaction of polynuclear aromatic hydrocarbons (PAHs) with soil is an important issue for bioremediation of contaminated sites. PAHs have a very low solubility in water but as they are dissolved in water an active bacterial culture can degrade them. An important stage in bioremediation is to overcome the solubility and mass transfer problems. Interactions of PAHs with soil would be a decisive factor affecting their solubility in water.

Soil as a complex mixture of minerals and organics which may have different interactions with organic compounds. Three different mechanisms are recognized: 1) adsorption to the mineral surface 2) partitioning into soil organic matter 3) catalytic reactions with mineral surfaces. The composition of the soil, water content and age of the soil will be parameters deciding the dominant mechanism.

This study has focused on catalytic reactions of polynuclear aromatic hydrocarbons with soils and clays. Anthracene was selected as a model compound and its interaction with three different soils (C horizon, Malmo soil and shale) and montmorillonite, as a pure clay, was studied in room temperature. The effect of time and moisture content as key factors in interactions were studied and a kinetic model was presented to predict the behavior of the system. In time course studies anthracene solutions in methylene chloride were contacted with soil. After the required time was elapsed methylene chloride was evaporated and anthracene was analyzed by GC.

The results showed that only a fraction of anthracene loaded onto the soil was extractable and the amount of extractable anthracene decreased with time. Further experiments revealed the fact that anthracene reacted with soil to give higher molecular weight compounds, and water could inhibit the reaction by competitive adsorption on mineral surfaces. GC-MS identified 9-9 bianthracene as one of the products. The mechanism for polymerization of anthracene is proposed to be free radical reactions in which transition metals act as terminal electron acceptors. These reactions are expected to be of particular importance in remediation of soil by thermal desorption, where water content would be reduced by drying and reactions would be accelerated at higher temperatures.

ACKNOWLEDGEMENT

I would like to take this opportunity to express my sincere appreciation to those who assisted me and made this work enjoyable. In particular:

Dr. Murray Gray for support, guidance and constant encouragement.
Dr. Mike Pickard for fruitful discussions.
Dr. Marvin Dudas for valuable discussions and cooperation in selecting the soil samples .
Ms. Atsumi Hashimoto for her valuable assistance in some experiments.

I would like to acknowledge the financial support from:

The Ministry of Culture and Higher Education of Islamic Republic of Iran for providing funds to complete this degree.
The Institute of Chemical Science and Technology for funding the project.

I am also grateful to my friends, Clara Qualizza, Norman Sawatsky, Clive Figueiredo, Dr. Dwijen Banerjee and Birgitte Willumsen for their help in different ways.

TABLE OF CONTENTS

Chapter Page

Chapter **Page**

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Increasing use of organic compounds in industrial and agricultural applications and use of chemical and petrochemical products have led to contamination problems in soil, water and air.

The fate of polynuclear aromatic hydrocarbons (PAHs) in soil has drawn increasing concern due to their toxic, carcinogenic and mutagenic effects. These compounds permeate the soil when solvents carrying PAHs are spilled to the surface. Depending on the characteristics of chemicals, soils and hydrology of the site they may remain as surface contamination or migrate down to become subsurface and ground-water contamination. The interaction between PAHs and soil is particularly important and decisive in distribution of the contaminants.

Soil is a complex mixture of mineral and organic material that can have different interactions with PAHs. Many studies have been conducted simulating aqueous systems, and some data indicating adsorption of organics from the vapor phase by soil is available, but none of these systems can demonstrate the interactions following surface contamination by spilling. When PAHs are spilled on the surface in a carrier solvent, the solvent may evaporate or undergo degradation, leaving behind the PAHs which may interact directly with soil components.

Previous studies, in which sorption of organic compounds to mineral surfaces or soil organic matter have been investigated, are limited to phenomenological descriptions of the process and do not explain the consequent results of this kind of interactions.

To investigate the interactions of PAHs with soils and sediments, anthracene, a three ring aromatic hydrocarbon, with molecular weight of 178, and solubility in water 45 part per billion, was selected as a model compound. A limited number of experiments were carried out with phenanthrene and pyrene.

Three soil samples with different characteristics, and one sample of montmorillonite were employed in this study.

The method employed to introduce the chemicals into the soil in these series of experiments (described in chapter 3), helped to mimic the real conditions in creosote contaminated sites. The interaction between the contaminant and the soil constituents in the unsaturated zone may be dramatically different from uptake of organics in aqueous solutions, which would occur at or below water table.

In contrast to adsorption from gas phase (tertiary system), and partitioning (quaternary system), a real contaminated soil is a 5-component system, consisting of soil mineral, soil organic matter, water, solvent and the PAHs. The interactions of these five components make a complex system, which needs vast and fundamental studies to be understood. Time can be considered as the sixth dimension affecting the overall behavior of the system.

In this study, the results of laboratory batch experiments are presented in which sorption of anthracene onto different types of soils was studied as a function of time and moisture content. The major goals of this study were:

1- To identify the type of interactions which are responsible for the irreversibility of sorption of anthracene on soils and clays.

2- To study the influence of water content on interactions between anthracene and soil.

3- To present a kinetic model representing the behavior of anthracene in soils and clays.

4- To investigate the bioavailibility of anthracene after interaction with soil.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Soil is an effective adsorbent for polar organic compounds as well as nonionic organics. The complex structure of soil and the heterogeneous characteristics of the surface have led to different hypotheses on the impact of each constituent of soil on sorption phenomena. The interaction of organics with soil influences the physical, chemical and biological properties of soil, hence, it is important to recognize the mechanism of such interactions. The accepted theories which have strong background are adsorption of organics on mineral surfaces and partitioning into soil organic matter. A few investigators have proposed the mutual effect of soil organic matter and mineral surface and its contribution on sorption of organics by soil organic matter (Murphy et al., 1990).

The sorption capacity is influenced by soil organic matter, the kind and amount of minerals, soil moisture content and the solvent system. Exchangeable cations, which are recognized as active sites of adsorption, and crystal edges having acidic characteristics may interact in electron donor acceptor reactions leading to complexion of organics on clay surfaces, or polymerization.

2.2 Characterization of Soil

Soil is a porous mixture of mineral particles, organic matter, water and air. Examination of a vertical cross section of soil reveals the presence of distinct horizons which have different characteristics due to biochemical decomposition, weathering, and development processes of the soil.

The A horizon, which is sometimes called the topsoil, corresponds to the zone of maximum biological activity. Frequently it is enriched in organic matter.

Underlying soil horizons (B and C) contain comparatively less organic matter than the topsoil. The B horizon is a zone characterized by geochemical reactions which alter the mineral assemblage and mineral characteristics in comparison to the C horizon, which is distinguished by accumulation of iron and aluminum oxides, clays, gypsum and calcium carbonate, with small amounts of organics. However the transition between different layers is so gradual, that establishment of boundaries is rather difficult.

In general soils are classified by texture, mineral structure, and organic matter.

2.2.1 Soil Texture

Soil texture is one of the important physical properties of soil. Soil texture is concerned with the size of mineral particles, and it refers to relative proportions of particles of various sizes in a given soil. When the mineral particles predominate, the soil is gravely or sandy, and when the mineral colloids are dominant the soil has clayey characteristics. The amount of organic matter is comparatively small in quantity and is removed by oxidation before particle-size analysis. A particle-size analysis gives a general picture of the physical properties of soil and is the basis for assigning the textural class name.

Table 2.1 illustrates the size range for different textural classes in international system (Brady, 1984).

Table 2.1 Classification of soil particles according to size by International Society of Soil Science (mm)

< 0.002	0.002 ~ 0.02	0.02 ~ 0.2	0.2 ~ 2.0	>2.0
Clay	Silt	Fine Sand	Coarse Sand	Gravel

The importance of soil texture relates to the relationship between texture and:

1) surface area
2) ion exchange capacity
3) sorption sites
4) water holding capacity
5) mineralogy

Sand particles are not sticky, and have very low water-holding capacity. They have good drainage and aeration capacity, but they do not have the capacity to be molded.

In contrast, clay particles are sticky and they can be easily molded. They have high water holding capacity and high surface area which ranges from 10-1000 m^2/g for colloidal clay (Brady, 1984). This high surface area provides a high ability for adsorption, which is a surface phenomena.

The characteristic of silt is defined as being intermediate between sand and clay. The surface area of silt is about 1 m^2/g.

2.2.2 Mineralogy of Soil

The principle building elements of the clay minerals are the silica tetrahedron and the alumina and /or magnesia octahedron, which are linked by planes of oxygen and hydroxyl anions. In the silicon-oxygen unit the silicon atoms are coordinated with four oxygen atoms at the four corners of a regular tetrahedron with the silicon atom at the center. Three of these oxygens are shared by neighboring tetrahedrons making a silica sheet.

In the alumina / magnesia sheet the Al or Mg atoms are located at the center and coordinated with six oxygen atoms or hydroxyl groups at the six corners of a regular octahedron. The sharing of oxygen atoms by neighboring octahedrons results in a sheet that oxygen atoms and hydroxyl groups lie in two parallel plates.

The tetrahedral and octahedral sheets are associated in different structural arrangements. The specific combination of sheets is called a layer and the combination of layers would lead to a crystal. There are two major classifications of layers due to the number of silica sheets and alumina / magnesia sheets. 1:1 layer clay is referred to one silica sheet bonded to one alumina sheet, as in kaolinite. The layers are held together by hydrogen bonding. The 2:1 layer clays have two silica sheets and one alumina sheet located in between. Smectite, vermiculite and illite fall in this category.

Isomorphous substitution of Mg^{2+} or Fe^{2+} for Al^{3+} in an octahedral sheet, and Al^{3+} for Si^{4+} in a tetrahedral sheet provide a net negative charge at the surface of clay. These negative charges comprise the cation exchange capacity and are satisfied by adsorption of exchangeable cations such as H^+, Ca^{2+}, K^+, etc.

Exchangeable cations and associated water molecules are attracted to the internal surfaces as well as external surfaces of clays causing expansion of the crystal lattice. Movement of water and cations to the interlayer spaces of crystals exposes a very large internal surface that exceeds the external surface area. For example, the specific surface area for kaolinite, which is a non-expandable clay, is 15 m^2/g while montmorillonite, an expandable clay, has a specific surface area of 700-800 m^2/g (Brady, 1984).

Unsatisfied negative charges, associated with oxygen and hydroxyl groups exposed at the broken edges and flat external surfaces of minerals, are other sources of negative charge which involve non expandable clays, such as kaolinite. The charges originating from isomorphous substitution are permanent and independent of pH. In contrast charges arising from dissociation of hydroxyl groups on octahedral sheets are variable and strongly depend on pH.

2.2.3 Soil Organic Matter

Soil organic matter (SOM) is a complex mixture of humic materials which are the result of microbial, or chemical degradation, and undecomposed residues of plants and animals. The amount of organic matter in soils varies widely from a trace to 20-30% and even higher for organic soils.

The structure of SOM is an area of active research. In general, the chemical composition of soil organic matter strongly depends on the origin of the initial biological material, age, and development stages the soil has been exposed (Behar and Vandenbroucke, 1987).

Soil organic matter is classified in two major groups: **1-** nonhumic substances consist of unaltered residues of plants and animals. This group is represented by well known classes of compounds including carbohydrates, fats, waxes and proteins. Nonhumic substances contribute 5-20% of soil organic matter. **2-** Humic substances which are altered or transformed components of plants and animals. Transformation may be biological or chemical. Humic substances are relatively resistant to further degradation, and are divided into three main classes:

1- Fulvic acid
2- Humic acid
3- Humins

The three classes are similar in structure, but different in molecular weight and functional groups. Fulvic acid is soluble in acids and bases, has lower molecular weight, and contains more oxygen containing functional groups than humic acid. Fulvic acid and humic acid are strongly polar and aromaticity increases from fulvic acid to humic acid.

Humins are non-extractable humic materials. They contain high molecular weight aromatic compounds and are relatively nonpolar. The structure of humins strongly depends on the evolutionary stage. As explained by Behar and Vandenbroucke (1987), different types of kerogen tend to higher aromaticity, with decrease in molecular weight and gradual decrease in H/C and O/C ratios as the development progresses.

2.3 Fate of Organic Chemicals in Soil

The fate of organic compounds in different soil environments is affected by the characteristic of the compounds, the presence of adsorbents, and the chemical potential of the solvent system in which the organic pollutant is transported and distributed. In this section I review the recently proposed theories and models relevant to organic solute sorptive behavior, and, in some cases chemical transformation which may or may not be influenced by biological activities.

The heterogeneous characteristics of soil dictate that soil behaves as a dual sorbent in which the mineral surfaces function as conventional solid adsorbents and the organic phase as a partitioning medium. In dry soils, and soils with very low organic content, adsorption to mineral surfaces is dominant. In contrast, uptake of solutes from aqueous solutions is considered as partitioning into the soil organic matter.

Most studies investigating the fate of organic compounds in soil are devoted to partitioning phenomena, though this phenomena is limited to transfer of pollutants to subsoil, where soil is fully saturated with water, and it may not apply to topsoil and unsaturated soils. In soils contaminated with PAHs, contaminants permeate the soil in carrier solvents such as coal tar and creosote. In these conditions PAHs may be adsorbed directly to the soil, or may accumulate as the solvent evaporates or undergoes biological decomposition. The interaction of pollutants with soil in these conditions is dramatically different from partitioning.

2.3.1 Adsorption

Adsorption is defined as condensation of gases on solid surfaces and internal micropores, or the fixation of solutes from a solution on the surface of a solid. The process of adsorption on solid surfaces can be divided into three general steps:

1- External diffusion
2- Internal diffusion
3- Adsorption on the surface

Adsorption is a consequence of the forces at the surface of solid which attract the molecules of solutes. The attraction forces may be physical to cause physical adsorption (van der Waals forces) or chemical causing chemisorption. The van der Waals interactions are weak and decrease rapidly with the distance between interacting particles.

Hydrogen bonding and ion exchange are other indications of adsorption. Hydrogen bonding may happen between protons of adsorbed water and oxygens of clay surfaces. Ledoux and White (1966) observed that the hydrogen bonding between water molecules and oxygen of the kaolinite surface was weaker than intermolecular hydrogen bonding of water. Organic compounds with carbonyl or carboxylic groups are adsorbed to the clay surfaces as a result of hydrogen bonding between the double-bonded oxygen of carbonyl group and hydrogen atoms of the clay lattice. Phenols, acids, alchohols, and compounds which contain π-electron, such as aromatics, and unsaturated hydrocarbons are capable of interacting by hydrogen bonding mechanism (Solomon and Hawthorne, 1983).

Adsorption on mineral surfaces may occur by uptake of organic gases (Chiou et al., 1985), or uptake of solutes from non-polar solvents (Hance, 1965; Mills and Biggar, 1969). However significant sorption of anthracene, carbazole and dibenzothiophene on kaolinite and hematite in aqueous solutions has been reported by Murphy et al. (1990).

The adsorption of solutes on mineral surfaces is affected by competition of solvents for occupation of free sites. Solvents such as water, methanol etc. with high polarity can compete successfully for the adsorbing sites on mineral surfaces. In this case, sorption of solute most likely happens in the soil organic matter. In the absence of polar solvents, solutes are adsorbed on active sites of minerals.

The results published by Hance (1965), Mills and Biggar (1969), Yaron and Saltzman (1972) and Chiou et al. (1985), confirm the fact that sorption by dry and partially hydrated soils from nonpolar organic solvents is determined by soil minerals.

2.3.1.1 Models for Adsorption Isotherms

The amount of uptake of solute by a soil mineral phase is a function of specific surface and charge density. When a solid is exposed to a gas (or solution) in a closed system, at constant temperature, the solid begins to adsorb the gas (or solute from solution), and gradually the pressure of the gas (concentration of solute) will drop until equilibrium is reached. The amount of uptake can be calculated from the drop in pressure of the gas (drop in concentration of solution) or by increase in weight of the solid.

Adsorption isotherms are proposed to explain the ability of different solids to adsorb various types of organic compounds. The most common applied isotherm is Langmuir isotherm. This isotherm is based on monolayer adsorption theory and is proposed for adsorption of gases on solids. The Langmuir isotherm is based on three basic assumptions:

1- All the adsorption sites are equivalent.
2- Interactions between the molecules bonded to these sites are negligible.
3- Only one adsorbing molecule can be bonded to each site on the solid.

The application of Langmuir isotherm is restricted because of heterogeneities in the adsorbing sites and the fact that interactions between adsorbed molecules cannot be neglected in many systems (Gates 1992), so it should be considered strictly empirical when applied to heterogeneous systems. The Langmuir isotherm equation is expressed as follows:

$$\frac{q}{q_m} = \frac{bP}{1+bP} \qquad (2.1)$$

where

q = amount of uptake, [g of solute/g of soil]

q_m= maximum uptake in the first layer, [g of solute/g of soil]

P = partial pressure, atm

b = Langmuir intensity coefficient (related to enthalpy of adsorption), atm^{-1}

In the Langmuir model, the sorbed phase concentration asymptotically approaches the maximum limit. At low concentration the isotherm simulates linear adsorption, which tends to higher non-linearity at high concentrations. Adsorption of nitroaromatic compounds on kaolinite (Haderlein and Schwarzenbach 1993) and parathion and lindane on woodburn soil (Chiou et al 1985) follow the Langmuir isotherm .

The Freundlich isotherm as an empirical model has become well established in the adsorption literature. Many investigators have used this model to interpret the experimental results in batch systems. Sorption of diuron from water and hexane (Hance 1965), is an example of Freundlich isotherm. Freundlich isotherm is expressed by following equation:

$$q = K_f C_e^{1/n} \tag{2.2}$$

where

q = amount of uptake, [g of solute/g of soil]

C_e = equilibrium concentration, [g of solute/cm^3]

K_f = Freundlich capacity coefficient, [(g of solute/g of soil)/(g of solute/cm^3)$^{1/n}$]

$1/n$ = Freundlich exponent

$n > 1$

The Freundlich model is the type of isotherm expected for low coverage on a nonuniform surface and it has been used in analyzing data from partitioning experiments as well as adsorption on minerals.

The BET (Brunauer, Emmett, Teller) isotherm is based on the same assumptions made by Langmuir, but assuming that multiple layers of adsorbate will accumulate on the surface of adsorbent, i.e. each adsorbed species in the first layer will be an adsorbing site for the next layer. In this model, enthalpy of adsorption for the second and higher layers is equal to the enthalpy of condensation. This model is represented by the following equation:

$$\frac{q}{q_m} = \frac{cP}{(P_0 - P)(1 + \frac{(c-1)P}{P_0})} \qquad (2.3)$$

where

q = amount of uptake, [g of solute/g of soil]
q_m= maximum uptake in the first layer, [g of solute/g of soil]
P = partial pressure, atm
P_0= saturation pressure, atm
c = temperature dependent constant (related to enthalpy of adsorption)

The BET model is widely used to represent the experimental results. Chiou and Shoup (1985) have successfully applied this model for adsorption of water, benzene and chlorobenzene on Woodburn soil.

2.3.1.2 Parameters Affecting Adsorption

2.3.1.2.1 Soil Clay Content

The major adsorbent components in soils are clay particles. Clays have a high surface area (2 orders of magnitude larger than silt, and 3 orders of magnitude larger than sand) to provide a large capacity of adsorbing sites for organic compounds to be adsorbed. This idea is supported by Call (1957), who studied the adsorption of ethylene dibromide on different types of soils and clays. He observed a wide range of sorption in different dry samples varying from 10 mg/g for Field Station Soil (4.15% OM, 7.4% clay) to 55 mg/g for Boston Silt (4.58% OM, 46.2% clay).

Sawhney and Gent (1990), investigating the adsorption of 1,2-dibromoethene and trichloroethene on different types of clays, noticed that adsorption of chemicals per gram of soil increased with increasing BET surface area (with the exception of smectite). However adsorption per unit surface area does not follow the same trend, implying that not only the surface area is important, but the structure of sorbent surface plays a major role in adsorption.

In general, it is accepted that the sites where cation exchange takes place determine the adsorption of organic compounds on mineral surfaces. Theng (1974) suggests that polar organic compounds interact with exchangeable cations rather than silicate surfaces and uncharged organic compounds can interact with cations directly or indirectly by means of water bridging.

The cations are held to the surface by different energies as the sites of negative charge are located at different distances from the surface. The polarization power of cations is significantly different and causes different bonding strength in the order Na < K < Rb < Cs (Cremers and Landelout, 1966).

Haderlein and Schwarzenbach (1993), in their studies looking for adsorption of nitroaromatic compounds (NACs) to mineral surfaces and particularly to various homoionic kaolinites saturated with monovalent cations, found that adsorption of NACs particularly takes place on the negatively charged surfaces of silica sheet, and the strength of adsorption depends on the structure of the compound. The results suggest that the sites at which cation exchange takes place have an important role on adsorption of NACs. The results also suggest that the nature of the cation adsorbed on the surface of silica sheet had a dramatic effect on adsorption. Strongly hydrated cations (Li^+, Na^+, Mg^{2+}, Ca^{2+}, Al^{3+}), had no significant effect on adsorption. In contrast, with weakly hydrated cations such as NH_4^+, K^+, Rb^+, Cs^+, the adsorption increased with decreasing free energy of hydration of cations.

2.3.1.2.2 Humidity

The effect of humidity on adsorption of organic compounds on mineral surfaces has been investigated by several research groups. Call's work (1957), on adsorption of ethylene dibromide on moist soils is one of the earliest publications in this area. He observed that increasing relative humidity reduces sorption of ethylene dibromide, and a type II BET isotherm for 0% relative humidity shifts to type III for over 20% relative humidities. The same type of change in isotherm behavior is reported by Thibaud et al. (1993), for adsorption of chlorobenzene and toluene on a standard EPA soil (0.8% OM, 56.4 sand, 14.7% silt, 28.9% clay).

Chiou et al. (1985) reported that adsorption of benzene and its chlorinated derivatives on Woodburn soil (1.9% OM, 21% clay) is suppressed by the presence of water. Their results also suggest higher adsorption for polar compounds than relatively nonpolar compounds. The effect of water is not restricted to adsorption from the gas phase. Yaron and Saltzman (1972) reached similar conclusions on the uptake of parathion and lindane from hexane solutions.

The results published by Call (1957), Chiou et al. (1985), Yaron and Saltzman (1972) and Thibaud et al (1993) all confirm that adsorption of solutes on mineral surfaces is affected by the polarity of solute, and water with the high polarity is a powerful competitor for adsorbing sites. In saturated soils, all the available sites are occupied by water molecules, but in dry soils or partially hydrated soils removing some of the water creates vacant sites for adsorption of organic compounds.

Walker's (1961) finding about the existence of different layers of water around exchangeable cations probably would be the best analysis for the effect of water on adsorption. Walker, using X-ray diffraction, reported two distinct layers of water in vermiculite crystals. Type I water corresponds to water molecules directly coordinated to exchangeable cations, while type II water forms the outer layer of hydration water and is indirectly linked to exchangeable cations. The latter is not strongly held and it is more labile, consequently polar molecules can displace type II water.

Mortland (1970) proposed that water associated with metal cations forms a bridge which allows hydrogen bonding to polar organic compounds. Adsorption of ketones, amides, benzoic acid and nitrobenzene on montmorillonite have been reported to be as a result of water bridging. Humic acid and fulvic acid are adsorbed on clay surfaces by hydrogen bonding to a water molecule in the primary hydration shell of the adsorbed cation on the exchange complex (Theng, 1976).

2.3.1.2.3 Time

Persistence of some chemicals, such as 1,2-dibromoethene, in soil long after their application cast doubt on the assumption that adsorption is a rapid and reversible process, and raises the question whether adsorption/desorption of organic contaminants in soil is rate limiting and to what extend.

The conventional sorption experiments are usually carried out in limited times. Adsorption from gas phase is reported to reach equilibrium in a few hours (Chiou et al., 1985), and in short term techniques the equilibrium time for sorption from solutions is 24-48 hours (Enfield et al., 1989; Chiou et al., 1988). However there is growing evidence that sorption/desorption may not reach equilibrium in the time scale the experiments are done (Sawhney and Gent, 1990; Sawhney and Brown, 1989), and they may require a much longer time to reach equilibrium.

Ball and Robert (1991) have reported that adsorption of tetrachloroethene and tetrachlorobenzene on a sandy aquifer material required a contact time of 20 and 159 days respectively to equilibrate. The organic carbon partition coefficient in this experiment is one order of magnitude higher than the published correlation would predict.

Desorption of 1,2-dibromoethene and trichloroethene on different clay types is suggested to have two different rates (Sawhney and Gent, 1990). The fast desorption which occurs in the first few hours of desorption process, and the slow rate, which may take longer time (100 hours), due to entrapment of solutes in interaggregate micropores. Based on these observations they have proposed a two desorption compartment model with two independent rates.

Pignatello and Huang (1991) and Pignatello et al. (1993) have indicated the effect of aging as the main reason for slow desorption in old contaminated soils compared to the freshly contaminated soils. In a column study, comparing the desorption of atrazine and metolachlor from one of the contaminated field samples (the sample was collected 7 months after the last herbicide application), with a freshly contaminated soil, they noticed that in the aged soil half of the contaminant leached in 3.5 months with most coming out in the first 3 weeks. Comparing to the common adsorption/desorption studies, after the first 24 hours, 87% of atrazine and 92% of metolachlor were still remaining in the soil. Their results in batch studies, confirm the idea that slow rate adsorption may take a few months to achieve equilibrium, and the fact that apparent partition coefficient increases with time.

2.3.2 Partitioning

Partitioning is defined as uptake of solutes from a solution by soil organic matter. Permeation of solutes through the network of organic compound is the characteristic of partitioning which distinguishes it from adsorption. Partitioning into soil organic matter is assumed to be the primary interaction of hydrophobic organic compounds with soil in aqueous systems (Chiou et al., 1983; Garbarini and Lion, 1986; Karickhoff et al., 1979; Murphy et al., 1990). Sorption through partitioning into soil organic matter is considered to be homogeneous throughout the entire volume of organic phase.

Chiou et al. (1990) suggested that soil organic matter has a low BET surface area of about 1 m^2/g (using N^2 as adsorbate), which is consistent with the hypothesis that sorption of organic solutes in SOM is not surface adsorption, but partitioning.

Chiou et al. (1981, 1983, 1988, 1989) and Schwarzenbach and Westall (1981) have investigated the experimental data in aqueous systems and presented the partitioning hypothesis for nonionic organic compounds. In their predictive model, migration of nonionic organic compounds from solute and its accumulation in soil organic matter is described by the distribution coefficient, K_{om}:

$$K_{om} = \frac{\text{Conc. of solute in soil organic phase}}{\text{Conc. of solute in aqueous phase}} \qquad (2.4)$$

The distribution coefficient to the soil organic phase can be expressed in terms of the distribution coefficient for the organic carbon:

$$K_{oc} = \frac{K_{om}}{f_{oc/om}} \qquad (2.5)$$

where

$f_{oc/om}$ = mass fraction of organic carbon in soil organic matter
K_{oc} = organic carbon partition coefficient

The nature of soil organic matter and its characteristic in various soils is different according to its source, age and development conditions to which it has been exposed (Behar and Vandenbroucke, 1987; McKeague et al., 1986). Despite this variation, investigators working in this area have found good correlation between K_{oc} and K_{ow} (octanol water partition coefficient) which is one of the thermodynamic properties of the solutes.

$$\mathrm{Log}(K_{oc}) = A\mathrm{Log}(K_{ow}) + B \qquad (2.6)$$

where

K_{ow} = octanol water partition coefficient
A, B = regression coefficients

For nonionic organic compounds which have relatively low solubility in water, octanol water partition coefficient is related to solubility in water by following equation:

$$\mathrm{Log}(K_{ow}) = -C\mathrm{Log}(S_w) + D \qquad (2.7)$$

where

S_w = solubility in water
C, D = regression coefficients

A complete review of partition modeling and predicted values of K_{OW}, A, B, C and D is provided by Chiou et al. (1982, 1989), Karickhoff et al. (1979, 1981), Schwarzenbach and Westall (1981).

The solubility of solutes in soil organic matter can be evaluated, by the following equation:

$$S_{om} = K_{om}S_w \qquad (2.8)$$

where

S_{om} = solubility in organic matter

Chiou et al. (1983) suggested that the K_{om} values are approximately one order of magnitude lower than K_{ow}. They mentioned that "if one finds S_{om} to be greater than, or nearly the same as, the solute solubility in a good solvent (such as octanol), the validity of the assumed partitioning with soil organic matter should then seriously be questioned." Assuming partitioning, lower solubility of nonionic organic compounds in water results in higher K_{om} values, which alters the solubility in soil organic matter.

However uptake of organic compounds from aqueous solutions can not always be attributed to soil organic matter. For soils with very low organic matter such as aquifer solids, adsorption to mineral surfaces can be dominant (Haderlein and Schwarzbach, 1993; Ball and Roberts, 1991). Haderlein and Schwarzbach (1993) have reported significant adsorption of nitroaromatic compounds on homoionic kaolinite from aqueous solutions. Murphy et al. (1990) reported sorption of anthracene and dibenzothiophene on kaolinite and hematite from aqueous solutions.

Rutherford et al. (1992) studied the effect of soil organic matter composition on the partition of benzene and carbon tetrachloride. They found that polarity of organic matter has a strong effect on partitioning. Qualizza (1993) has reported the same observations correlating the polarity of soil organic matter to the age of soil. Adsorption of nonionic organic compounds such as anthracene, naphthalene and naphthol was greatly increased in aged soils having low polarity. In contrast, young soils with high polarity showed very low tendency to partition nonpolar organic compounds.

2.3.3 Reactions

2.3.3.1 Biological Reactions

Organic materials in soil can undergo biological reactions as well as chemical reactions. Soil, as a source of nutrients, provides a favorable environment for large populations of microorganisms with a variety of species. Indigenous organisms in contaminated soils are capable of degrading organic compounds to some extent, and upon isolating and incubating on a specific carbon source their capability to degrade the specific organics will increase.

Organic compounds may be transferred in soil by microbial degradation, either as a source of energy or as cometabolism. Degradation of organic compounds in soil is catalyzed by enzymes for oxidation, reduction, hydrolysis and dehalogenation. Degradation pathways are often different for each class of organic compounds and microbial species.

Biological reactions are the major concern in bioremediation of soils, contaminated with different types of hydrocarbons. In this regard, aromatic hydrocarbons are of particular interest because of their low solubility in water that influences the bioavailability of hydrocarbons dramatically.

2.3.3.2 Chemical Reactions

The application of clays for removing color has been well established, but perhaps the increasing use of pesticides and herbicides, the fast growth of chemical and petrochemical products, and commercial use of minerals as catalysts for synthetic organic and polymerization reactions has persuaded the scientists to look for possible reactions of organics with clay minerals. Mortland et al. (1971) and Pinnavaia and Mortland (1971) have shown that transition metals ion-exchanged with clays can make complexes with aromatic hydrocarbon on layer silicates. Two distinct types of copper II-arene complexes were identified when benzene bound to dehydrated montmorillonite. Type I, a green colored complex, was formed by hydrated mineral and Cu^{2+} ions coordinated with the edge of benzene ring, retaining the planar structure and aromaticity. Type II, a redish brown complex, was formed by dehydration of mineral in the presence of benzene by electron transfer between the benzene and Cu^{2+}, forming benzene cations. As proposed by Rooney and Pink (1962), type II complexes can be formed by aromatic ring components such as biphenyl, anthracene, naphthalene and thiophene.

Solomon et al. (1965, 1967, 1968) have proposed that the mechanism of polymerization of styrene by sodium montmorillonite follows radical and ionic reactions, which can be activated by the presence of cobaltous ions, giving rise to electron transfer initiated polymerization. Polymerization of benzene and phenol (Mortland and Halloran, 1976; Fenn and Mortland, 1972) and dimerization of anisole (Fenn et al. 1973) have been shown to follow electron transfer mechanisms. Transition metals definitely play a major role in these redox reactions. They can act as electron donor or acceptor sites depending on their oxidation state. Dragun and Helling (1985), in a review of soil-catalyzed free radical oxidation, mention that iron, aluminum and trace metals within layered silicates and adsorbed oxygen are identified as factors promoting free radical reactions.

McKnight et al. (1992), investigating the removal of dissolved organic carbon in two streams, found that 40% of DOC was removed from solution by sorption onto aluminum and iron oxides. Molecules with higher aromaticity, carboxylic acid groups, N- and S- containing groups, and amino acid residues were preferentially sorbed by oxides. The ligand exchange-surface complexation mechanism has been proposed as a conceptual model for sorption of organic solutes by metal oxides.

Haderlein and Schwarzenbach (1993) proposed that electron donor-acceptor complexes between surface oxygens of the siloxane surface and nitroaromatic compounds are responsible for adsorption of NACs. The effect of exchangeable cations, as proposed by these authors, is that strongly hydrated cations such as Li^+, Na^+, Ca^{2+} may block the electron donor sites and prevent electron donor-acceptor complexes. However cations which have weak hydration energy such as NH_4^+, K^+, Rb^+, Cs^+ yield better adsorption and complexation.

Although all the reported results explain reactions of specific organics with particular mineral surfaces, we can conclude that there are variety of active groups on mineral surfaces that have intrinsic potential to initiate and catalyze reactions. Bronsted acid, Lewis acid, and transition metals are identified as these active sites.

2.4 Discussion:

The interactions of organic compounds in soil can be classified as:

-Adsorption
-Partitioning
-Catalytic reactions

The first two classes are investigated in parallel, considering the differences in mechanisms and possible mutual influences, but not very well correlated to the third class which specifically focuses on reactions.

The prior research done on the criteria of sorption failed to include possible reactions. Adsorption/desorption experiments done with radiolabelled chemicals do not specify that decrease in activity of solution (in case of sorption), and increase in activity (in case of desorption), corresponds to the original solute or other possible transformed materials. As an example, Ball and Robert (1991) have reported strong sorption of tetrachlorobenzene on sandy aquifer materials, having organic content of 0.013% - 0.099%. The organic carbon partition coefficient in this long term study is reported to be one order of magnitude larger than published sources. Although the explanation for this phenomena, as proposed by the authors, is physical adsorption to the mineral surfaces even in aqueous systems, in the case of any strong unexpected adsorption or irreversible sorption we need to consider whether a chemical reaction is happening.

CHAPTER 3

Materials and Methods

3.1 Materials

3.1.1 Sampling

Three different samples of soils were chosen for these series of experiments. Soil A was taken from the C horizon of a site south of Edmonton. The C horizon was sampled at a depth of 75+ cm. It has a dark grayish brown color, sandy clay loam texture, moderately calcareous with a very low content of organic matter (0.62%). The soil contained 50.6% sand, 25.5% silt and 23.9% clay. The surface area was 348 m^2/g (the surface area for montmorillonite is 810 m2/g and for kaolinite 20-40 m2/g by ethylene glycol), and the pH in distilled water was 8.3. The clay composition was dominated by smectite and kaolinite.

Malmo soil with a relatively high organic content was chosen to reflect the characteristics of a high organic content soil. The A horizon of a Black Chernozem was sampled from a field at The University of Alberta Ellerslie Research Station south of Edmonton. The soil was acidic at the time of sampling and the pH in distilled water was 5.4. The Malmo soil consisted of 16% sand, 62% silt and 22% clay. The organic carbon content was 4.43% and the clay surface area was 454 m^2/g (ethylene glycol).

Shale was sampled from a site on the North Saskatchewan River near Devon. This soil contained 9% sand, 48% silt, 43% clay with organic matter content of 1.6%. Montmorillonite was the dominant phyllosilicate in the shale, comprising 80% of the clay.

Montmorillonite # 23 from Arizona (Ward's Natural Science Establishment, Inc.) was used as a reference pure clay to compare the mineralogy effects on interactions. An ideal montmorillonite crystal consists of an expandable layered structure that contains octahedrally coordinated Al^{3+} and tetrahedrally coordinated Si^{4+} in a 2:1 stoichiometric ratio.

All soils and montmorillonite were air-dried, ground and sieved with 1.7 mm sieve.

Tables 3.1 and 3.2 demonstrate the physical and chemical characteristics and mineralogy of the three types of soils used in these series of experiments.

Table 3.1 Physical and chemical characteristics of soil and clay samples

	% Sand	% silt	% Clay	pH*	% Organic Matter	CEC me/100 g
Soil A[a]	51	25	24	7.8	0.62	12.1
Malmo[b]	16	62	22	7.3	4.4	35.1
Shale[b]	9.0	48	43	8.3	1.6	
montmorillonite[c]			100			120

[a] From Abder-Ruhman , 1980

[b] From Qualizza, 1994

[c] Data handbook by Olphen and Fripiat, 1979

* pH in 0.01 N $CaCl_2$

Table 3.2 Mineralogy of soils

	Montmorillonite Vermiculite %	Kaolinite %	Mica %	Quartz %	Muscovite %
Soil A	33 + 9	26	24	7	
Malmo	60	20			20
Shale	80	10			10

3.1.2 Chemicals

- Anthracene and Phenanthrene were purchased from Sigma Chemical Co. (St. Louis, USA) with purities of 98% and 96% respectively.

- Pyrene was a sample from Eastman Kodak.

- Methylene chloride was purchased from Fisher Scientific (New Jersey, USA) in HPLC grade.

- [9 - ^{14}C]Anthracene from Amersham Corporation (Oakville, Ontario) was used for the labeled experiments. The purity was 98% and specific activity was 15.1 mCi/mmol. Radioactive concentration was reported as 500 µCi/mL.

- Buffer solution consisted of 1.33 g/L KH_2PO_4, 2.67 g/L K_2HPO_4, 1 g/L NH_4Cl, 2 g/L Na_2SO_4, 2 g/L KNO_3, 0.05 g/L $FeSO_4.7H2O$, and 1 mL of trace metal per one liter of solution. After autoclaving at 120°C for 20 minutes, $MgSO_4.7H2O$ was added to a concentration of 0.2 g/L (Fedorak and Grbic-Galic, 1991).

3.1.3 Anthracene Utilizing Culture

The microbial population was a mixed culture enriched from a creosote contaminated soil (Gray et al., 1994). The mixed culture was incubated using anthracene as the sole carbon source. Seven grams. of soil and 0.1 g of anthracene were added to 200 mL of buffer solution and shaken at 27°C. The culture was maintained by monthly transfers of 10% by volume , and growth was monitored by plating and counting. After the population was stabilized the culture was harvested by centrifugation. The precipitate was suspended in 20% glycerol to a concentration of 10^8 CFU/mL and kept frozen in 1 mL vials for further use.

3.2 Experimental Methods

3.2.1 Preparation of Anthracene Impregnated Soil

To remove water, soil was oven dried at 140°C for a period of 48 hours, then placed in a desiccator for two hours to reach room temperature before loading of the anthracene. These conditions allowed the soil to release surface water and part of interlayer water. The interlayer water was not directly coordinated to the cations and most cations started to release a large portion of interlayer water at 140 °C, but they still retained small amounts of structural water which were tightly associated with soil minerals.

To prepare soil containing 600 ppm (mg anthracene/kg soil), a measured weight of anthracene was dissolved in methylene chloride at a concentration of 3 mg/L and mixed with soil at a ratio of 200 mL solution per kg soil (Gray et al., 1994). This ratio provided a very good mixing condition and uniform distribution of anthracene in soil. The anthracene-loaded soil was kept for the experiments on the effect of time. The flasks containing loaded soil were covered by aluminum foil to exclude light to prevent photo-oxidation of anthracene. After the required time had elapsed, the methylene chloride was evaporated using a rotary-evaporator with water bath at 60°C and the dried soil was analyzed for anthracene.

For the experiments done with radiolabeled anthracene, the same procedure was followed except that 1 mL of ^{14}C-anthracene solution with total activity of 2376×10^3 dpm/µL was added to cold anthracene before loading the soil.

3.2.2 Preparation of Soil with Different Water Content

Oven dried soil was used in these experiments. The soil was heated at 140° C for 48 hours to remove moisture. These conditions are considered as a base for 0% water content. For the experiments with variable water content, after drying the soil the required amount of water was added to the soil. To get a uniform distribution of water, the resulting humid soil was thoroughly shaken and left for 24 hours before impregnation with anthracene.

3.2.3 Thin Layer Chromatography

Soil extract was analyzed to separate anthracene from other compounds that had produced in the process of loading the soil. Silica gel plates (Kodak chromatogram sheet / 13181) were used in this experiment. The plates were 20 × 20 cm. in dimension and 100 µm thick coating of silica gel covered the inert polyethylene terephthalate. The samples were applied to the plates at the starting point, 2.5 cm from the lower edge and the side of the plate. The spots (0.2-0.3 cm) were placed 2 cm apart. Separation was carried out at room temperature in a 27×27×9 cm developing chamber. The plates were placed in glass chamber filled with solvent to a depth of 0.5 cm. The height of solvent on the plate at the time of exposure was carefully designed not to cover the spots. The chamber was covered by a glass plate and plastic wrapping to allow the solvent to saturate the space inside the chamber. The length of run was normally between 10-15 cm from the starting point. After the separation was completed, the plates were taken out and air dried at room temperature for a few minutes.

3.2.3.1 Continuous and Multiple Development

Continuous and multiple development were employed to separate different components of the soil extract. In multiple development the same solvent was used several times, and between each development the plate was taken out and the solvent evaporated completely. A solution of benzene (85 mL), acetone (15 mL), and acetic acid (5 mL) was used as the solvent system.

3.2.3.2 Stepwise Development

Stepwise development was achieved by using two different solvents. In the first stage a solution of benzene (85 mL), acetone (15 mL) and acetic acid (5 mL) was used as solvent system. After completion of the first step, the plate was taken out and dried. The second stage was carried out with the less polar solvent, hexane (70 mL), diethyl ether (30 mL), acetic acid (2 mL).

3.2.3.3 Two Dimensional Separation

In this method the sample was applied to the plate in one corner. After developing with the first solvent system (benzene, 85 mL, acetone, 15 mL, acetic acid, 5 mL), the plate was air dried, turned through 90°, and development was carried out in the second dimension with the second solvent system (hexane, 70 mL, ether, 30 mL, acetic acid, 2 mL).

3.2.3.4 Visualization of Chromatogram

To evaluate the chromatogram, all the separated substances should be identified. For colorless substances, the following methods are available:

- UV lamps
- Sprayers for atomizing or spraying suitable reagents

The first method was successfully employed in these series of experiments. Long wave UV light (around 365 nm), was applied to the treated silica gel plates and different colored spots were identified.

3.2.3.5 Autoradiography of Chromatogram

Radioactive substances were detected on an ordinary clinical X-ray film. The TLC plates were pressed against the X-ray films and kept in plate holder. The holder was wrapped in black cloth and kept in freezer at -60°C. The contact time varied between 1-7 days, depending on the activity of the compounds. After the required time had elapsed, the film was developed.

3.2.4 Bioreactor Operation

An aluminum rotating drum, 31 cm long and 24 cm in internal diameter with a volume of 14 L was used for the experiment. The drum was equipped with 8 lifters 2.4 cm high. Upon rotation the lifters picked up the slurry and showered it at the top, providing a high level of mixing. The rotation speed was 3 rpm. Oxygen was supplied to the reactor by passing air at a flow rate of 50 mL/min. The inlet air was saturated with water. More details of bioreactor and its operating conditions is explained by Banerjee et al. (1994).

Soil was loaded into the reactor in the ratio of 60% soil and 40% mineral salts medium, after Banerjee et al. (1994). The volume of slurry occupied 18% of the total volume of the reactor.

3.3 Analytical Methods

3.3.1 Analysis of Anthracene

Anthracene was recovered from the soil by Soxhlet extraction for four hours (in some experiments the extraction was done over a period of 16 hours), using methylene chloride as solvent (EPA method # 3540). The solvent was evaporated and the residue was redissolved in methylene chloride to a known volume in a volumetric flask. The extracted anthracene was analyzed by gas chromatography (EPA method # 8000), using phenanthrene as the internal calibration. A Hewlett Packard 5890 gas chromatograph, equipped with a DB-1 capillary column and flame ionization detector, was used to measure the concentration of anthracene.

3.3.2 Biooxidation Analysis

Biological oxidizer OX 300 was employed to measure radiolabeled anthracene present in soil. 0.1 g of soil together with 0.025 g cellulose were combusted at 900°C for 1.5 minutes. The CO_2 evolved was trapped in vials containing scintillation solution for further measurement of activities.

3.3.3 Scintillation Counter

A known volume of the soil extract was added to the scintillation solution which consisted of xylene and methanol, purchased from Amersham Canada Ltd. A Beckman LS 3801 liquid scintillation counter was employed to measure the activity. The scintillation counter displayed the number of decays per minute directly.

3.3.3 Analysis of Carbon Dioxide

CO_2 evolution from mineralization of anthracene was measured by a Dycor Quadrupole mass spectrometer connected to the bioreactor. The analyzer was calibrated with 5% CO_2, 2% argon, 10% oxygen, and the balance nitrogen. The response factor for this known concentration was used to calculate the concentration in the effluent gas stream based on argon in the effluent as internal standard. An IBM computer connected to gas analyzer displayed the time average of effluent composition every 5 minutes.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Effect of Time on Recovery of PAHs in Dry Soils

4.1.1 Recovery of Anthracene

The soil was loaded to achieve a concentration of 600 ppm (mg anthracene/ kg soil) and kept in a flask in the presence of solvent (25 ml of methylene chloride/100 g of soil). After the desired time had elapsed anthracene was extracted and the concentration was measured as described in chapter 3. For this experiment different batches of soils were prepared.

4.1.1.1 Soil A

The data in Fig 4.1 shows that soil A continues to interact with anthracene for several days. These results clearly reveal the fact that with increasing loading time the losses in anthracene increases and the concentration of extractable anthracene decreases dramatically. The rate of interaction was initially rapid, but decreased gradually with time until no anthracene was detected after 7-8 days. In the first 30 minutes 50% of anthracene was lost and the losses gradually increased to the concentration of 597 ppm after 7 days.

The reproducibility of the results was examined by repeating the experiment using one batch of loaded soil. The results are presented in Fig 4.1. Sampling from one batch gave more consistency in the results, suggesting that the results may be sensitive to minor variations in preparing the soil.

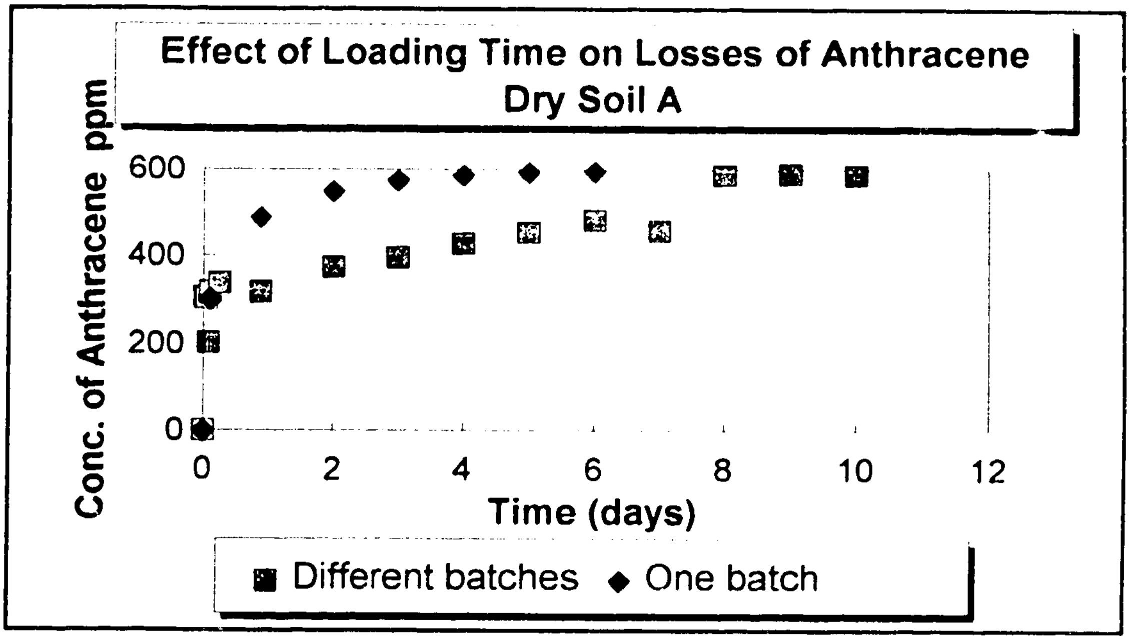

Fig 4.1 Losses of anthracene as a function of time in soil A. (Soil was initially loaded 600 ppm).

4.1.1.2 Malmo Soil

Another experiment was conducted with Malmo soil which has a high organic carbon content (4.43%), to investigate if the phenomena observed in the first experiment with soil A was specific for that soil or if it can be generalized to different types of soils. Fig 4.2 demonstrates the results of this experiment. The Malmo soil was loaded with 600 ppm of anthracene as described previously. All samples were from one batch of loaded soil. Recovery of anthracene was determined after time intervals ranging from 3 hours to 9 days. The results clearly suggested that the same phenomena was taking place, but the rate of disappearance of anthracene was slower compared to the experiment conducted with soil A. In this sample 140 ppm of anthracene was lost in the first 3 hours and a gradual increase in losses continued to a concentration of 390 ppm after 9 days.

4.1.1.3 Shale

Similar experiment was set up using a shale, with a very high clay content (43%). The results are presented in Fig 4.3. After 3 hours 250 ppm of anthracene was lost and the concentration of recoverable anthracene dropped from 600 ppm to 350 ppm and then gradual increase in losses continued to 535 ppm after 8 days. The rate of disappearance of anthracene for shale was intermediate between Soil A and Malmo.

4.1.1.4 Montmorillonite

To examine the contribution of pure clay to the phenomenon observed with different soils, montmorillonite from Arizona was selected as a reference clay. The characteristic of this clay was described in chapter 3. Montmorillonite was ground and sieved with 1.7 mm sieve, then loaded with 600 ppm anthracene. After 4 days of loading, the solvent was evaporated and montmorillonite was extracted and analyzed for anthracene. As illustrated in Fig 4.4, montmorillonite exhibited similar behavior to soil A. The concentration of extractable anthracene dropped to 15 ppm after 4 days.

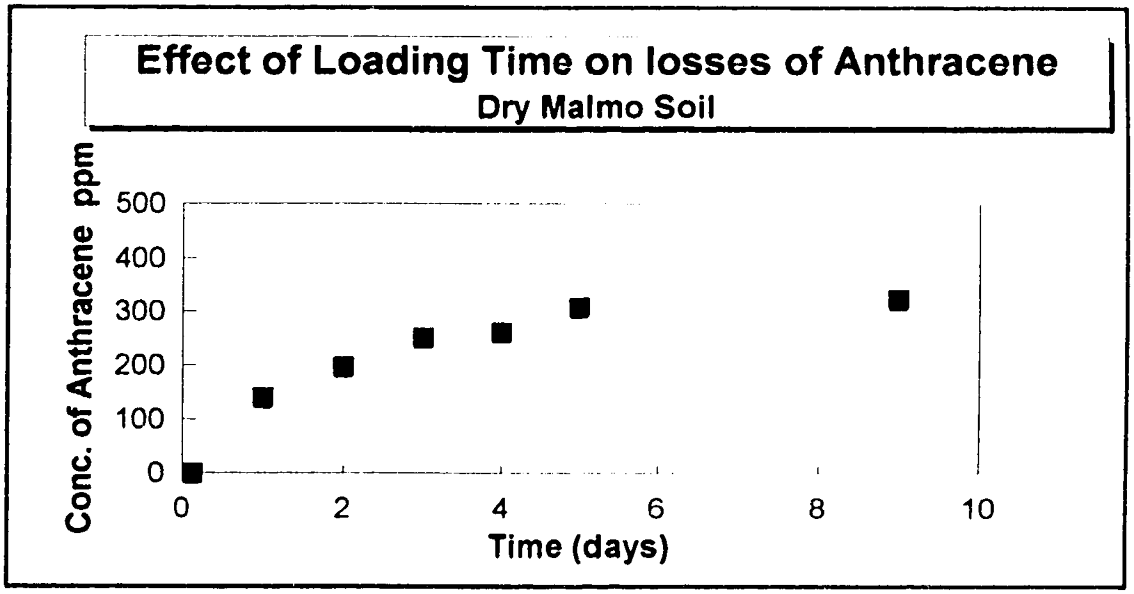

Fig 4.2 Losses of anthracene as a function of time in Malmo soil. (Soil was initially loaded 600 ppm).

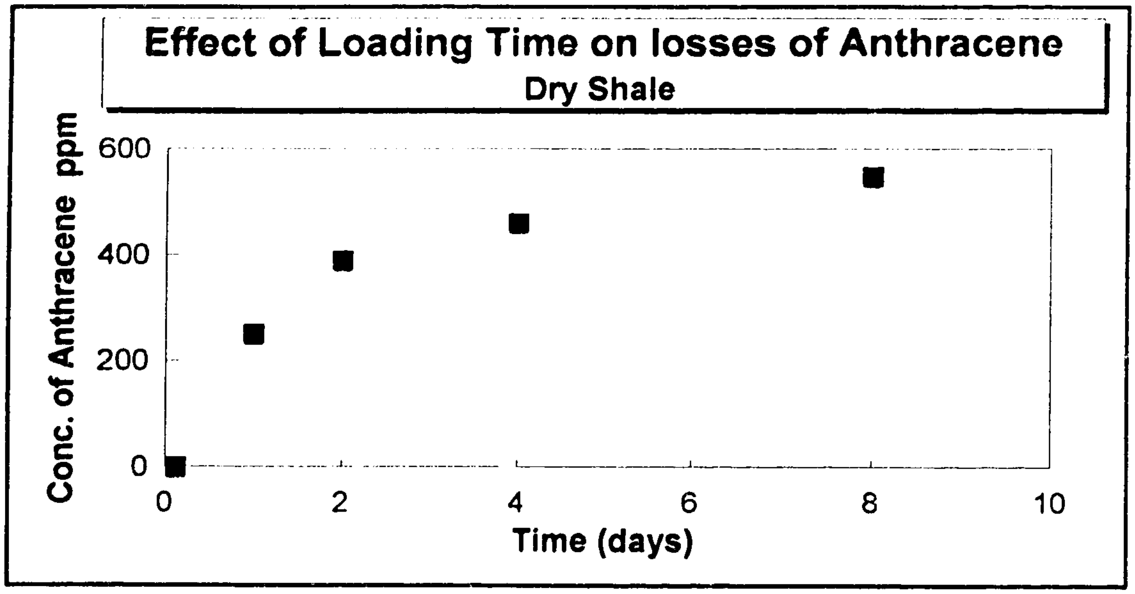

Fig 4.3 Losses of anthracene as a function of time in Shale. (Soil was initially loaded 600 ppm).

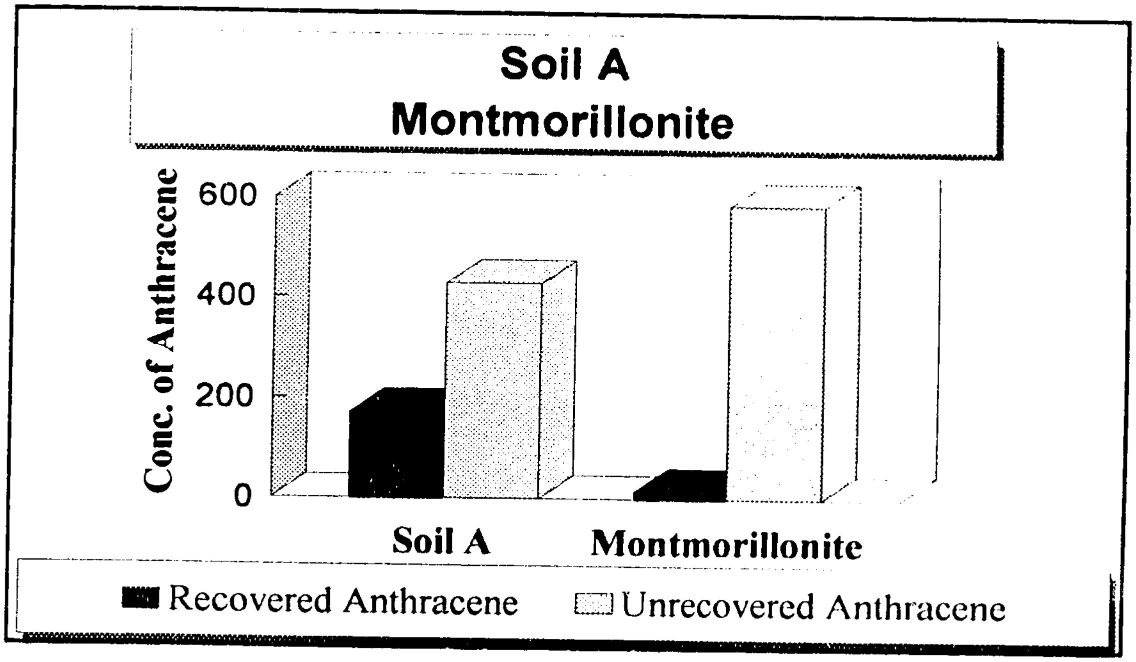

Fig 4.4 Recovery of anthracene after 4 days in Montmorillonite and soil A.

4.1.2 Effect of Particle Size on Recovery of Anthracene

A sample of soil A was loaded with 600 ppm of anthracene. After 10 days the soil was dried and sieved into 5 different fractions and each fraction was analyzed for anthracene. The results are illustrated in Fig 4.5. The results imply that there is no significant difference in recovery of anthracene in different aggregate fractions.

4.1.3 Recovery of Phenanthrene and Pyrene

To investigate and generalize the phenomena observed in the interaction between anthracene and soil, phenanthrene and pyrene were selected for further experiments. The loading for phenanthrene and pyrene followed exactly the same method as described in chapter 3. The experiments were carried out with soil A.

Fig 4.6 demonstrates the results of recovery of phenanthrene after 1, 4 and 9 days of loading. Phenanthrene was more recoverable than anthracene, but 6-10% of phenanthrene was recovered as other compounds, including quinones and other detectable compounds by GC. The amount of unrecovered phenanthrene was 20-25% of input.

In the experiment carried out with pyrene, even after one day pyrene was not detectable in the soil extract by GC. This experiment was repeated 3 times and all the results were consistent.

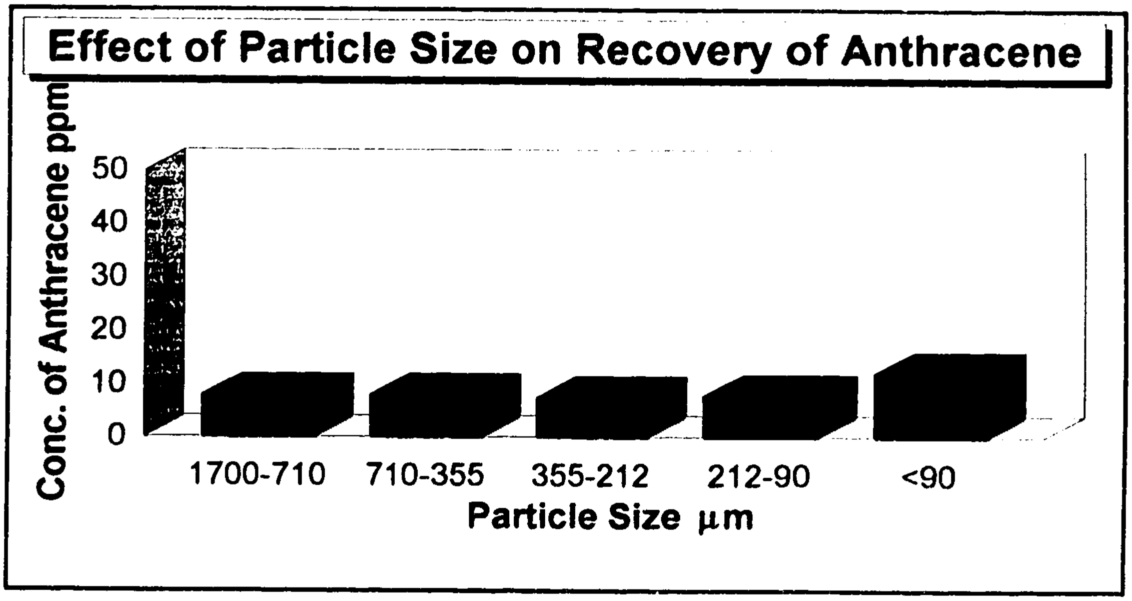

Fig 4.5 Recovery of anthracene as a function of particle size in soil A.

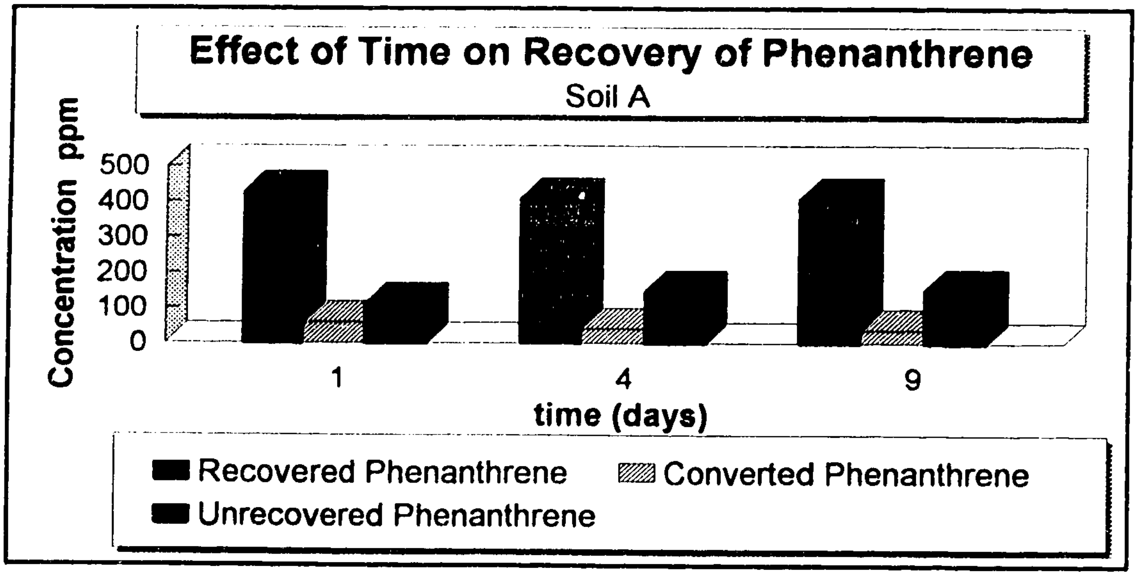

Fig 4.6 Recovery of phenanthrene as a function of time in soil A.

4.1.4 Discussion

Fig 4.7 illustrates the recovery of anthracene in the three oven dried soil samples comparatively as a function of time. Malmo showed higher recovery than the two other samples. In contrast, soil A showed the least recovery of anthracene. Although in the literature, any deficit of solute concentration after contact with soil has been attributed to adsorption (Yaron and Saltzman, 1972; Chiou et al., 1985) or partitioning (Chiou, 1989; Lee et al. 1992), in this text, we will refer to losses as interaction. The results of this experiment are in agreement with what Ball and Roberts (1991) and Sawhney and Gent (1990) have reported as nonequilibrium sorption, emphasizing that interactions between soil and organic compounds are not instantaneous and time is a decisive factor to be considered.

The uniqueness of these series of experiments was that methylene chloride was utilized in both processes, loading the soil by anthracene and recovering anthracene from soil by soxhlet extraction method. Because of the high solubility of anthracene in methylene chloride, this solvent was assumed to be a proper choice and has been used in the whole series of experiments. The possible contribution of methylene chloride in interaction between soil and anthracene is not clear.

The low recovery of anthracene cannot be attributed to biological activities. Although the soil was not sterilized, heating at 140°C for 48 hours was sufficient to inhibit the majority of microbial population in soil and decrease their activity. The soil was in contact with methylene chloride during the experiments, which did not provide a favorable environment for microbial growth. A more careful analysis of data shown in Fig 4.1 suggests that the sharp drop in concentration of recoverable anthracene in 3 hours can not be the result of biological activity.

Comparing the soil organic matter, Malmo had the highest organic content (4.43%) vs. soil A which has the lowest organic matter (0.62%). Comparing the soil organic matter in three different samples with the data in Fig 4.7, there was no quantitative relation between soil organic matter and the rate of interaction of anthracene. The soil organic matter did not, therefore, contribute positively in the interaction between anthracene and soil in dry conditions.

A comparison between table 3.1 and the data presented in Fig 4.7 indicates that there was no systematic relation between the clay content and the rate of interaction of anthracene with the three soil samples. The same conclusion was achieved in considering the type of clay which constituted each soil. The data in table 3.1 and 3.2 show that there was no consistency between the type of clay, the amount of clay and the rate of interaction. The fact that montmorillonite, as a pure clay, demonstrated the same behavior was convincing proof that the mineral fraction contributes in the interaction between anthracene and soil. The specific sites on mineral surfaces, such as Bronsted acid and Lewis acid sites, as well as metals in exchangeable position or in oxide forms may interact with organic compounds. The results imply that interaction between anthracene and Malmo was significantly lower than the two other soils. Considering the organic content of Malmo (4.43%), one may infer that part of the active mineral surfaces were already occupied by soil organic matter so that anthracene did not have access to all sites.

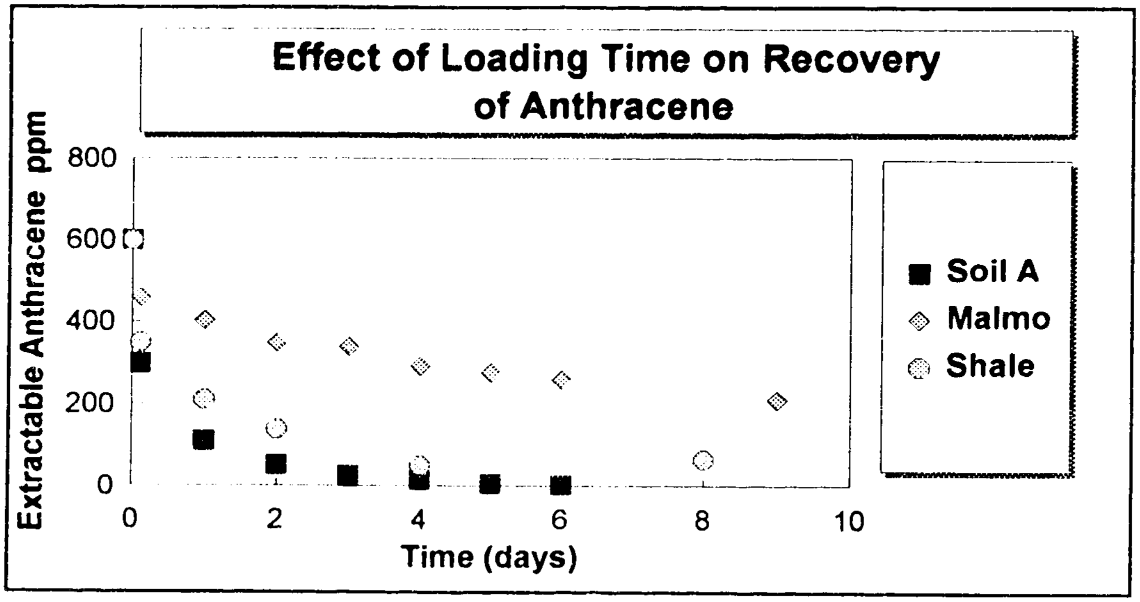

Fig 4.7 Recovery of anthracene as a function of time in soil A, Malmo and shale.

The strong interaction of pyrene with soil, and the weaker interaction of phenanthrene with soil, suggest the generality of the phenomenon in polynuclear aromatic hydrocarbons. However, more studies are required to characterize the parameters affecting these interactions and the contribution of the structure of aromatics that may affect the intensity of interaction.

4.2 Effect of Moisture on Recovery of Anthracene

4.2.1 Distribution of water in soil

For the experiments carried out in different water contents, it was very important to distribute water homogeneously and to allow enough time for water to distribute evenly in the whole volume of the soil.

To evaluate the effect of time on rehydration, 5 samples with 2% water content (2 ml of water / 100 g. dry soil) were prepared, varying the loading time for water from one day to 21 days and fixing the loading time for anthracene on 4 days. The extractable anthracene was in the range of 513±21 ppm. The results suggested that one day of contact time with water was sufficient to give a homogeneous distribution of water in soil.

4.2.2 Recovery of Anthracene From Soil A

To investigate the effect of humidity on the phenomena observed in dry soils, a series of experiments were set up in soils with water contents of 0.05% up to 20% (g of water/100 g. of soil). Oven dried soil (140°C) was selected as the base for 0% water content and the required amount of water was added to get soils with the desired moisture content. After equilibrating for 24 hours, 600 ppm anthracene was loaded to the soil as described in chapter 3, and after 4 days the recoverable anthracene was measured. By changing the water content, we were able to vary the chemical potential of water and study its effect on sorption of organic compounds under different moisture contents.

Fig 4.8 illustrates the recovery of anthracene in different water contents for soil A. Anthracene recovery showed a rapid increase with increasing water content, indicating the sensitivity of soil interaction with anthracene when the water content was less than 1%. The results suggested that water competes successfully with anthracene for mineral surfaces, and in hydrated soils there were not enough sites available for anthracene. This result was in agreement with data presented by Chiou and Shoup (1985) on benzene and chlorobenzene and Thibaud et al. (1993) on toluene.

Fig 4.9 illustrates the recovery of anthracene as a function of time in soil A with different water contents. Obviously, in dry samples the recovery of anthracene was dramatically lower than in moist samples. In dry samples, the recovery of anthracene dropped sharply in the first 3 hours from 600 ppm to 300 ppm and then gradually decreased to 3 ppm after 6 days. The soil with 0.1% water content exhibited the same trend with a lower rate. As the water content was increased, the curves for recovery of anthracene as a function of time shifted up, implying that the rate of interaction was very low for higher water contents.

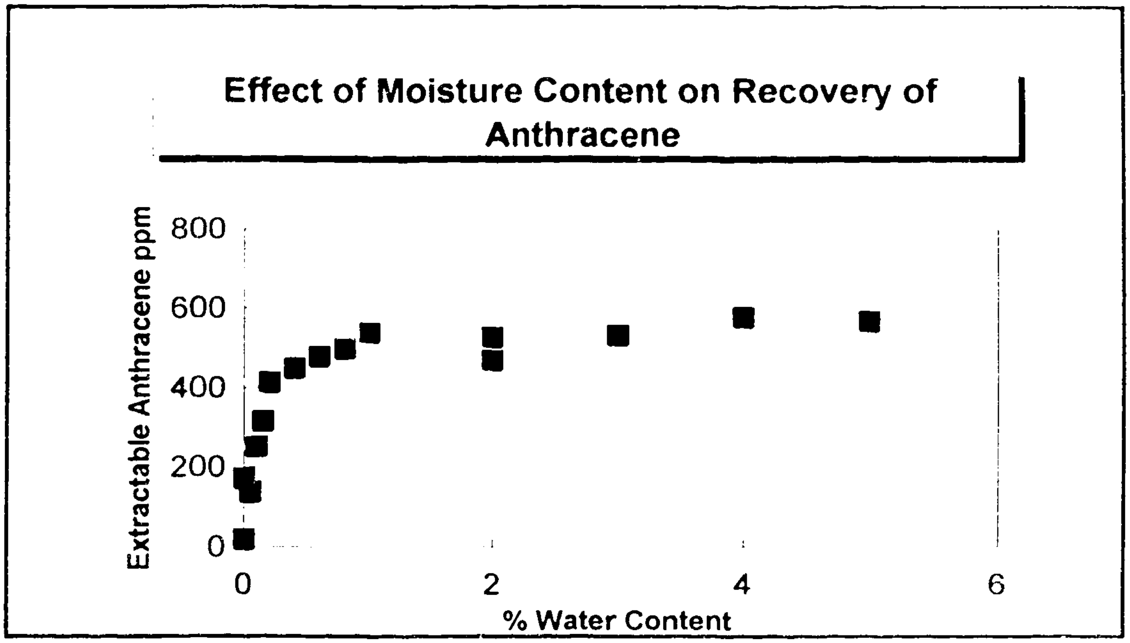

Fig 4.8 Recovery of anthracene as a function of water content in soil A.

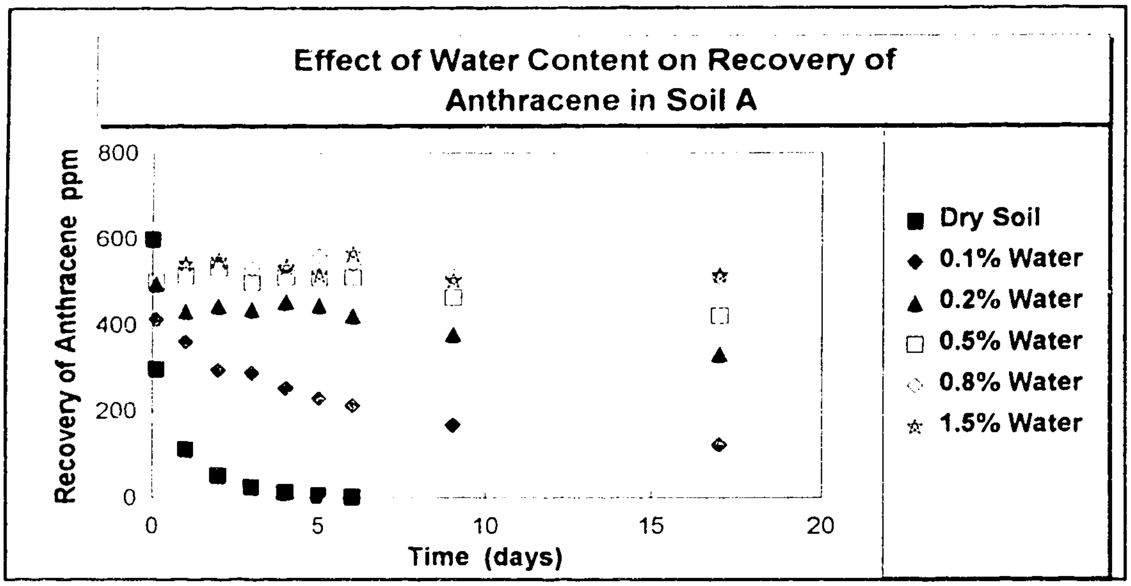

Fig 4.9 Recovery of anthracene as a function of time and water content in soil A.

4.2.3 Recovery of Anthracene from Malmo

Similar experiments with different water contents were carried out with Malmo soil, which had a high organic content (4.43%). As in the previous experiment a range of water contents (0%, 0.1%, 0.2%, 0.5%, 0.8% and 1.5%) were selected to study the effect of moisture. The results were similar to the results of soil A. In this sample the rate of interaction for dry soil was the highest showing a sharp drop in the first 3 hours and further decrease in recoverable anthracene up to 211 ppm after 9 days. The results are demonstrated in Fig 4.10. As the water content increased, the curves shifted up demonstrating higher recovery of anthracene.

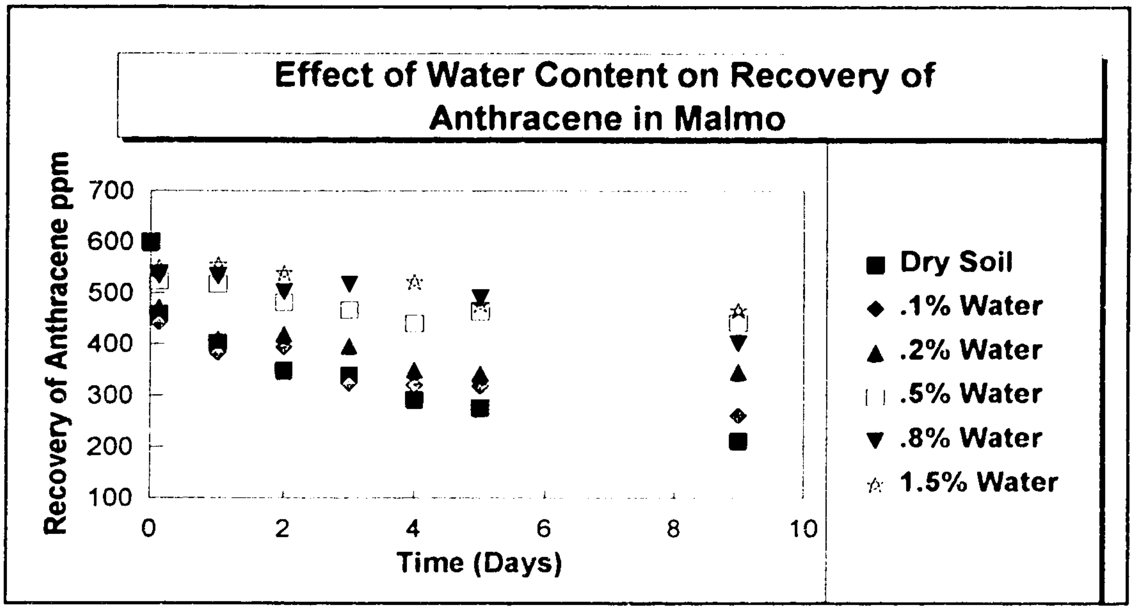

Fig 4.10 Recovery of anthracene as a function of time and water content in Malmo soil.

4.2.4 Discussion

The results in Fig 4.9 and 4.10 revealed the significance of water content in recovery of anthracene. At low water content, the recovery was very low implying strong interaction between soil and anthracene. As the water content increased the recovery of anthracene increased, indicating the reduction in the exposed mineral surfaces in hydrated soils. Comparing Fig 4.9 and 4.10, however both soils demonstrated similar behavior. A more careful analysis of data revealed the fact that Malmo soil exhibits lower recovery at high water content compared to soil A. To get a better image of the behavior of two soils in different water contents, assuming a first order kinetic for interaction of anthracene with soil, Fig 4.11 illustrates the apparent rate constants as a function of water content for soil A and Malmo. The strong inhibitory effect of water is clearly indicated by a sharp decrease in the rate of interaction. The interesting point to be mentioned is that, a comparison of the rate constants at different moisture contents, indicated that in dry conditions soil A had a higher rate constant than Malmo. However, by increasing water content, Malmo showed a higher rate constant than soil A.

In a 5-component system consisting of soil organic matter, soil minerals, water, solvent and organic compound, the major interactions of organics with other constituents of the system would be:

1- Direct interaction with mineral surfaces as in dry soils
2- Partitioning into soil organic matter
3- Dissolution into adsorbed water, which may cause consequent partitioning or interacting with mineral surfaces.

In this system, direct partitioning into soil organic matter was not significant because the solvent (methylene chloride) is classified as a nonpolar solvent (Weast et al., 1984).

Direct interaction with mineral surfaces may be classified as: 1) Adsorption on mineral sufaces as Chiou and Shoup (1985) have reported. 2) Chemical interaction with active mineral surfaces. Strong interactions in dry soils and low water content soils (0.1% and 0.2%) can be attributed to direct interaction of anthracene with exchangeable cations in mineral surfaces. Exchangeable cations adsorb and retain water in two different shells. The water in the inner shell is directly coordinated to the cation, but the water in outer shell is indirectly linked to the cation and has higher mobility. The number of complete layers depends on the nature of exchangeable cations. Davis and Warrall (1971) suggested that the physical characteristic of third and fourth layer of water is the same as liquid water. Heating soil at 140°C removes the water in the outer shell, exposing the inner shell and the open active sites to anthracene. As the water content increases, more layers of water surround the inner shell increasing the thickness of the water layer, which in turn reduces the accessibility to the active mineral surfaces. However, Anderson et al. (1967) suggested that adsorbed water can dissolve organic compounds and permit their diffusion, but at a lower rate than in bulk water.

The data in Fig 4.8 confirm the results suggested by Goss (1992). He suggested that at moisture contents below one layer of adsorbed water, the sorption coefficient is very high and reduces rapidly as the monolayer of water establishes. Above monolayer, sorption occurs on the adsorbed water film decreasing exponentially with increasing moisture content.

The behavior of the hydrated and partially hydrated soils may be related to the acidic characteristic of mineral surfaces. The surface acidity would develop by removal of water adsorbed on the surface (Theng, 1974; Solomon and Hawthorne, 1983). Adsorbed water is the most important source of acidity on clay minerals. The strength of the acidity depends on the polarizing power of the cations in the clay as well as the amount of water. In dehydrated clays, the polarizing power of the cations acts on a few coordinated water molecules, causing strong dissociation which leads to stronger acidity. Increasing the amount of water may mask the acidity of mineral surface, since the polarization power is distributed among large number of molecules of water and dissipated.

Although the nature of active surfaces is not well established, the results of the experiments suggest that the active surfaces are highly influenced by water and their activity is suppressed by moisture content, consistent with the results of Chiou and Shoup (1985) for adsorption of benzene and chlorobenzenes on mineral surfaces. In the present study, however, recovery of anthracene was so poor that simple adsorption cannot account for the observations.

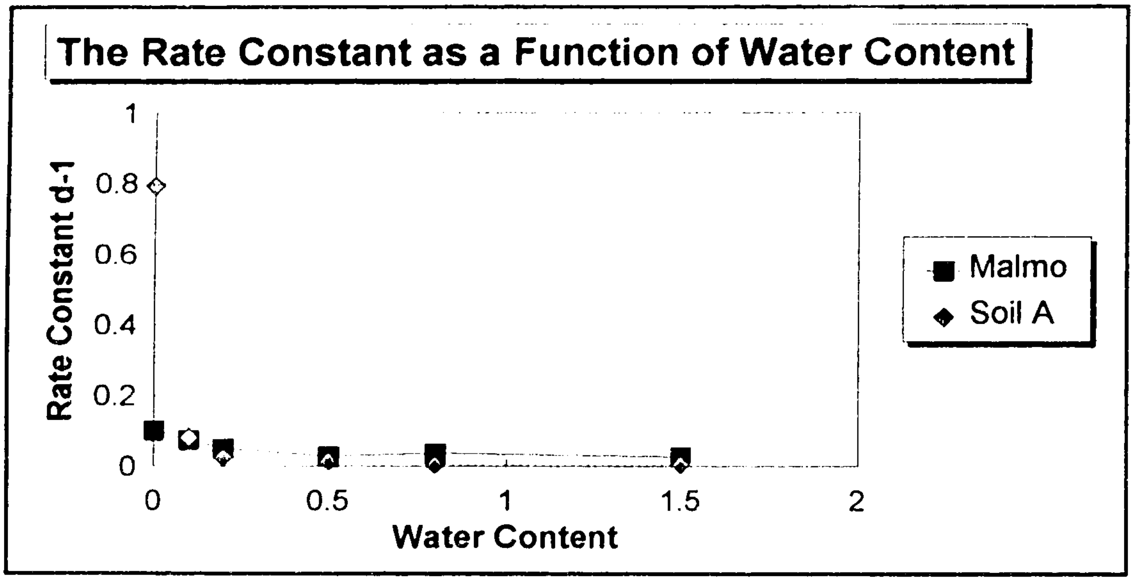

Fig 4.11 The rate constant as a function of water content for soil A and Malmo.

4.3 Transformation of PAHs in Soil

4.3.1 Characterization and Identification of Transformed Anthracene

The results of the first series of experiments showed that anthracene had interacted with soil and was not extractable with methylene chloride. The question that arose was whether the unextractable anthracene was bound to the soil, or in the soil extract but not detectable with GC. If it was in the soil, had it maintained its characteristics or was it transformed to other compounds?

To answer these questions, an experiment was set up using ^{14}C labeled anthracene. The solution of anthracene in methylene chloride was prepared and ^{14}C anthracene with the total activity of 47520 dpm/g of soil was added to the solution. The loading process was exactly the same as the experiments with cold anthracene. After 12 days methylene chloride was evaporated and soil was extracted to identify anthracene and other possible compounds which might be produced. To get more conclusive results several methods were employed in parallel to identify and characterize the compounds that possibly were present in the soil extract.

4.3.1.1 Results of ^{14}C-Anthracene Experiments

Soil (15.7 g.) was loaded with ^{14}C-anthracene and extracted for a period of 4 hours with methylene chloride. The volume of soil extract was decreased to 10 ml by evaporating the excess methylene chloride. A known volume of soil extract was added to scintillation solution and the activity was calculated. In this soil extract 5923 dpm/g of activity was extracted which consisted 12.4% of the total initial activity.

The sample was further extracted 3 consecutive times, each time for a period of 4 hours. The amount of activity extracted each time were 9696, 2520 and 940 dpm/g respectively.

To get a mass balance on ^{14}C-anthracene, the activity in soil before extraction and the residue after 4 extractions were both measured by the bio-oxidation method.

Table 4.1 Mass balance on ^{14}C-anthracene based on 1.5 min. combustion

Sample	dpm/g	dpm/g	dpm/g	%
Load	40505			100
1st. extract		5923		
2nd. extract		9696		
3rd. extract		2520		
4th. extract		940		
Total extract		19079		47
Residue			21480	53

As illustrated in table 4.1 the total activity extracted in 4 consecutive extractions was 47%, and the residue contained 53% of the total initial activity.

The initial activity measured by bio-oxidation was slightly different from the input activity (40505 dpm/g vs. 47520 dpm/g), suggesting that 1.5 min. of combustion was only enough to release 85% of the total activity of the soil. However, as the soil and the residue were both measured the same way the error will not affect the mass balance significantly. Another bio-oxidation test was combusted for 3 min. and 46030 dpm/g activity in soil was detected which was closer to the input value. Similar programming showed the activity of the residue to be 24070 dpm/g which was 52% of the initial activity based on analysis using the 3 min. combustion time.

To investigate the effect of aging of anthracene, the soil was again extracted after two months of loading (methylene chloride was evaporated after 12 days). 8.5 g. of soil was extracted 3 times and the activity was measured. The results are listed in table 4.2. In comparison with the first sets of extractions, this time only 31% of total initial activity (based on analysis with 1.5 min. combustion) was extractable.

Table 4.2 Mass balance on ^{14}C-anthracene after two months

Sample	dpm/g	dpm/g	dpm/g	%
Load	40505			100
1st. extract		11750		
2nd.extract		777		
3rd. extract		394		
Total extract		12921		31.9

4.3.1.2 Results of UV Absorption

An experiment was set up to analyze the soil extracts by UV spectrophotometry to find out if the activities in soil extracts were due to anthracene, or whether chemical transformation had taken place.

Anthracene has absorption at 256 nm (corresponding to E1 band) and 375 nm (corresponding to E2 band) (Silverstein et al., 1981). The characteristic peaks of anthracene appears in the range of 300-380 nm, as illustrated in Fig 4.12a.

Fig 4.12b illustrates the absorption spectrum for the first extract. The characteristic peaks of anthracene between 300-380 nm are clearly observed in this sample (the sample was diluted to get the absorption spectrum in the absorption range). Figs 4.12c and 4.12d indicate the absorption spectrum for the 2nd and 3rd extracts. The lack of anthracene spectrum in the 2nd and 3rd extracts of soil reveals the fact that transformation of anthracene has taken place. However, absorption of 255 nm shows that the new compound has maintained its aromaticity.

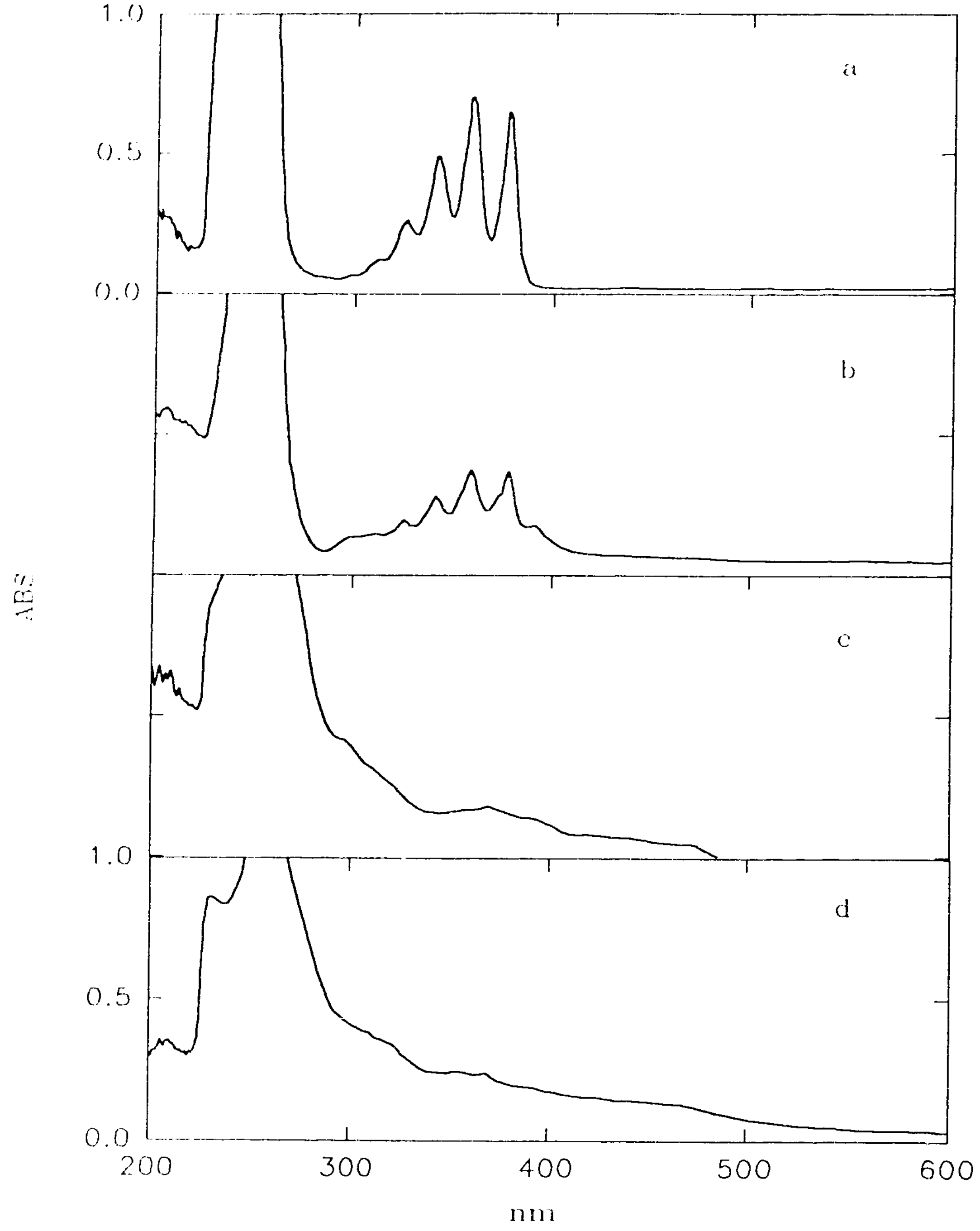

Fig 4.12 The UV spectrum of a) anthracene b) first extract c) second extract d) third extract

4.3.1.3 Results of Thin Layer Chromatography

Thin layer chromatography was employed to characterize the compounds present in the soil extract. Two different solvent systems were used, as described in chapter 3. Among the different methods of thin layer chromatography, stepwise development and two-dimensional separation using both solvents gave the best separation. To confirm that the compounds extracted in 2nd, 3rd and 4th extract were not anthracene, ^{14}C-anthracene was added to the plate at the time of applying the soil extract.

Continuous development using each of the solvents separately was not successful in separating ^{14}C-anthracene from soil extract. Stepwise development using solvent 1 in the first stage and solvent 2 (the composition of solvents is described in chapter 3) in the second stage yielded considerable separation and two clear spots appeared in autoradiograms. The spots both migrated with the front solvent in solvent 1, but in solvent 2 anthracene was slightly ahead, leaving behind the compounds from soil extract. As explained by Stahl (1969), for polynuclear aromatic hydrocarbons a linear relation exists between the Rf values and the size of molecule, expressed as the log of molecular weight or as the number of carbon atoms in the molecule. The conclusion from this experiment was that nonpolar compounds with higher molecular weight than anthracene were produced in the soil. In two dimensional separation with two solvent systems, at least 5 different components were distinguished under long-wave UV light.

4.3.1.4 Results From GC-MS

In the typical experiments to analyze anthracene by GC, the oven temperature was 180°C, the injector 320°C and the detector was 350°C. At these conditions, no peaks corresponding to high-molecular weight compounds were detected. Increasing the oven temperature to 300°C and concentrating the sample gave a peak which was identified by GC-MS analysis, as bi-anthracene (C28H18). Fig 4.13 indicates the chromatogram showing the peak due to bi-anthracene that appeared after 27 minutes. However there might be compounds with higher molecular weight and higher degree of polymerization that could not be detected by GC-MS.

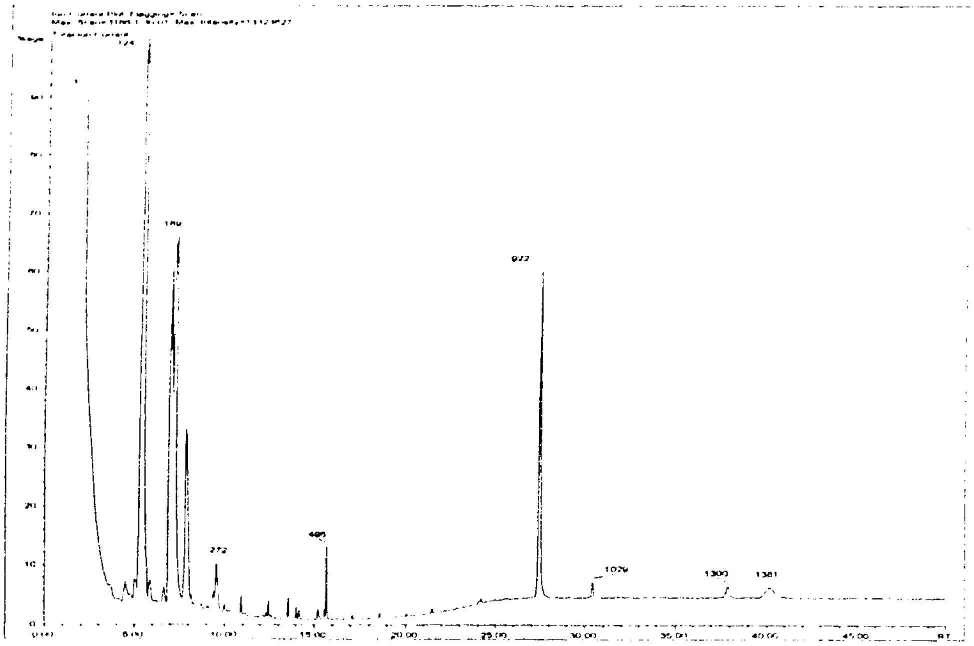

Fig 4.13 Chromatogram from GC-MS indicating peak of bi-anthracene at 27 min.

4.3.2 Transformation of Phenanthrene and Pyrene

In the limited experiments done on phenanthrene a high percentage of phenanthrene was recoverable. Part of phenanthrene was converted to other compounds which were detectable by GC, and partly not detectable. Fig 4.14 shows the chromatogram of soil extract of phenanthrene.

In the experiments done on pyrene a very strong interaction was observed. After one day of loading, pyrene was not detectable by GC. Inspecting the UV spectrum of soil extract the same behavior as anthracene was observed. Fig 4.15 demonstrates the characteristic peaks for pyrene and the spectrum of extracted soil. As in the case of anthracene, the UV spectrum suggests oligomerization to form an aromatic product of higher molecular weight.

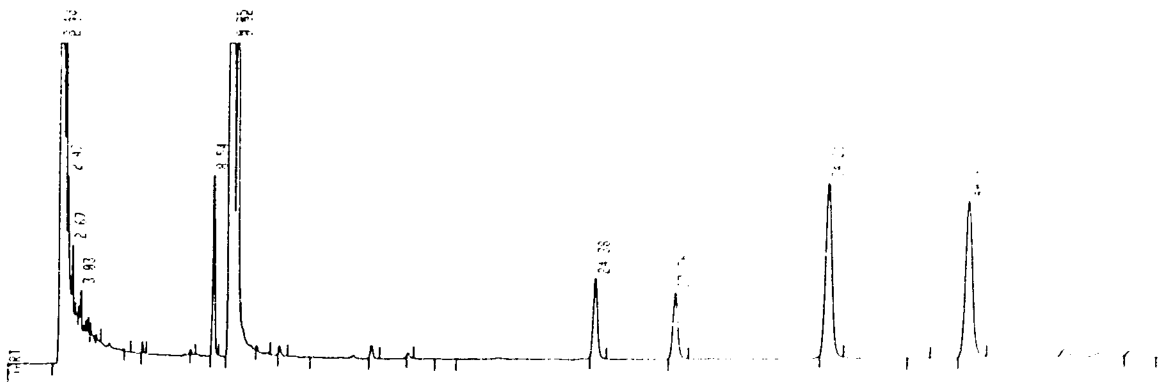

Fig 4.14 Chromatogram from GC indicating degradation of phenanthrene in soil.

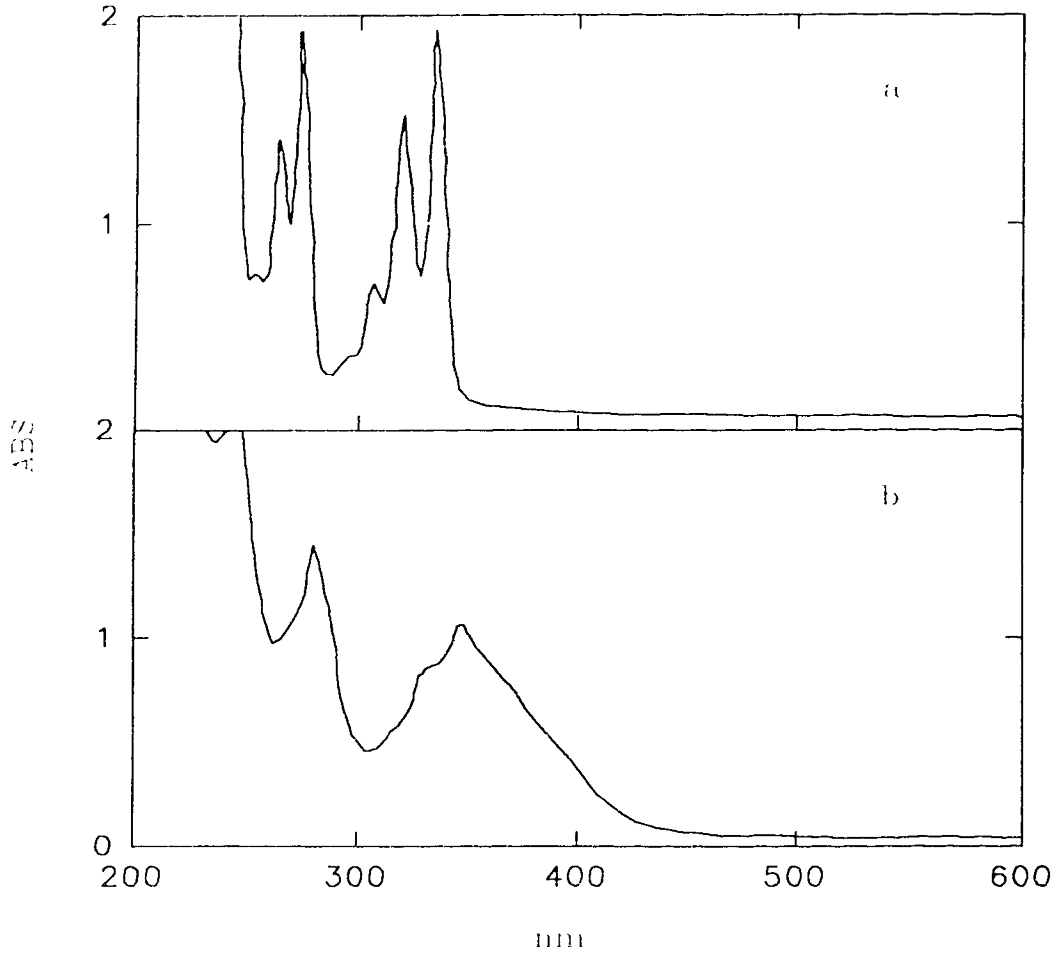

Fig 4.15 The UV spectrum of a) pyrene b) soil extract

4.3.3 Mechanism of Reaction

The results of the ^{14}C-anthracene experiment, thin layer chromatography, ultraviolet absorption and GC-MS were consistent in showing that molecules with higher molecular weight than anthracene were produced. Bi-anthracene was identified as one of these products.

The nature of oxidizing sites on mineral surfaces is not well established. The potential electron acceptors may include Lewis acid sites, transition metals in exchangeable positions or within the crystal lattice and adsorbed oxygen (Solomon and Hawthorne, 1983). Transition metals, such as manganese in amorphous form, are also considered very active (Dixon and Weed, 1989) and may contribute in the reactions . The oxidizing sites on mineral surfaces such as transition metals can undergo redox reactions with aromatic hydrocarbons. The electron transfer may be from electron-rich centers of π-electrons of aromatics to electron acceptor sites (transition metals) in mineral surfaces. As long as the electron transfer is not complete, the oxidation process may result in charge transfer complexes in which the transferred electron is shared between organic species and the mineral sites. A complete electron transfer will result in radical cations. The radical cations may undergo chain reactions to produce high molecular weight compounds such as polymers.

The following mechanism was proposed by Mortland and Halloran (1976) for oligomerization of benzene on smectite containing Fe(III) or Cu(II):

$$Ar + M^{n+} \longrightarrow Ar^{+}. + M^{(n-1)+}. \qquad (4.1)$$

$$Ar^{+}. \rightarrow \text{Chain Reaction} \rightarrow \text{Chain Polymerization} \qquad (4.2)$$

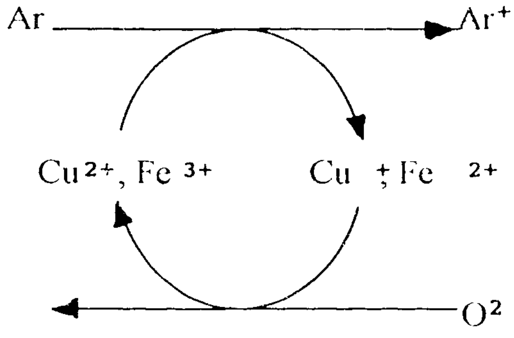

Fig 4.16 Reaction mechanism for aromatics on mineral surfaces.

The proposed mechanism in Fig 4.16 is in agreement with observations of Rooney and Pink (1962) that anthracene forms free radical cations in benzene, carbon disulfide and carbon tetrachloride solutions. The active sites for radical production were described as Lewis acid sites on silica-alumina catalysts. Mortland and Halloran (1976) have confirmed the production of high molecular weight compounds following adsorption of benzene and phenol on smectite containing Fe (III) and Cu (II) on exchange complex. They have suggested that radical cations of benzene and phenol were formed by electron transfer to transition metals. Since soils contain clays and metals such as Fe(III), this mechanism likely accounts for the interaction of anthracene with dry soils.

4.3.4 Bioavailibility of Transformed Anthracene

Most petroleum constituents which are considered as contaminants in soil are biodegradable, but the rate of degradation is different for different compounds. Polynuclear aromatic hydrocarbons (PAHs) are reported by Weissenfels et al. (1992) to be biodegradable. The main factor which limits the availability of PAHs to microorganisms is their low solubility in water that controls the rate of desorption. Degradation of anthracene has been investigated by Gray et al. (1994) and later continued by Banerjee et al. (1994), expanding the studies for degradation of phenanthrene and pyrene. They have reported a very high degradation of anthracene using a rotating drum bioreactor, employing the mixed culture enriched from creosote contaminated soil.

To investigate whether or not the transformed anthracene was biodegradable, an experiment was set up using the same bioreactor and microbial culture used by Banerjee et al. (1994). The rotating drum provided an effective mixing for a high solid content slurry (Masliyah et al. 1992) and high rate of oxygen transfer (Gray et al. 1993). The effluent gas from bioreactor was connected to a mass spectrometer to monitor carbon dioxide evolution from mineralization of anthracene.

Oven dried soil A (1200 g.) was loaded to obtain 600 ppm anthracene by the same method used in previous experiments and described in chapter 3. The soil extract showed only 17 ppm extractable anthracene at the time of experiment. 20% of the slurry from the previous run of the reactor was recycled to provide adapted micro-organisms to the newly added soil. 0.8 liter of salt media was added to provide nutrients and to balance the slurry as 60% concentration of dry soil.

Evolution of CO_2 was measured and compared to CO_2 evolution from a typical bioreactor cycle as described by Banerjee et al. (1994). As illustrated in Fig 4.17, in a typical bioreactor cycle an initial peak appears right after the fresh soil is added to the bioreactor and lasts for 3 hours. The CO_2 production then declines and after one day increases again. The maximum peak appears in 1.5 days and the whole period of second peak is between day 1 and day 2. This peak belongs to anthracene degradation while the former peak is attributed to activities of native micro-organisms at the time they were provided by mineral salts and aeration. Utilization of volatile compounds in the freshly added soil by micro-organisms present in bioreactor may contribute to the first peak.

In the second run, oven dried soil A was used. This soil was loaded for a long time and exhibited only 17 ppm extractable anthracene. A burst of CO_2 appeared in the first few hours and later the concentration of CO_2 decreased. There was no peak appearing at the time expected for anthracene, nor was CO_2 evolved later. The operation of bioreactor was continued for 5 days.

The results of this experiment implies that the transformed anthracene was not available for micro-organisms. The lack of biological degradation may be due to potential affinity of high molecular weight compounds for soil mineral surfaces following solubility and desorption limitations. These results were comparable with the findings of Edwards (1975) and Lu et al. (1975) that aldrin and heptachlor transform to dieldrin and heptachlor epoxide respectively, which are more persistent in soil than their parent chemicals.

Another important point which arises here is the possible toxicological effects of high-molecular weight compounds, and their leachability and release into the environment.

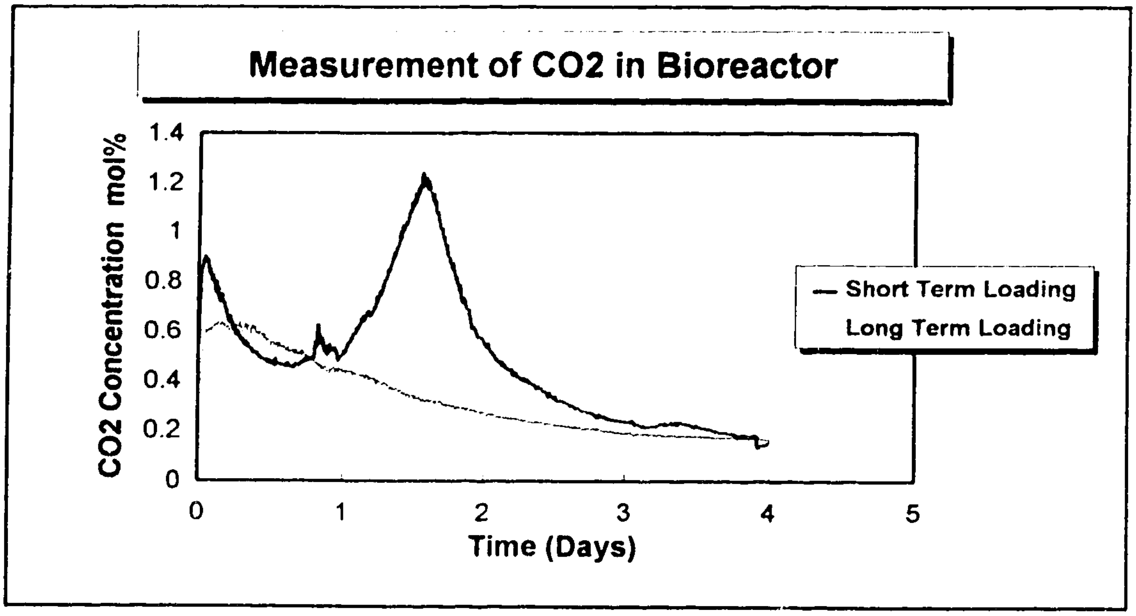

Fig 4.17 CO_2 evolution in bioreactor corresponding to degradation of anthracene.

4.3.5 Discussion

In sorption studies from aqueous or non-aqueous systems, the deficit in the concentration of a solution or decrease in activity of the solution (in case of using radiolabelled materials) is assumed to be due to sorption. Similar assumptions are made in desorption studies. The activity released from soil is assumed to be due to adsorbed compounds, without chemical transformations.

In these series of experiments we characterized the adsorbed materials and identified them by employing different analytical methods, so that the results of each experiment could support the results from other methods.

The consistency in the results of UV absorption and thin layer chromatography were supportive to the hypothesis that reaction is taking place in the soil. Lack of the characteristic peaks for anthracene in the samples demonstrating high activities was clear evidence that the activity extracted in ^{14}C-anthracene experiment was not due to anthracene. The GC-MS analysis clearly identified 9-9 bi-anthracene which is one of the 6 possible isomers of bi-anthracene, however, there may be higner molecular weight polymers which are not extractable, or if extractable they may not be detectable by GC-MS.

Although there has been some controversy in discussions in the literature about the active sites responsible for polymerization, the proposed mechanism and contribution of transition metals in reactions is consistent with findings of Rooney and Pink (1962), Solomon and Rosser (1965), Mortland and Halloran (1976), Solomon and Hawthorne (1983) and Issacson and Sawhney (1983). Contribution of transition metals as active sites can explain the variation in the behavior of two soils in dry and hydrated conditions. In dry conditions, interaction with active sites is very strong, consequently, soil A with organic content of 0.62%, shows higher interaction compared with Malmo which has 4.43% organic content. Although Malmo may have lower intensity of active sites, it is more likely that the active sites in Malmo are partly occupied by 4.43% organic matter, and some of them are not available for anthracene. This behavior can be explained as deactivation of transition metals by the chelating characteristic of soil organic matter. As water content increases, the active sites are covered by layers of water, but some sites covered by soil organic matter are protected from water and can contribute in interactions in a non-aqueous solvent. Such hydrophobic characteristics would explain why the rate of interaction for soil A drops so fast, but Malmo with 4.43% organic content compared with 0.62% organic content of soil A has enough active sites to continue to catalyze the reaction.

The possibility of formation of charge transfer complexes as an intermediate step for polymerization should be considered. This idea arises from the fact that almost 50% of anthracene interacts with soil very rapidly. The results of the first experiment, presented in Fig 4.1, indicated that in the first 30 min. 50% of anthracene was unrecoverable. One explanation for this behavior could be rapid adsorption and interaction of anthracene with the active sites of mineral surfaces, leading to the formation of free radical cations. As the contact time increases, the reaction develops as completion of electron transfer process and polymerization.

The reactions which give rise to polymeric products proceed under moderate conditions of room temperature. Increasing temperature will develop the reaction and increase the reaction rate. This phenomena may conflict with some analytical methods such as thermal desorption. It may also be important in commercial soil remediation processes based on thermal desorption. The conditions in these processes combine elevated temperature with low moisture content due to drying (Lighty et al., 1989).

High-molecular weight polymers are more likely present in the residue of the extract composing the 53% of non-extractable activities in the ^{14}C-anthracene experiment. Desorption of polymers from mineral surfaces is slow. As explained by Kipling (1965), polymers are adsorbed to the surface by different anchor-segments and in thick layers. Statistically, the probability of simultaneous removal of all the anchor-segments from the mineral surface will decrease by increasing the molecular weight of polymer and subsequent increase in anchor-segments.

The desorption of strongly anchored polymers is a major limiting step in bioremediation. As the results of bioreactor experiment indicated, bi-anthracene and other high-molecular weight materials formed in the process of loading were not available for micro-organisms known as anthracene degraders. However this is not unexpected as biological treatment is enhanced by solubilization of organic contaminants into the water phase where micro-organisms can metabolize the contaminants. If the contaminant does not dissolve into the water phase, then, it will not be available for micro-organisms.

The fact that only 47% of the initial activity was extractable, and this amount decreased to 31% within two months, was consistent with observations of

Pignatello et al. (1993). They observed that aged herbicides were more persistent in the soil than freshly added herbicides.

Toxicology effects may be a main concern regarding the slow desorbing materials, and the conditions that may accelerate the leaching process are of particular importance. In contrast to the belief that if a compound present in soil is not leaching out then is not harmful to the environment, the long term studies of the leaching process may be an important issue and worthwhile to investigate in detail.

4.4 Kinetic Modeling

4.4.1 General Characteristics of Soil-Anthracene-Water System

Anthracene reacts with dry soil to produce bi-anthracene and other higher molecular weight compounds. In dehydrated soils the rate of reaction is high and 600 ppm anthracene can disappear in 6 days. Two different stages were distinguished in the interaction of anthracene with soil. The first stage had a high rate constant, and the reaction occurred at the time anthracene solution was added to the soil. The reaction seemed instantaneous. The second stage had lower rate and was time dependent. Increasing the water content influenced the reaction and exhibited a strong inhibitory effect. The inhibitory effect likely arises when water competes successfully with anthracene for mineral surfaces. Adsorption of water on mineral surfaces would prevent any further reaction of anthracene with that site.

The focus of developing a kinetic model, was on the second stage of interaction between anthracene and soil.

4.4.2 Kinetic Models

4.4.2.1 Empirical Equation

An empirical equation which was developed to demonstrate the inhibitory effect of water on catalyst activity was examined.

$$-r_{anth} = \frac{k_0 C_{anth}}{1 + Kh^n} \tag{4.3}$$

where

C_{anth} = initial concentration of anthracene, [mg of anthracene/kg of soil]
k_0 = Rate constant at h = 0, day^{-1}
h = Water content, [g of water/100 g of soil]
K = constants, [g of water/100 g of soil]$^{-1}$
n = constant

4.4.2.2 Competitive Inhibition Model

To develop a model that could well represent the behavior of the system a competitive inhibition model (Bailey and Ollis, 1986) was employed to approximate the interaction of water as inhibitor, anthracene as substrate and soil as the active sites.

$$S + Anth \xleftrightarrow{K_s} S - Anth \qquad K_s = \frac{[S - Anth]}{[S][Anth]} \tag{4.4}$$

$$S + H_2O \xleftrightarrow{K_i} S - H_2O \qquad K_i = \frac{[S - H_2O]}{[S][H_2O]} \tag{4.5}$$

$$S - Anth \xrightarrow{k} S + P \tag{4.6}$$

The inhibitory effect of water in the above sequence is due to its interaction with the active sites. In the presence of water, all the active sites are not available for anthracene, so that the rate of reaction decreases. The reaction rate for this mechanism is as follows:

$$-r_{anth} = \frac{kC_sC_{anth}}{C_{anth} + \frac{1}{K_s}(1 + K_i h)} \tag{4.7}$$

Assuming a very high affinity of anthracene for the sites implies the inequality:

$$C_{anth} \ll \frac{1}{K_s}(1+K_i h) \qquad (4.8)$$

Simplifying equation (4.7) gives:

$$-r_{anth} = \frac{k_0 C_{anth}}{1+K_i h} \qquad (4.9)$$

where:

k_0 = Rate constant at h = 0, day^{-1}
h = Water content, [g of water/100 g of soil]
K_i = Equilibrium constant for inhibitory reaction,$[g$ of water/100 g of soil$]^{-1}$
C_{anth} = Concentration of anthracene, [g of anthracene/kg of soil]
K_s = Equilibrium constant for anthracene soil reaction,
[mg of anthracene/kg of soil$]^{-1}$

4.4.3 Results and Discussion

Fig. 4.18 and 4.19 represent the empirical equation used to correlate the data points for soil A and Malmo. The rate constants are listed in table 4.3.

Table 4.3 Kinetic parameters of empirical model for degradation of anthracene.

	k_0	n	K_i
Soil A	0.78	1.28	216
Malmo	0.097	0.80	3.37

Table 4.4 Kinetic parameters of competitive inhibition model for degradation of anthracene.

	k_0	n	K_i
Soil A	0.78	1	191.867
Malmo	0.097	1	3.025

Fig 4.20 and 4.21 represent the competitive inhibition model. The parameters are listed in table 4.4. The large difference in K_i values for two soils indicates that the inhibitory effect of water for soil A is much higher than Malmo. This result may be because soil A had a low organic content (0.62%) and, therefore, the mineral surfaces had more affinity to water, compared to Malmo (4.43% organic content) that mineral surfaces are partially isolated by soil organic matter.

The proposed model described the behavior of the system quite well. The largest deviation of the model from experimental data appeared in soil A when the water content was 0.1%. The experimental data were below the model prediction. This error likely arose from the accuracy of measurement at such a low water content and the sensitivity of the soil to moisture at dry conditions.

Fig 4.22 illustrates the prediction of model for soil A and Malmo in different moisture contents. As the model predicts to achieve 10% conversion for soil A and Malmo with 2.5% moisture content (air dried soil), it will take 62 and 9 days respectively. Table 4.5 represents more predictive data for both soils in different moisture contents.

Table 4.5 Prediction of model for 10% conversion

	Soil A	Malmo
Moisture Content %	Time (years)	Time (years)
2.5	0.17	0.025
10	0.70	0.093
20	1.45	0.187
50	3.58	0.450

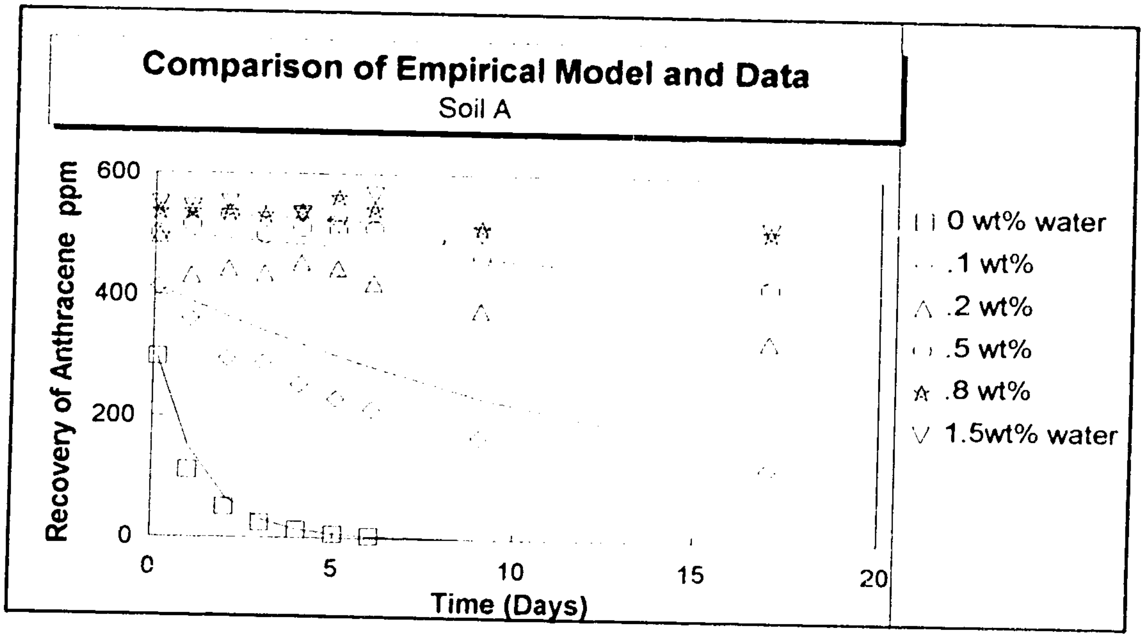

Fig 4.18 Empirical model for prediction of soil-anthracene-water system in soil A. (Equation 4.3 was used for calculating reaction rate for t >3 h)

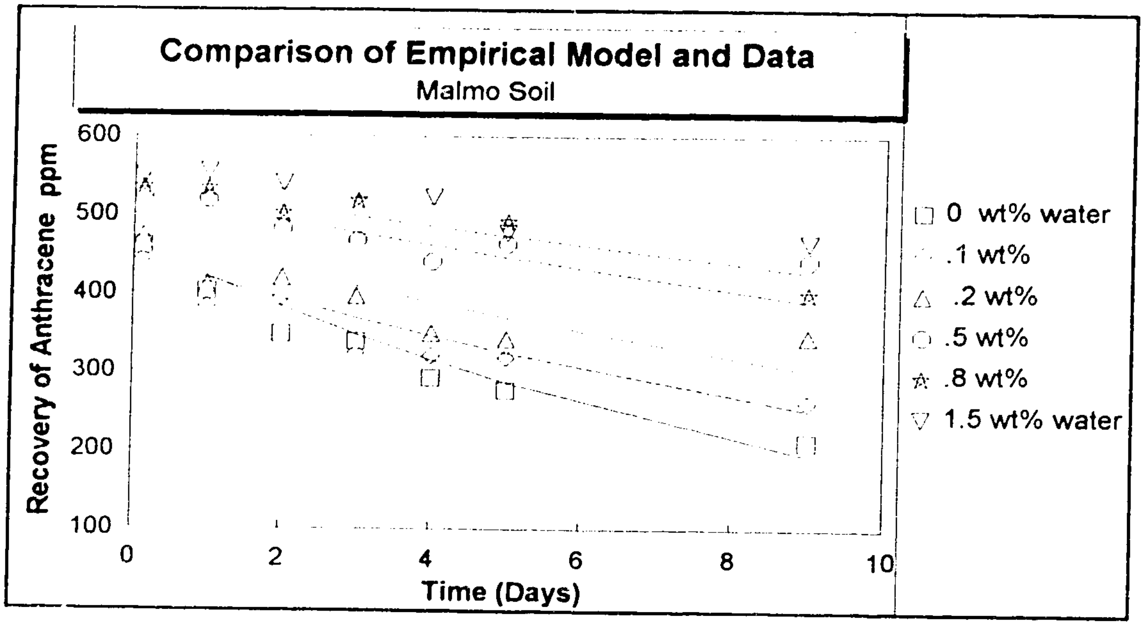

Fig 4.19 Empirical model for prediction of soil-anthracene-water system in Malmo. (Equation 4.3 was used for calculating reaction rate for t >3 h)

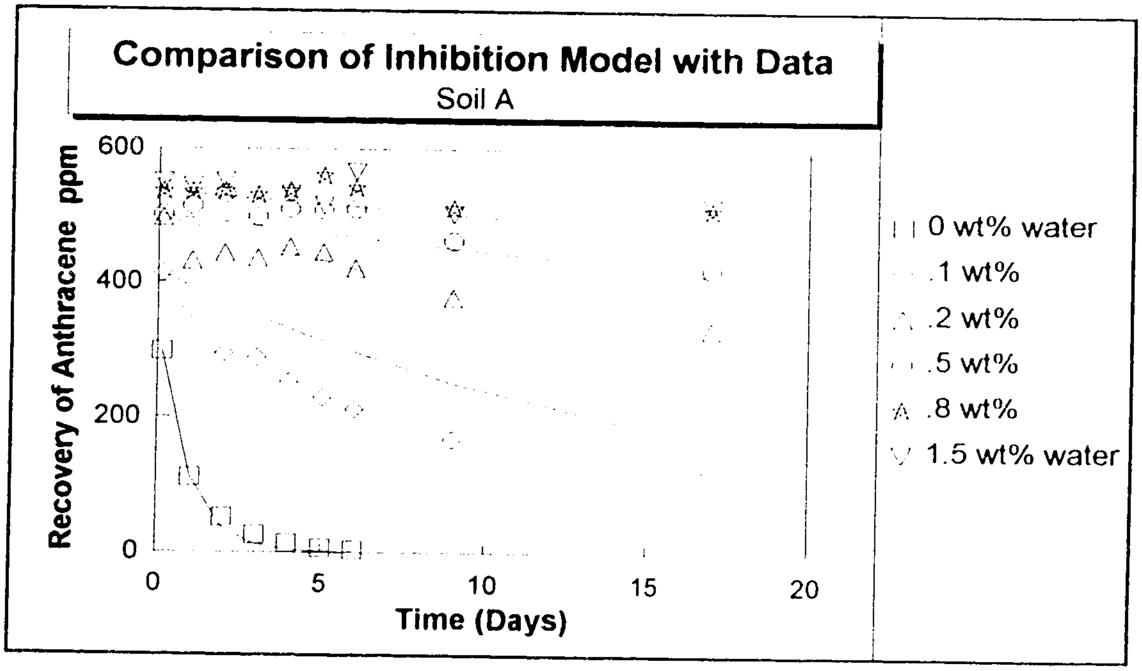

Fig 4.20 Competitive inhibition model for prediction of soil-anthracene-water system in soil A. (Equation 4.9 was used for calculating reaction rate for 1 -3 h)

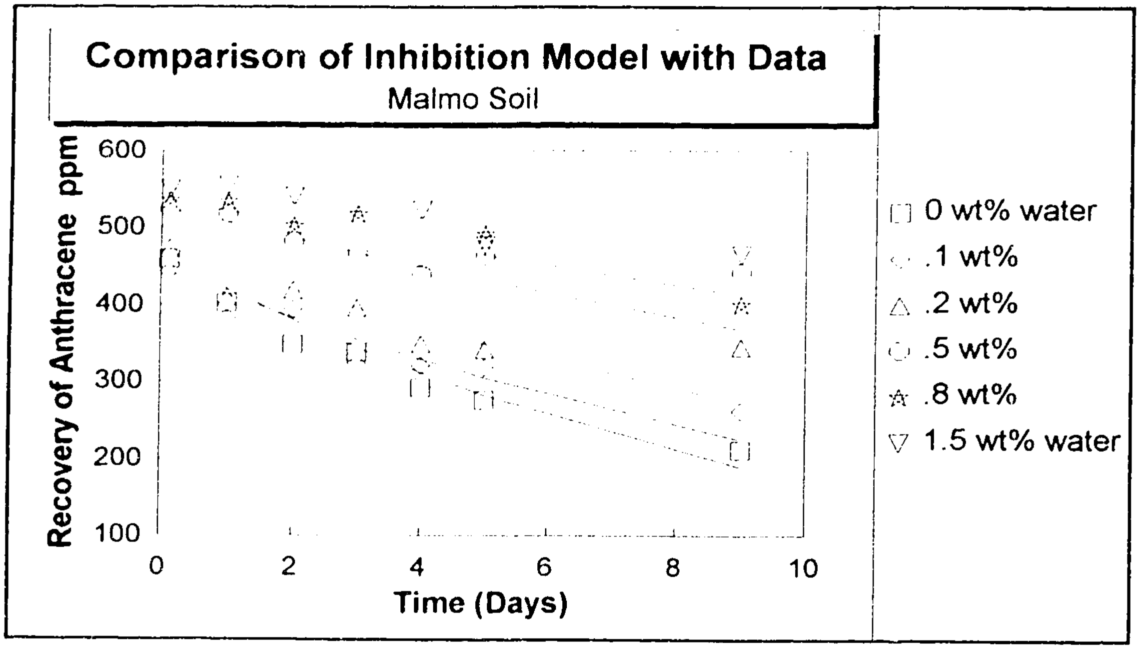

Fig 4.21 Competitive inhibition model for prediction of soil-anthracene-water system in Malmo. (Equation 4.9 was used for calculating reaction rate for t >3 h)

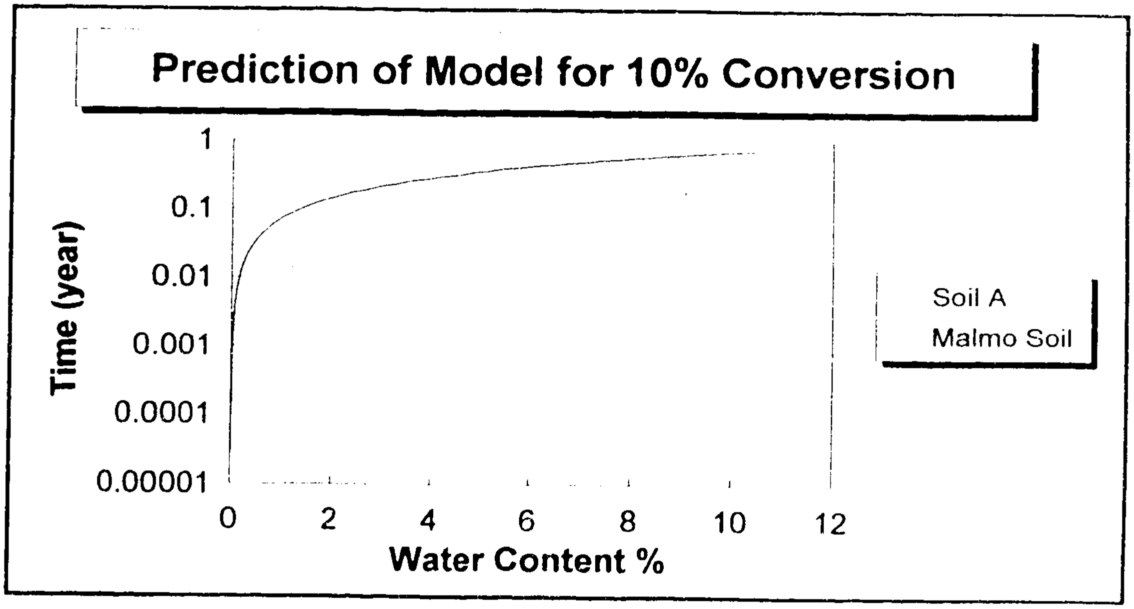

Fig 4.22 Prediction of competitive inhibition model for 10% conversion in soil A and Malmo (Equation 4.9 was used for calculating reaction rate for t <3 h)

CHAPTER 5

CONCLUSIONS

In dry soils the recovery of anthracene was very low, and for the three soils was in the order of soil A< shale < Malmo. In soil A after approximately 6 days, anthracene was unrecoverable. The highest recovery was in the Malmo soil with the highest organic content. Montmorillonite demonstrated similar behavior to the soils. The improved recovery of anthracene in soils containing high organic content. can be attributed to physical isolation of metal cations by soil organic matter that prevents the interaction of anthracene with those sites. The initial activity of the mineral sites may also be a major factor.

Moist soils exhibited different behavior than dry soils. Anthracene recovery was improved by increasing water content of the soil. The increased recovery of anthracene in hydrated soils demonstrated the inhibitory effect of water in interaction between soil and organic compound. This behavior suggested that water competes successfully with anthracene for active sites on mineral surfaces.

The results of the experiments using radiolabelled anthracene confirmed that after 4 series of extractions only 47% of the initial activity was extractable and 53% was still persistent in soil. This experiment also demonstrated that aged anthracene was more persistent in soil. The UV spectrum showed that anthracene was present only in the first extract, which constituted 12.4% of activity. The characteristic peaks of anthracene did not appear in the later extractions. however they showed strong activities, implying that anthracene was transformed to other compounds which had strong absorption at 255 nm, reflecting the aromaticity of the compounds.

GC-MS identified 9-9 bi-anthracene, with a molecular weight of 354 as one of the products. The results suggested that oligomers with higher molecular weight were produced. Part of these products were not extractable and the extractable fraction was not detectable by GC.

Similar phenomena were observed in interaction of pyrene and phenanthrene with soil. The strong interaction of pyrene with soil, and weaker interaction of phenanthrene suggest the generality of the phenomena in polynuclear aromatic hydrocarbons. However, more studies are required to characterize the parameters affecting these interactions and contribution of the structure of the aromatics that may affect the intensity of interaction.

The proposed mechanism for polymerization of aromatics in soil is formation of free radical cations via an electron transfer mechanism. The oxidizing sites would be transition metals and lewis acid sites which can undergo redox reactions with aromatic hydrocarbons through π-electron centers. The inhibitory effect of water on these reactions can be explained as dissipating the polarization power of cations by distributing it through the increased number of water molecules surrounding the cation.

The reactions leading to polymeric products are expected to be of particular importance in soil remediation processes based on thermal desorption, where elevated temperature and low moisture content would accelerate the reactions.

Bioavailibility of transformed anthracene was studied in a rotating drum bioreactor. This experiment confirmed that the products of transformation were not available for micro-organisms. Persistence of high molecular weight products in soil and desorption limitations explains the failure of bioremediation to remove the organic contaminants.

A kinetic model was developed, based on the inhibitory effect of water in the soil-anthracene-water system. This competitive-inhibition model can represent the kinetic behavior of the anthracene as a function of moisture content.

REFERENCES

Anderson, D.M.; Brown, R.L.; Buol, S.W.; 1967. "Diffusion of the dyes, Eosin yellowish, Bromophenol blue and Naphthol green bluish in water adsorbed by montmorillonite." Soil Sci. 103:281-287.

Bailey, J. E.; Ollis, D. F.; 1986. "Biochemical Engineering Fundamentals." McGraw-Hill. New York.

Ball, W.P.; Roberts, P.V.; 1991. "Long Term Sorption of Halogenated Organic chemicals by Aquifer materials 1- Equilibrium." Environ. Sci. Technol. 25:1237-1249.

Ball, W.P.; Roberts, P.V.; 1991. "Long Term Sorption of Halogenated Organic chemicals by Aquifer materials 2- Intraparticle Diffusion." Environ. Sci. Technol. 25:1223-1236.

Banerjee, D.K.; Fedorak, P.M.; Hashimoto, A.; Masliyah, J.H.; Pickard, M.A.; Gray, M.R.; "Monitoring the Biological Treatment of Anthracene-Contaminated Soil in Rotating Drum Bioreactor." Submitted to Applied Microbiol. Biotechnol.

Behar, F.; Vanderbroucke, M.; 1987. "Chemical Modelling of Kerogens." Org. Geochem. 11:15-24.

Block , R.; Stroo , S.; Swett , G. H. 1993. "Bioremediation-Why Doesn't it work Sometimes?" Chem. Eng. Prog. 8:44-50.

Brady, N.C.; 1985. "The Nature and Properties of Soils." Macmillan, New York.

Call, F.; 1957, "The Mechanism of Sorption of Ethylene Dibromide on Moist Soils." J. Sci. Food Agric. 8:630-639.

Chiou, C.T.; Lee, J.F.; Boyd, S.A.; 1990. "The Surface Area of Soil Organic Matter." Environ. Sci. Technol. 24:1164-1166.

Chiou, C.T.; Shoup, T.D.; 1985. "Soil Sorption of Organic Vapors and Effects of Humidity on Sorptive Mechanism and Capacity." Environ. Sci. Technol. 19:1196-1200.

Chiou, C.T.; 1989. "Theoretical Considerations of the Partition Uptake of nionic Organic Compounds by Soil Organic Matter". Reactions and Movement of Organic Chemicals in Soils. B.L. Sawnhey and K. Brown ed.'s. SSSA Special Publication. Number 22.

Chiou, C.T.; Shoup, T.D.; Porter, P.E.; 1985. "Mechanistic Roles of Soil Humus and Minerals in the Sorption of Nonionic Organic Compounds from Aqueous and Organic Solutions." Org. Geochem. 8:9-14.

Chiou, C.T.; Porter, P.E.; Schmedding, D.W.; 1983. "Partition Equilibria of nonionic organic Compounds Between Soil Organic Matter and Water." Environ. Sci. Technol. 17:227-231.

Chiou, C.T.; Kile, D.E.; Malcolm, R.L.; 1988. "Sorption of Vapors of Some Organic Liquids on Soil Humic Acid and its Relation to Partitioning of Organic Compounds in Soil Organic Matter." Environ. Sci. Technol.. 22:298-303.

Cloos, P.; Vande P.D.; Camerlynck,J.P.; 1973. "Thiophene Complexes on Montmorillonite Saturated with Different Cations." Nature Physical Sci. 243:54-55.

Davis, G.A.; Worrall, W.E.; 1971. "The Adsorption of water by Clays." Trans. Brit. Ceramic Sci. 70:71-75.

Dragun, J.; Helling, C.S.; 1985. "Physicochemical and Structural Relationships of Organic Chemicals Undergoing Soil- and Clay-Catalyzed Free radical Oxidation." Soil Sciene. 139:100-110.

Fedorak, P.M.; Grbic Galic, D.; 1991. "Aerobic Microbial Cometabolism of Benzothiophene." Appl. Environ. Microbiol. 57:932-940.

Fenn, D.B.; Mortland, M.M.; 1972. "Interlamellar Metal complexes on Layer Silicates. II. Phenol Complexes in Smectites." Proc. Int. Clay Conf., Madrid. 591-603.

Garbarini, D.R.; Lion, L.W.; 1986. "Influence of the Nature of Soil Organics on the Sorption of Toluene and Trichloroethylene." Environ. Sci. Technol. 20:1263-1269.

Gates, B.C.; 1992. "Catalytic Chemistry." John Wiley & Sons. New York.

Goss, K.U.; 1992. "Effect of Temperature and Relative Humidity on the sorption of Organic Vapors on Quartz Sand." Environ. Sci. Technol. 26:2287-2294.

Gray, M.R.; Mehta, B.; Masliyah, J.H.; 1993. "Liquid Side Mass Tansfer Coefficients for Liquid and Slurries in a Rotating Drum." Chem. Eng. Sci. 48:3442-3446.

Gray, M.R.; Banerjee,D.K.; Fedorak,P.M.; Hashimoto,A.; Masliyah,J.H.; Pickard,M.A.; 1994. "Biological Remediation of Anthracene- Contaminated Soil in Rotating Bioreactors." Applied Microbiol. Biotechnol. 40:933-940.

Haderlein, S.B.; Schwarzenbach, R.P.; 1993. "Adsorption of Substituted Nitrobenzenes and Nitrophenols to mineral Surfaces." Environ. sci. Technol. 27:316-326.

Hance, R.J.; 1965. "Observations on the Relationship Between the Adsorption of Diuron and the Nature of the Adsorbent." Weed Res. 5:108-114.

Huang, P.M.; Schnitzer, M.; 1986. "Interactions of Soil Minerals with natural Organics and Microbes." SSSA Special Publication. No. 17.

Isaacson,P.J.; Sawhney,B.L.; 1983. "Sorption and transformation of phenols on Clay Surfaces: Effect of Exchangable Cations." Clay Minerals. 18:253-265.

Kipling,J.J.; 1965."Adsorption from Solutions of non-Electrolytes." Academic Press. London.

Ledoux, R.L.; White, J.L.; 1966. "Infrared Studies of Hydrogen Bonding: Interactions between Kaolinite Surfaces and Intercalated Potassium Acetate, Hydrazine, Formamide and Urea." J. Colloid Interface Sci. 21:127-152.

Lighty, J.S.; Silcox, G.D.; Pershing, D.W.; Cundy, V.A.; Linz, D.G.; 1989. "Fundamental Experiments on Thermal Desorption of Contaminats from Soils." Environ. Prog. 2:57-61.

McKnight, D.M.; Bencala, K.E.; Zellweger, G.W.; Alken, G.R.; Feder, G.L.; Thorn, K.A.; 1992 "Sorption of Dissolved Organic Carbon by Hydrous Aluminum and Iron Oxides Occuring at the Confluence of Deer Creek with the Snake River, Summit County, Colorado." Environ. Sci. Technol., Vol. 26, No. 7.

McKeague,J.A.; Cheshire,M.V.; Andreux,F.; Berthelin,J.; 1986, "Organo-Mineral Complexes in Relation to Pedogenesis.", Interactions of Soil Minerals with Natural Organics and Microbes. SSSA Special Publication., No. 17, chapter 15.

Mills, A.C.; Biggar, J.W.; 1969. "Solubility Temperature Effect on The adsorption of Gamma and Beta-BHC from aqueous anh hexane Solutions by Soil Materials." Soil Sci. Soc. Amer. Proc. 33:210-216.

Morrill , L.G.; Mahilum , B.C.; Mohiuddin , S.H.; 1982. "Organic Compounds in Soils: Sorption, Degradation and Persistence." Ann Arbor Science. Michigan.

Mortland ,M.M.; Pinnavaia ,T.J.; 1971. "Formation of Copper (II) Arene Complexes on the Interlamellar Surfaces of Montmorillonite." Nature Physical Science. 229:75-78.

Mortland ,M.M.; Halloran ,L.J.; 1976. "Polymerization of Aromatic molecules on Smectite." Soil Sci. Soc. 40:367-370.

Mortland ,M.M.; Raman, K.V.; 1968. "Surface Acidity of Smectites in Relation to Hydration, Exchangeable Cation, and Structure." Clays and Clay Minerals. 16:393-398.

Murphy, E.M.; Zachara, J.M.; Smith, S.C.; 1990. "Influence of Mineral-Bound Humic Substances on the Sorption of Hydrophobic Organic Compounds." Environ. Sci. Technol. 24:1507-1516.

Pawluk, S.; Dudas, M.J.; 1982. "Floralpedoturbations in Black Chernozemic Soils of the Lake Edmonton Plain." Can. J. Soil Sci. 62:617-629.

Pinnavaia,T.J.; Mortland, M.M.; 1971. "Interlamellar Metal complexes on layer Silicates. I. Copper(II)-Arene Complexes on Montmorillonite." The journal of Physical Chemistry. 75:3957-3962.

Pignatello, J.J.; Huang, L.Q.; 1991. "Sorptive Reversibility of Atrazine and Metolachlor Residues in Field Soil Samples." J. Environ. Qual. 20:222-228.

Pignatello, J.J.; Ferrandino, F.J.; Huang, L.Q.; 1993. "Ellution of Aged and Freshly Added Herbicides from a Soil." Environ. Sci. Technol. 27:1563-1571.

Qualizza, C.V.; 1993. "The Influence of the Nature of Soil Organic Matter on the Sorption of Anthracene, Naphthalene and α-Naphthol." MSc. Dissertation. University of Alberta.

Rooney ,J.J.; Pink ,R.C.; 1962. "Formation and Stability of Hydrocarbon radical-Ions on a Silica-Alumina Surface." J. Phys. Chem. 77:1632-1641.

Rutherford, D.W.; Chiou, C.T.; Kile, D.E.; 1992. "Influence of Soil Organic Matter Composition on the Partition of Organic Compounds". Environ. Sci. Technol. 26:336-340.

Rutherford, D.W.; Chiou, C.T.; 1992. "Effect of Water Saturation in Soil Organic Matter on the Partition of Organic Compounds." Environ. Sci. Technol. 26:965-970.

Sawhney, B.L.; Gent, M.P.N.; 1990. "Hydrophobicity of Clay Surfaces: Sorption of 1,2-Dibromoethane and Trichloroethene." Clays and Clay minerals. 38:14-20.

Silverstein, R. M.; Bassler, G. C.; Morrill, T. C.; 1981. "Spectrometric Identification of Organic Compounds." John Wiley & Sons. New York.

Solomon, D.H.; Rosser, M.J.; 1965. "Reactions Catalyzed by Minerals. Part I. Polymerization of Styrene." J. of Applied Polymer Science. 9:1261-1271.

Solomon, D.H.; Swift, J.D.; 1967. "Reactions Catalyzed by Minerals. Part II. Chain Termination in Free-Radical Polymerization." J. of Applied Polymer Science. 11:2567-2575.

Solomon, D.H.; Loft, B.C.; 1968. "Reactions Catalyzed by Minerals. Part III. The Mechanism of Spontaneous Interlamellar Polymerization in Aluminosilicates." J. of Applied Polymer Science. 12:1253-1262.

Solomon, D.H.; Loft, B.C.; Swift, J.D.; 1968. "Reactions Catalyzed by Minerals. IV. The Mechanism of the Benzidine Blue Reaction on Silicate Minerals." Clay Minerals. 7:389-397.

Solomon, D.H.; Loft, B.C.; Swift, J.D.; 1968. "Reactions Catalyzed by Minerals. V. The Reactions of Leuco Dyes and Unsaturated Organic Compounds with Clay Minerals." Clay Minerals. 7:399-409.

Solomon, D.H.; Hawthorne, D.G.; 1983. "Chemistry of Pigments and Fillers." John Wiley & Sons. New York.

Stahl, E.; 1969. "Thin Layer Chromatography." Springer-Verlag. Berlin.

Theng, B.K.G.; 1974. "Chemistry of Clay Organic Reactions." Halsted Press. New York.

Thibaud, C.; Erkey, C.; Akgerman, A.; 1993. "Investigation of the Effect of moisture on the Sorption and Desorption of Chlorobenzene and Toluene from Soil." Environ. Sci. Technol. 27:2373-2380.

Van Olphen, H.; Fripiat, J. J.; 1979. "Data Handbook for Clay Materials and Other Non-metalic Minerals." Pergamon press. Ontario. Canada

Walker, G.F.; 1961. "X Ray Identification and Crystal Structures of Clay Minerals." Mineral. Soc. London.

Warren, D.S.; Clark, A.I.; Perry, R.; 1986. "A Review of clay Aromatic Interactions with a View to Their Use in Hazardous Waste Disposal." The Science of Total Environment. 54:157-172.

Weast, R. C.; Astle, M. J.; Beyer, W. H.; eds. 1984. "CRC Handbook of Chemistry and Physics." CRC Press, Inc. Boca Raton, Florida.

Weissenfels, W. D.; Klewer, J.; Langhoff, J.; 1992. "Adsorption of Polycyclic Aromatic Hydrocarbons (PAHs) by Soil Particles : influence on biodegradability and biotoxicity." Applied Microbiol. Biotechnol. 36:689-696.

Yaron, B.; Saltzman, S.; 1972. "Influence of Water and Temperature on Adsorption of Parathion by Soils." Soil Sci. Soc. Amer. Proc. 36:583-587.