University of Alberta

Sorption of anthracene from non-aqueous solutions and subsequent degradation

by

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science.

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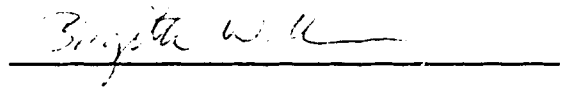

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled Sorption of anthracene from non-aqueous solutions and subsequent degradation submitted by Birgitte Willumsen in partial fulfillment of the requirements for the degree of Master of Science.

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ABSTRACT

It was hypothesized that the availability of anthracene for biological degradation would be dependent on how anthracene was deposited into the soil. The experimental design varied the parameters that were expected to influence the availability of anthracene in soil, including soil organic matter, clay content, moisture content, solvent polarity and time of contact between the soil and the anthracene in carrier solvent.

The data showed evidence for long-term abiotic degradation of as much as 78% of the anthracene even at a moisture tension of 15 bars, which would be high enough to prevent bacterial growth. The extent of degradation increased with increasing content of soil organic matter.

The availability of anthracene for biodegradation was high, and it was only reduced after a contact time of 180 days between the soil and the solvent solution. Anthracene in hexane solution crystallized in the samples with a contact time of 180 days, and these samples showed the highest residual concentration of anthracene, 22-100 ppm, after biodegradation over a 4 week period.

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CHAPTER 1

INTRODUCTION

Increasing amounts of soil contaminated with organic chemicals are being catalogued around the world causing increasing concern because of the carcinogenic, mutagenic and toxic effects of organic contaminants like polynuclear aromatic hydrocarbons (PAHs).

The interacting components in a contaminated site are soil organic matter, soil minerals, soil moisture and an organic contaminant. Non-volatile contaminants such as PAHs, pentachlorophenols and chlorinated biphenyls are often spilled as a solution in a carrier solvent. Contaminated soil, therefore, is a four or five component mixture, which needs to be studied more to be understood better. Time can be added as a sixth dimension affecting the interactions between the components.

Depending on the characteristics of the site, i.e. soil characteristics and hydrology, and the characteristics of the organic contamination, the contamination can remain as surface contamination or it can migrate down to cause subsurface soil and groundwater contamination. The distribution of the contaminants in the soil and groundwater will depend on the interactions with the soil.

When organic contaminants are introduced to the environment in an organic solvent or a mixture of solvents, a non-aqueous environment is created in the area of the contamination. As the solvent evaporates or undergoes degradation, an aqueous environment is left behind.

Investigations of sorption of organic compounds to mineral surfaces and soil organic matter from aqueous solution or vapour phase have been conducted previously to study the chemical interactions, but without applying the results to a real contamination situation (Chiou et al., 1985, Call, 1957). Few studies have been concerned with sorption of organic chemicals onto soil from non-aqueous solutions (Chiou et al., 1985, Gerstl and Yaron, 1978, Hance, 1965). Several sorption studies have found that the sorption was not reversible and there was a loss of the organic chemical with time (Hatzinger and Alexander, 1995, Lotfabad, 1994, Weissenfels et al., 1992, Pavlostathis and Mathavan, 1992, Bollag, 1992, Gamble and Khan, 1990). These losses have been attributed to strongly bound organic chemical in most of the studies. Very few studies have considered the possibility that the loss might be caused by a chemical transformation (Lotfabad, 1994).

Studies on biological degradation (Weissenfels et al., 1992, Gray et al. 1994) have shown a low availability of the organic chemical in some cases i.e. the compounds could be extracted from the soil, but they were not biodegraded. The interactions between soil and organic chemicals that cause a

low availability for biological degradation are poorly understood, but of high importance for the cleaning of contaminated soil by bioremediation. Therefore more research is still needed to identify the type of interactions that are happening between soil and organic contaminants.

In the present study, it was hypothesized that the availability of a compound for biological degradation would depend on the way the compound was deposited in the soil. Anthracene, a three ring aromatic hydrocarbon with a molecular weight of 178 and a water solubility of 45 ppb, was chosen as a model compound to investigate the interactions between soil and organic contaminants. Two soils and a pure clay were used in the study to reflect different levels of soil organic matter.

The method used for introducing the contaminant into the soil (described in Chapter 3) was designed to simulate a contamination situation in an unsaturated soil zone. The interactions between soil and organic contaminants in the unsaturated zone may be different from the interactions occurring in the saturated zone below the water table.

This study presents the results of laboratory batch experiments in which sorption of anthracene onto different soils was studied as a function of soil moisture content, carrier solvent and contact time. The samples with anthracene sorbed were also used for studying the availability of anthracene for biological degradation. The main goals of this

study were:

1. To study the influence of soil moisture content, carrier solvent and contact time on the interactions between soil and anthracene.

2. To study the availability of anthracene for biodegradation after interaction with the soil under different conditions.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Soil is a complex structured solid which acts as an adsorbent for both nonionic and polar organic contaminants. The heterogeneity of soil has led to different theories on the interactions taking place between the organic contaminant and the different constituents of the soil. Adsorption/ desorption onto mineral surfaces and partitioning into soil organic matter are the most investigated among the theories, but recently more researchers have acknowledged the importance of diffusion into macro- and micropores within soil particles.

2.2 Soil characterization

2.2.1 Soil organic matter

Soil organic matter is the result of microbial or chemical degradation of plant or animal parts (Brady, 1984). The amount of soil organic matter present in the soil can vary from close to 100% in organic soils to less than 1% in most subsoils. The soil organic matter is often involved in forming aggregates and coating the mineral grains with a membrane-like coating (Wershaw, 1993).

Soil organic matter is divided into recognizable compounds, which account for 5-25% of the soil organic matter,

and amorphous polymers, which are the remaining 75-95% of the soil organic matter. The recognizable compounds are compounds like polysaccharides, lignins and polypeptides. The amorphous polymers, which are high molecular weight compounds, are divided further into fulvic acid (10%), which is acid soluble and has a molecular weight of 10^3-$3 \cdot 10^4$, humic acid (40%), which is base soluble and has a molecular weight of 10^4-10^5, and humins (50%), which are insoluble and have a molecular weight of more than 10^5 (Paul and Clark, 1989).

The exact structures of the amorphous polymers are not clear, but it is known that they contain aromatic rings. The soil organic matter gets more and more aromatic as it gets older, i.e. the soil organic matter in a topsoil will be less aromatic than the soil organic matter in a subsoil.

The amorphous polymers have a high surface area (560-800 m^2/g as measured with ethylene glycol (Chiou et al., 1990)), which means that they have a high water-holding capacity (Brady, 1984).

2.2.2 Soil texture

The soil texture or the particle size distribution divides the soil minerals into three fractions : 1- the 2 mm to 50 μm fraction, which consists mainly of sand with a spherical shape. 2- the 50 μm to 2 μm fraction, which consists mainly of silt with a spherical shape. 3- the less than 2 μm fraction, which consists mainly of clay shaped as flat

platelets.

There are different types of clay. A clay like montmorillonite has a swelling or expandable structure, whereas a clay like kaolinite is non-expandable (Brady, 1984).

Expandable clay has an interlamellar surface that can be accessible to polar molecules like water, because of the negatively charged silicate layers this clay is build of (Brady, 1984).

The non-expandable clay kaolinite has also got silicate layers in the structure, but these are held together by hydrogen bonds, therefore there is no accessible internal surface in this clay type (Brady, 1984).

The different clays are different in size, thus the sizes of montmorillonite and kaolinite are 0.01-1.0 μm and 0.1-5.0 μm respectively (Brady 1984).

2.2.3 Surface area

There are different methods for measuring the surface area. The BET (Brunauer-Emmett-Teller) method, which is used in catalysis, calculates the surface area based on the uptake of N_2. This method will not include the interlamellar surface of expandable clays, so the more commonly used method for soil samples calculates the surface area based on the uptake of a polar compound like ethylene glycol or ethylene glycol monoethyl ether. This method will include the interlamellar surface of clays and it will also include uptake by soil

organic matter if present.

The surface area measured with ethylene glycol is 700-800 m^2/g and 5-20 m^2/g for montmorillonite and kaolinite respectively (Brady, 1984). The surface area of soil organic matter is 560-800 m^2/g measured with ethylene glycol and less than 1 m^2/g measured with N_2 (Chiou et al., 1990). It is questionable for soil organic matter if the measurement with a polar compound is giving a true surface area, since the polar compound can partition into the soil organic matter, and thereby cause a higher uptake than required for coverage of the surface only.

2.2.4 Soil moisture tension

The soil moisture tension expresses the tension or the suction needed to remove water from the soil at a given soil moisture content. The water activity at the soil moisture content corresponding to a tension of 15-40 bars gets too low for bacteria to be active, whereas fungi can remain active down to a soil moisture content corresponding to a tension of 50 bars (Brady, 1984). Both bacteria and fungi can survive through spores down to a tension of 100-400 bars.

The relationship between soil moisture tension and soil moisture content is affected by the particle size distribution of the soil. A soil with a high clay content will hold more water than a sandy soil at a given moisture tension. The degree of granulation of the soil also affects the

relationship between soil moisture tension and soil moisture content at a moisture tension below 15 bars as used in the present study. A compacted soil will have a reduced pore space compared to a granulated soil leading to a lower moisture content at a given low tension.

2.3 Distribution of organic contaminants in soil

The distribution of organic contaminants in soil may be affected by both kinetic factors and thermodynamic equilibrium. The latter defines the equilibrium state for distribution between phases, such as adsorption on soil minerals and partitioning into soil organic matter. Kinetic processes include transport through macropores and micropores and degradation, biological as well as chemical. These processes are influenced by several parameters and characteristics of both the organic contaminant, the carrier solvent and the soil.

At high concentrations the organic chemical can also be present in the soil matrix as a crystal (Piotrowski, 1991) or a liquid droplet (Block et al., 1993). In these circumstances, the contaminant can be considered as a separate phase within the soil matrix.

2.3.1 Diffusion

Diffusion can take place when a gradient in chemical potential for the organic contaminant is present in the soil.

The contaminant can enter the water phase of the soil and then continue by diffusion into the micropores within the soil particles. The rate of diffusion can be decreased due to aggregation of the soil particles. A change in aggregation of the soil gives a change in the pore size distribution, which can have a considerable effect on diffusion rates.

2.3.2 Sorption

Two major components of the soil can interact with non-polar organic contaminants; the soil organic matter and the minerals.

There is some debate as to what kind of interaction occurs between organic contaminants and soil organic matter. Some literature refers to uptake by soil organic matter as adsorption onto the surface of soil organic matter and some as partitioning into soil organic matter. Some researchers refer to the mechanism as sorption, without further specification.

In contrast, the interaction between the nonpolar organic contaminant and minerals proceeds via adsorption onto solid surfaces. Adsorption is defined as the attachment of species (adsorbates) onto the surface of a solid (adsorbent). This can occur either by physical adsorption or by chemisorption. The interaction between adsorbates and adsorbent in the case of physical adsorption is a result from dispersive forces like van der Waals forces or dipole interactions. This means that it is possible to have more than one layer of attached species

(multilayer coverage). Chemisorption results from electronic interactions (like in a chemical reaction) between adsorbates and adsorbent, therefore chemisorption can result in a monolayer coverage where one adsorbate is adsorbed to every active site of the adsorbent.

Because soil is a mixture, the overall interaction will be a sum of the interaction with specific components of the soil, which will be referred to as sorption hereafter.

2.3.2.1 Dependence of sorption on soil organic matter

An organic contaminant will partition into the soil organic matter because most nonpolar organic compounds have a greater affinity for organic phases compared to hydrated mineral surfaces, but the extent of partitioning will depend on chemical interactions.

Partitioning of compounds between soil organic matter and water is often expressed by the partition coefficient (K_{OM}) or alternatively the organic carbon normalized partition coefficient (K_{OC}) which both expresses the concentration of organic contaminant associated with soil organic matter to concentration of organic contaminant in aqueous solution at equilibration.

The amount of partitioning taking place will be affected by the polarity of both the contaminant and the soil organic matter.

Several researchers (Liu and Amy, 1993, McCarthy and Jimenez, 1985, Schlautman and Morgan, 1993, Wershaw, 1993) have suggested that nonpolar organic contaminants can bind to the hydrophobic interior of humic material, due to the micelles that humic material will form in the presence of water. The binding will depend on the hydrophobicity and the size of the organic contaminant and on the ability to fit into the hydrophobic interior of the humic material (Schlautman and Morgan, 1993). Schlautman and Morgan also found that the dimension and the hydrophobicity of the voids in the humic acid were sensitive to variations in pH, salt concentration and the valence of cations present. Liu and Amy (1993) found the binding to the hydrophobic interior to be reversible suggesting a weak physical interaction.

Mackay and Powers (1987) suggested a loose sorption of hydrophobic organic compounds on the surfaces of soil organic matter in aqueous solution. They also suggested that sorption would occur on internal surfaces of the organic matter, which is "like a sponge saturated with water".

Means et al. (1980, 1982) reported that sorption of PAHs and amino- and carboxy-substituted PAHs onto soils and sediments from aqueous solution was correlated to the amount of organic carbon content, but not to any other soil/sediment characteristic.

Kile et al. (1995) investigated the partitioning of carbon tetrachloride and 1,2-dichlorobenzene from water into

soil organic matter and sediment organic matter for a wide range of soils and sediments. They found the partitioning to be dependent on the polarity of the soil organic matter, though very little variation in the organic carbon normalized partition coefficient (K_{OC}) within the soil samples or within the sediment samples was observed. The K_{OC} found for sediments was twice the K_{OC} found for soils, which they explained was due to a lower polarity of the sediment organic matter compared to the soil organic matter. When using suspended solids from rivers they found K_{OC} values comparable to the ones found for soil samples. They therefore suggest the difference in K_{OC} for soil and sediment to be caused by the more polar fraction of organic matter to be dissolved in the river water thus leaving the less polar organic matter in the sediment. The very little variation in K_{OC} for the soil samples or the sediment samples indicated a similar polar to nonpolar ratio for all the samples compared even though they were sampled from very different places (USA and China) and had different contents of organic matter.

Rutherford et al. (1992) found that the partitioning of benzene and carbon tetrachloride into soil organic matter was dependent on the polarity of the soil organic matter. Rutherford and Chiou (1992) observed a reduced uptake of benzene, carbon tetrachloride and trichloroethylene with water-saturated soil organic matter, possible due to preferential sorption of water on the polymer molecules or an

increase in polarity of the soil organic matter due to the associated water.

Xing et al. (1994) suggested a new method for predicting partition coefficients for nonionic organic contaminants. They suggested an equation describing the relation between polarity index [(O+N)/C], octanol-water partition coefficient (K_{OW}) and measured K_{OC}. The polarity index was calculated from elemental composition for selected pure compounds such as lignin, chitin, and cellulose. K_{OC} was measured for benzene, toluene and o-xylene using two lignins, chitin, cellulose and humic acids extracted from two different soils. The polarity index, calculated from the equation by the use of measurements of K_{OC} for one compound, and K_{OW}s from literature can be used in the same rearranged equation to predict K_{OC} for other compounds. They found a better agreement between measured K_{OC} for two compounds, and predicted values from their equation, than predicted from other equations in the literature only based on K_{OW}, when using measurements of K_{OC} for a third compound to calculate the polarity index for five soils. They also found a linear relationship between polarity index for the five soils and the fraction of carbon present as aromatic carbon in the soils, thus suggesting that sorption is also influence by the aromaticity of the soil organic matter not just the polarity. Since their correlations are based on a low number of organic contaminants and soil samples the equations needs to be confirmed for more contaminants and soils.

Hance (1965) reported comparisons of sorption of diuron onto soil organic matter which had been pretreated to change the structure of the soil organic matter. In contradiction to other researchers (Kile et al., 1995, Rutherford et al., 1992, Rutherford and Chiou, 1992, Xing et al., 1994) he did not find any relationship between the treatments, and thus the structure of organic matter, and the sorption.

2.3.2.2 Kinetics of sorption

Most studies have found the uptake of contaminants to reach equilibrium in a short time, within hours to a few days. These laboratory studies have all been conducted with a high solution to soil ratio relative to what would be expected at a contaminated site.

Chiou et al. (1985) reported the equilibrium to be reached in less than 10 hours for nonionic compounds in aqueous solution or in hexane. This finding agrees with the findings of Gerstl and Yaron (1978) who reported an equilibrium time of several hours without reporting the number of hours, but they used 18 hours for their batch equilibrium studies for a pesticide in aqueous solution or in hexane.

Yaron and Saltzman (1972) reported that equilibrium was reached quickly for a pesticide in aqueous solution or in hexane, and they used an equilibrium time of 60 minutes in their batch studies.

Ball and Roberts (1991) used results from batch experiments for sorption of halogenated organic chemicals onto aquifer material to calculate the time required to reach equilibrium based on intraparticle diffusion. These calculations showed an equilibrium time ranging from less than one day up to two and a half years depending on which particle size fraction they were looking at. The calculated time to reach equilibrium was 7 days for the bulk solids, which is more in the range of results from the investigations mentioned earlier than the calculation of equilibrium times of years.

2.3.2.3 Role of soil moisture content

Water, with a high polarity, is an effective competitor for adsorption sites on mineral surfaces. Water molecules will preferentially cover the adsorption sites in a saturated soil, whereas a dry or partially hydrated soil will have adsorption sites that are available for adsorption of organic contaminants.

Thibaud et al. (1993) and Call (1957) both found a change in sorption from vapour phase as the relative humidity was increased. The change indicated a weaker interaction between the soil and the organic chemical at higher moisture levels.

Thibaud et al. (1993) hypothesized that the change could be caused by 1-reduced adsorption by soil minerals, 2-increasing importance of uptake by soil organic matter, 3-adsorption at the liquid-gas interface (the soil particles

will be covered by a liquid phase of water as the relative humidity increase). The effect of uptake from vapour phase due to dissolution of the organic chemical in the liquid phase and partition into the soil organic matter was examined together with the adsorption at the gas-liquid interface by theoretical calculations. The adsorption at the gas-liquid interface was shown to be an important part of the uptake by the soil, but very little dissolution and partitioning was found. Their equations might not have been describing the system well enough to account for dissolution and partitioning. Only a few of their calculated total uptake corresponded to the actual measured values.

Call (1957) ascribed the change in sorption to a change in mechanism for the adsorption of ethylene dibromide. He found a relative humidity of 10% to cause a monolayer coverage of water, and therefore above this relative humidity the adsorption of ethylene dibromide was no longer competitive with water.

Steinberg and Kreamer (1993) also found an effect of moisture content on the adsorption of volatile organic compounds. Their results indicated that additional water would reduce the available area by filling some of the soil pores.

This hypothesis was also used by Thibaud et al. (1993) when they calculated the uptake at the gas-liquid interface, though their calculations did not correspond to actual measurements of total uptake of toluene. This could also have

been due to the equations used to calculate the dissolution and partitioning, which might not have been adequate for describing the system.

2.3.2.4 Role of solvent

The presence of a solvent, as in many spills of organic compounds on soil, complicates the picture by introducing another phase. In addition to the interactions of water and contaminant with the soil, the interactions between soil and solvent must be considered. If the soil is fully hydrated, then interactions between soil and the liquid organic phase will be minimal since the soil particles will tend to be covered with water molecules. If there is free water present, though, a non . polar solvent could cause immiscible displacement. In a dry soil, however, the organic solvent will contact the soil surface directly without an intervening aqueous film. Partly hydrated soil would give an intermediate case.

Chiou et al. 1985 compared uptake of the nonionic organic compounds parathion and lindane (insecticides) from water and from hexane by dry, partially hydrated and fully hydrated soils. They found linear isotherms for the aqueous system indicating uptake by soil organic matter. The uptake from the non-aqueous solution showed a dependence on the soil moisture content. They observed competitive adsorption between water and the nonionic organic compounds on the soil minerals when

the soil was partially hydrated. The uptake for the fully hydrated soil showed partition into soil organic matter to be the most important mechanism. This result showed preferential adsorption of water onto the minerals, thus significantly reducing the mineral surface available to the chemicals.

Gerstl and Yaron (1978) also found the adsorption from non-aqueous solution to be dependent on how hydrated the clay was when they studied the adsorption of a pesticide from aqueous and hexane solutions onto clay. Hance (1965) found the minerals to adsorb more diuron from non-aqueous solution (petroleum spirit) than from aqueous solution, and the opposite was observed for uptake by soil organic matter (higher uptake from aqueous solution). This was also the findings of Mills and Biggar (1969) and Yaron and Saltzman (1972) for their comparisons of uptake of pesticide from aqueous and hexane solutions.

2.3.3 Desorption

Several investigators (Pavlostathis and Mathavan, 1992, Pignatello et al., 1993, Connaughton et al., 1993) have found the desorption to happen at an initial fast rate and thereafter at a slower rate. The slower rate could be due to slow diffusion from micropores within the soil. It has therefore been suggested (Pignatello et al., 1993) that experiments preformed in a laboratory may not reflect what is happening at a contaminated site since many investigations are

conducted over a very short time period (days) compared to the time frame for a contaminated site (years).

Pavlostathis and Jaglal (1991) studied the desorption of trichloroethylene from a soil contaminated for a long time. They used seven successive hourly or daily reequilibrations with distilled water, and found a higher total desorption with daily reequilibrations (75%) than with hourly reequilibrations (43%), but the desorbed amount after 7 hourly reequilibrations was higher than the desorbed amount after the first of the daily reequilibrations (33%). They measured their desorption as a fraction of trichloroethylene extractable with methanol. They found 72% of the trichloroethylene was desorbed from a short continuous flow soil column during a period of 90 hours. The total desorbed amount was 73% after two additional equilibration periods of 24 hours with no flow.

Wu and Gschwend (1986) investigated sorption and desorption of four chlorobenzenes and they found all sorbed material to be desorbed within 2 days. They also found the desorption rate to increase with decreasing particle sizes.

Steinberg et al. (1987) found that pulverization promoted the release of 1,2-dibromoethane which had been present in the soil for a minimum of 19 years, thus suggesting that the organic chemical was trapped in micropores in aggregates. This was also the findings of Hatzinger and Alexander (1995). They found an increasing mineralization of phenanthrene by active bacteria when the samples were dispersed compared to left as

aggregates.

Pignatello et al. (1993) investigated the influence of soil organic matter on desorption of aged herbicides from soil. They found the desorption to be slow and depend on the soil organic matter content. The same result was found when normalizing the concentration of herbicide to the content of organic carbon for all particle size fractions. They therefore suggested that the rate of desorption was diffusion limited with a diffusive length scale of 1 μm, since the desorption rate from the ≤ 2 μm fraction was the same as for the other fractions.

The findings of Steinberg et al. (1987), Hatzinger and Alexander (1995) and Wu and Gschwend (1986) showed that the desorption was diffusion limited as suggested by Pignatello et al. (1993). This is also in agreement with the findings of Pavlostathis and Jaglal (1991) since successive reequilibrations showed increasing amount desorbed with the number of reequilibrations. The diffusion limitation is most likely due to slow diffusion from micropores in the soil particles.

2.3.4 Biological degradation

Soil (containing nutrients, water and oxygen for surface soils) provides a growth environment for populations of different microorganisms, which can use organic contaminants as a carbon source or as a cometabolite. Many contaminants,

such as PAHs and other hydrocarbons, are degraded by the bacterial population within the contaminated soil. The rate of degradation can often be increased by increasing the availability of the carbon source, the contaminant, and by supplying other crucial metabolic needs such as oxygen, nitrogen and phosphorous.

The degradation of organic contaminants, which is catalyzed by enzymes for oxidation, reduction and hydrolysis, may follow different chemical pathways depending on the organic contaminant and the microbial species.

The biological reactions are used in bioremediation of soils contaminated with different types of organic contaminants that may have a low water solubility (hydrophobic compounds). The water solubility of the contaminant is very important, since the bacteria are believed to have access to contaminants in the aqueous phase. The desorption process is therefore of importance, because organic contaminants trapped in the macropores of soil aggregates can be inaccessible to microorganisms (Pavlostathis and Jaglal, 1991).

The availability of a contaminant for biological degradation will be limited by the desorption rate. When the desorption rate becomes lower than the supply of substrate required for maintenance energy for the bacteria, the biodegradation will cease and a residual concentration of a contaminant can be observed. This observation is not a result of microorganisms being unable to degrade the compound.

Chung et al. (1993) pointed out the importance of intraparticle diffusion and sorption for the rate of biodegradation. They developed a model to determine if the biodegradation is limited by intraparticle diffusion or sorption, since the biodegradation process can be retarded by these processes.

Manilal and Alexander (1991) found a lower mineralization rate of unlabelled and ^{14}C-labelled phenanthrene in soil compared to the mineralization in an inorganic salts solution. They found the mineralization rate, measured by evolution of $^{14}CO_2$, to be directly related to the amount of soil organic matter when comparing four soils with different soil organic matter content. The desorption was decreasing with increasing content of soil organic matter. They found no desorbable activity for the soil with the highest amount of soil organic matter (36.7%) after 24 hours of equilibration with sterile distilled water (25g soil to 100 mL water) but a similar sample still showed evolution of $^{14}CO_2$ when incubated with microorganisms (no addition of liquid is mentioned). The finding of no desorbable activity could be due to a transformation of phenanthrene into higher molecular weight compounds with a lower solubility than phenanthrene in water which could lower the activity of phenanthrene to a level below detectability. Phenanthrene could still be present in a high enough concentration, especially since the water content seems to have been low for the mineralization study, to cause

mineralization from the soil sample when incubated with microorganisms or the microorganisms could be able to mineralize transformed phenanthrene. Manilal and Alexander did not use any other analytical method to determine if the desorbed activity from the other soil samples was due to phenanthrene.

Miller and Alexander (1991) investigated the influence of clay in suspension on the degradation of benzylamine. They found the mineralization to be slower in the presence of montmorillonite, which they suggested was caused by an irreversibly bound fraction. The microorganisms could only utilize the soluble fraction of the organic contaminant. They did not consider that the lower mineralization could have been due to benzylamine transforming into other compounds in the presence of clay, compounds not available for degradation by the microorganisms.

Hatzinger and Alexander (1995) found the mineralization rate of phenanthrene to be dependent on the soil type and the age of the contaminant. They observed an increasing mineralization rate after dispersing the samples for both freshly added and aged phenanthrene, though the increase was smaller for the aged than for the freshly added phenanthrene. This observation supports the importance of slow desorption and intraparticle diffusion in biodegradation.

The results found by Weissenfels et al. (1992) supported this theory as well. They investigated degradation of

anthracene oil in decontaminated soil (by extraction with toluene), sand and an amberlite resin for PAH adsorption (XAD 2) in shake-flasks. They measured the degradation by extraction and HPLC analysis after different time intervals using the extractable amount of anthracene oil from a similar sample with $HgCl_2$ to inhibit microbial activity as a reference. They found the anthracene oil to be fully degraded in sand after 7 days, while 23% of the anthracene oil remained in the soil after 28 days and no or very little degradation was found after 28 days for XAD 2. This shows the influence of desorption on biodegradation because the PAHs are assumed to be strongly sorbed to XAD 2. They also found a bioavailability limit when they added toluene extracted material to the same sample as it was extracted from. They found the PAH concentration, measured as the total of PAHs degradable by the mixed culture, to decrease to 28% of the original concentration over a period of 42 days, with very little decrease from day 14 to day 42. An incubation of 8 weeks of the same contaminated soil (from a tar-oil refinery) showed no significant degradation of the target compounds, thus indicating a binding within the soil matrix which made the compounds less available to microorganisms. This could be due to slow desorption and diffusion from intraparticle pores into the aqueous phase where the microorganisms can degrade the compounds.

This could also explain the residual concentration of anthracene that Gray et al. (1994) found. They found that 90% of the anthracene added to a soil with a low soil organic matter content was degraded within the first 4 days in roller bottles, but a residual of anthracene of 30 ppm remained after biodegradation, showing a fraction not available for biodegradation.

2.3.5 Chemical degradation

Chemical reactions that can take place in soils can be divided into three groups; hydrolysis, oxidation and polymerization.

The hydrolysis of contaminants is believed to be catalyzed by acidic or basic species. In an aqueous environment the hydrolysis will be catalyzed by H_3O^+ or OH^-, but the hydrolysis in a moist soil may also be catalyzed by copper or calcium (Dragun, 1988). The carboxyl groups in undissolved humic acid, which as mentioned earlier is present in soil organic matter, is also believed to be able to cause hydrolysis (Gamble and Khan, 1990).

Iron, aluminum, adsorbed oxygen and trace metals within layer silicates in clay and soil have been identified as catalysts promoting free-radical oxidation (Dragun, 1988). The water content of the soil or clay has been found to be an important factor for the catalyzed oxidation (Dragun, 1988).

Inscoe (1964) found that some polycyclic aromatic hydrocarbons could undergo changes when exposed to ultra-violet light on thin layer chromatogram plates containing silica gel or aluminum oxide. Since clay particles can contain both silica layers and aluminum oxides this photochemical change might also happen in a top soil exposed to light.

Montmorillonite has been found to initiate polymerization of different organic compounds by acting as a reducing agent (Dragun, 1988). Lotfabad (1994) found that anthracene polymerized to higher molecular compounds at low soil moisture content, when the mineral surface of a clay rich soil was not covered with water. Lotfabad identified bianthracene as one of the reaction products.

2.4 Discussion

Many investigations on the interactions between organic contaminants and soil have been conducted over the years, but the interactions are still not fully understood. Most investigations did not consider the large number of parameters that can influence the interactions, for instance chemical reactions are not considered in most adsorption/desorption studies. More recent investigations tend to be more aware of the many parameters, and there is an increasing number of investigators pointing out the importance of intraparticle diffusion in the desorption process.

Parameters like soil moisture content and contact time have been shown to be of importance to the uptake of organic contaminants by soils. Whether the uptake is dominated by soil organic matter or by the minerals depends on the soil moisture content and on the kind of chemical contaminant. The effect of a nonpolar organic carrier solvent has been studied by comparisons to the uptake from aqueous solution. No data on the role of the polarity of the organic solvent have been reported in the literature.

The interactions between organic contaminants and soil is therefore still an area where more research is needed.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Sampling

Two soil samples and one clay sample were chosen for the experiments. The A horizon of a Black Chernozem was sampled at the surface of a field at The University of Alberta Ellerslie Research Station south of Edmonton. The C horizon of a sandy clay loam was sampled at a depth of 75+ cm at a site south of Edmonton (Cooking Lake area, Edmonton, Alberta, Canada). The two soil samples were chosen to reflect two different levels of soil organic matter, a high and a low level.

The A horizon was acidic at the time of sampling, the pH in distilled water was 5.9. The soil contained 13% sand, 67% silt and 20% clay by weight. The organic carbon content was 4.88% by weight (8.41% soil organic matter) and the surface area of the clay fraction was 447 m^2/g (ethylene glycol monoethyl ether).

The C horizon was moderately calcareous with a pH of 8.3 in distilled water (Abder-Ruhman, 1980). The soil contained 50.6% sand, 25.5% silt and 23.9% clay by weight (Abder-Ruhman, 1980). The content of soil organic matter was 0.62% by weight (Abder-Ruhman, 1980) and the surface area of the soil was 157 m^2/g (ethylene glycol monoethyl ether).

Ca-Montmorillonite ("Cheto") from Arizona (Source Clay Minerals Repository, University of Missouri, Columbia) was used as a pure clay reference, since montmorillonite was the dominant clay type in the two soils.

The soils were air-dried, ground and sieved through a 10 mesh sieve (1.70 mm). The clay was used as received.

The characteristics of the soils and clay are shown in Table 3.1, and the mineralogy of the soils is shown in Table 3.2. The measured surface area of Ca-Montmorillonite, which exceeds the values reported in the literature, is reported as measured for comparison with the soils.

Table 3.1 Characteristics of the soils and the clay.

	% Sand	% Silt	% Clay	pH*	% Organic Carbon	Surface area+ m^2/g
A horizon	13	67	20	5.0	4.88	111
C horizon	51[b]	25[b]	24[b]	7.8[b]	0.36[b]	157
Ca-Montmorillonite			100			1092

* pH in 0.01 M $CaCl_2$
\+ Surface area by ethylene glycol monoethyl ether
[a] From Qualizza, 1994
[b] From Abder-Ruhman, 1980

Table 3.2 Mineralogy of the soils.

	Montmorillonite + Vermiculite %	Kaolinite %	Mica %	Quartz %	Muscovite %
A horizon[a]	60	20			20
C horizon[b]	33 + 9	26	24	7	

[a] From Qualizza, 1994
[b] From Abder-Ruhman, 1980

3.1.2 Chemicals

-Anthracene and phenanthrene, with purities of 98% and 96%, were purchased from Sigma Chemical Co. (St. Louis, USA). They were used without further purification.

-Methylene chloride, hexane and toluene, all HPLC grade, cyclohexane, spectranalyzed grade, methanol, Karl Fisher grade (low water), and hexanes, CERTIFIED ACS grade were purchased from Fisher Scientific (Nepean, Ontario, Canada).

-[9-^{14}C]Anthracene, from Amersham Corporation (Oakville, Ontario, Canada) was used for labelled experiments. The purity was 98% and specific activity was 15.1 mCi/mmol. The radioactive concentration was 500 μCi/mL. At the time of use, the purity was 86% and the radioactive concentration was 270 μCi/mL determined by thin layer chromatography and scintillation counting.

-Buffer solution consisted of 1.2 g/L KH_2PO_4, 2.8 g/L K_2HPO_4, 1.0 g/L NH_4Cl, 2.0 g/L Na_2SO_4, 2.0 g/L KNO_3, 0.05 g/L $FeSO_4 \cdot 7H_2O$ and 1 mL trace metal solution (Fedorak and Grbić-Galić, 1991) per liter of buffer solution. Sterile $MgSO_4 \cdot 7H_2O$ was added to a concentration of 0.2 g/L after autoclaving at 120°C for 20 minutes.

-Water added to the soil or clay samples to obtain a certain known moisture level was tap-water filtered through a particle filter (5 μm) and an active carbon filter followed by a reverse osmosis treatment.

3.1.3 Anthracene utilizing culture

The anthracene utilizing culture was a mixed culture enriched from a creosote contaminated soil (Gray et al., 1994). The cells, which were stored in 20% glycerol solution at -75°C, were washed and resuspended in 1 mL sterile distilled water before use. Each milliliter contained $5 \cdot 10^8$ cells.

3.2 Experimental methods

3.2.1 Preparation of soil with different water contents

The soil or clay was heated to 105°C for 48 hours to remove surface water and a part of the interlayer water. The soil or clay was then placed in a desiccator for a minimum of 2 hours to cool down to room temperature. Samples at two

different moisture levels were prepared for each sample type. One level was a monolayer coverage based on the surface area, the other level was the moisture content corresponding to a soil moisture tension of 15 bars. The lower level was chosen to avoid chemical reaction as found by Lotfabad (1994), and the upper level was chosen to get a water activity low enough to avoid bacterial activity. The water levels for each soil type are shown in Table 3.3 and Table 3.4.

Table 3.3 Samples with different water content for adsorption experiments.

Soil type	wt% H_2O	Solvent
A horizon	14.2 13.9 14.2	Toluene Toluene Hexane
A horizon	5.4 5.9	Cyclohexane Methanol
A horizon	5.6 5.6	Methanol* Hexanes*
Clay	37.2 36.2 37.6	Toluene Toluene Hexane
Clay	29.5 29.1	Cyclohexane Methanol

* Labelled adsorption experiments

Table 3.4 Anthracene-impregnated samples with different water content for degradation experiments.

Soil type	wt% H_2O*	Solvent	Loaded ppm anthracene
A horizon	4.3 ± 0.4	Methanol	125
A horizon	13.7 ± 0.5	Methanol	100
A horizon	4.5 ± 0.5	Hexane	250
A horizon	13.7 ± 0.4	Hexane	150
C horizon	2.7 ± 0.4	Methanol	100
C horizon	6.2 ± 0.3	Methanol	100
C horizon	2.4 ± 0.3	Hexane	250
C horizon	6.0 ± 0.5	Hexane	150
Clay	30.5 ± 0.5	Methanol	125
Clay	38.7 ± 0.7	Methanol	100
Clay	29.8 ± 0.9	Hexane	200
Clay	37.7 ± 1.1	Hexane	200

* Mean ± standard error, n=7

The required amount of water was added to the soil or clay and the sample was thoroughly mixed to get an uniform distribution of water. The sample was then left for 72 hours to equilibrate before use in adsorption experiments or impregnation with anthracene for degradation experiments. It has been shown earlier by Lotfabad (1994) that 24 hours is sufficient to give a homogeneous distribution of water in similar soil samples.

3.2.2 Preparation of anthracene impregnated samples

A solution of anthracene in methanol or in hexane was added to the moist sample. The amount of solution added was designed to have enough solvent to wet the sample without having a free solvent phase. The added amount of solvent solution was varied with the soil type and the water content of the sample, thus giving different levels of loading concentration as shown in Table 3.4.

The anthracene-impregnated samples were kept in closed containers at 22°C in a dark place, to prevent photo-oxidation of anthracene to anthraquinone during the contact time.

The solvent was removed from the sample by evaporation in a vacuum-oven at 40°C and approximately 5 kPa after the required contact time had elapsed. The solvent was considered removed when a weight loss corresponding to the weight of added solvent was obtained. The sample was then kept in a freezer at -25°C until the time of use.

3.2.3 Adsorption experiments

Test tubes with a volume of 15 mL were used for adsorption experiments. The ratio of solid sample to solvent solution was 6 g of moist soil or 3.5 g of moist clay to 7 mL solvent solution containing 0 or 0.4 mg anthracene/L. The test tubes were placed in a CEL-GRO Tissue Culture Rotator, LAB-LINE, with a rotation speed of 33 rpm in a dark place at 22°C. One sample with and one sample without anthracene (blank) was

used at each time of measurement.

3.2.4 Labelled adsorption experiments

Adsorption of labelled anthracene onto the low moisture A horizon soil was studied at 3 concentration levels, 0.5, 1 and 5 mg/L in methanol or hexanes. A 6 g soil sample and 7 mL of solvent solution was placed in each test tube with a volume of 15 mL. The test tubes were covered with aluminium foil and placed in a New Brunswick Scientific TC-7 rotator, with a rotation speed of 24 rpm at 22°C.

Samples at each concentration level and a sample containing no anthracene (blank) were used at each time of measurement.

Test tubes containing 7 mL of the added solutions were placed in the rotator, and the activity of these solutions was measured at the same time as the last set of samples to make sure that adsorption onto the test tube was not occurring.

3.2.5 Biological degradation experiments

Roller bottles (1.7 L) were used for studies of the degradation of anthracene. The ratio of solid sample to buffer solution was 100 g of solid to 67 mL of buffer solution, after Banerjee et al.(1995). It was necessary to add up to 10 mL of sterile distilled water to some samples to obtain a slurry with good mixing. $CaCO_3$ (2.0 g) was added to each roller bottle containing the A horizon soil to keep pH in 0.01 M

$CaCl_2$ above 6.0. The roller bottles were inoculated with the anthracene utilizing culture and then placed on rollers with a roller bottle rotation speed of 1.7 rpm in a dark room at 22°C.

3.3 Analytical methods

3.3.1 Analysis of anthracene for adsorption studies

The concentration of anthracene in solution was measured on a Shimadzu UV-160 spectrophotometer after centrifugation at 2460 rpm (1100 RCF) for 10 minutes. The wavelength for each solvent was chosen to be where anthracene showed the highest absorbance (375-379nm), and the concentration was calculated using the slope of a standard curve for each solvent (shown in appendix A).

3.3.2 Analysis of anthracene for labelled studies

A known volume of solvent solution containing labelled anthracene was added to a scintillation solution containing xylene and methanol, purchased from Amersham Canada Ltd. A Beckman LS 3801 liquid scintillation counter was used to measure the activity, which was given directly as the number of decays per minute.

3.3.3 Analysis of anthracene for degradation studies

A 2.5-25 g sample was extracted with methylene chloride using a Soxhlet extraction unit (EPA method # 3540). An

extraction time of 4 hours was found to be sufficient by comparing samples extracted for 4, 8 or 12 hours (shown in appendix C).

The anthracene-impregnated soil was extracted directly without drying of the sample. Anthracene-impregnated clay samples and samples from roller bottles were dried with anhydrous $MgSO_4$ before extraction. These methods were based on a comparison of extraction of dried and undried anthracene-impregnated samples (appendix C). The recovery of anthracene from the A horizon soil was found to be higher for undried samples compared to dried samples. The same recovery was found for the C horizon soil with and without drying, and the recovery from the clay samples was higher for dried samples compared to undried samples.

The slurry samples from the roller bottle experiments had to be dried prior to extraction. A comparison of extraction following two drying methods was performed to make sure maximal amounts of anthracene were recovered. An A horizon sample from a roller bottle experiment which had been dried with anhydrous $MgSO_4$ was compared to a similar freeze dried sample. The recovery of anthracene was the same from both samples, therefore addition of magnesium sulphate did not impede extraction.

The extracted amount of anthracene was measured by gas chromatography using phenanthrene as an internal standard (an example of a calibration curve is shown in appendix B). A

Hewlett Packard 5890 gas chromatograph equipped with a HP-1 (30m*0.25mm*0.25μm) capillary column and a flame ionization detector was used for the analysis.

3.3.4 Moisture content

The moisture content was determined as the weight loss at 105°C after 24 hours divided by the amount of wet sample used.

3.3.5 Particle size distribution

The soil was fractioned into three particle size fractions by sedimentation and sieving after dispersion with ultra sound (Sheldrick and Wang, 1993).

3.3.6 Organic carbon

As a measure of total carbon, the CO_2 produced by combustion with oxygen at 1300°C was measured with a LECO Carbon Determinator CR12 equipped with an IR-detector. The inorganic carbon was subtracted from the total carbon to get the organic carbon content of the sample (Tiessen and Moir, 1993).

3.3.7 Surface area

The surface area of the samples was determined from the uptake of ethyl glycol monoethyl ether at approximately 5 kPa for 48 hours after grinding and drying of the sample at 105°C for 48 hours (Carter et al., 1986). The soil organic matter

had been removed by oxidation with sodium hypochlorite (Lavkulich and Wiens, 1970) prior to measurement of surface area for the A horizon soil.

3.3.8 Soil moisture tension

The sample was saturated with distilled water and then equilibrated between pressure plates for 48 hours at 15 bars. The remaining moisture was determined by the weight loss at 105°C after 24 hours (Topp et al., 1993).

3.3.9 pH

A 1 g soil slurry sample or soil sample was mixed with 9 mL 0.01 M $CaCl_2$ or 9 mL distilled water and left for 2 hours to let the solid settle. The supernatant was filtered through a 0.45 μm filter and the pH was measured with a Fisher Scientific accumet mode 10 pH meter equipped with a combined electrode.

3.3.10 Data

Results are given as mean ± standard error when more than one measurement was preformed, with n=1 when nothing else is mentioned. The standard error was calculated using the following equation

$$Standard\ Error = t_{0.975}(n-1) * \frac{std}{\sqrt{n}}$$

where std is the standard deviation and n is the number of measurements.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Adsorption of anthracene

The adsorption of anthracene from solvents with different polarities onto A horizon soil and clay was studied by spectrophotometric measurements and scintillation counting of anthracene in solution as a function of time to determine the time required to reach equilibrium. The uptake of anthracene by the solids was calculated as the difference between added and measured anthracene.

4.1.1 Experiments with unlabelled anthracene

Experiments with unlabelled anthracene were performed with high moisture content samples of the A horizon soil and the clay for two solvents (toluene and hexane) and low moisture content samples of the A horizon soil and the clay for two solvents (methanol and cyclohexane).

Methanol and toluene were found to extract material from the soil, thus creating a high background value (0.2 absorbance (abs) for toluene and 0.8 abs for methanol) and therefore the precision of the analytical method was lost due to subtraction of two large numbers to get the low absorbance caused by anthracene (approximately 0.02 abs). An apparent uptake of anthracene by the clay was found over a period of 30

days for toluene and 256 days for methanol. This apparent uptake was caused by an increase in background absorbance value.

An apparent uptake of anthracene by the A horizon soil was found over a period of 80 days for anthracene in hexane as shown in Figure 4.1. This result was an artifact caused by an increasing background absorbance value. The concentration of anthracene in hexane stayed the same for the first 30 days in the experiment with clay. A small decrease in concentration without changes in background value was found between each subsequent measurement, showing a slow uptake of anthracene over a period of 182 days as illustrated in Figure 4.1.

The A horizon soil showed an initial drop in concentration of anthracene in cyclohexane over the first 30 days and thereafter a stable concentration, whereas the clay still showed decreasing concentration at day 180. The results are shown in Figure 4.2. It was decided at day 180 to use the last clay sample to determine the recovery of anthracene after evaporation of cyclohexane and 72 hours of soxhlet extraction with methylene chloride. The characteristic peaks of anthracene (Figure 4.3 top) were not found, as shown in Figure 4.3 (bottom), when a spectrophotometric scan of the extract was performed. Scattering due to fine particles that could not be centrifuged out caused an increase in the baseline at all wavelengths. A wide peak was observed in the range of UV-wavelengths from 200 to 315 nm where the characteristic of

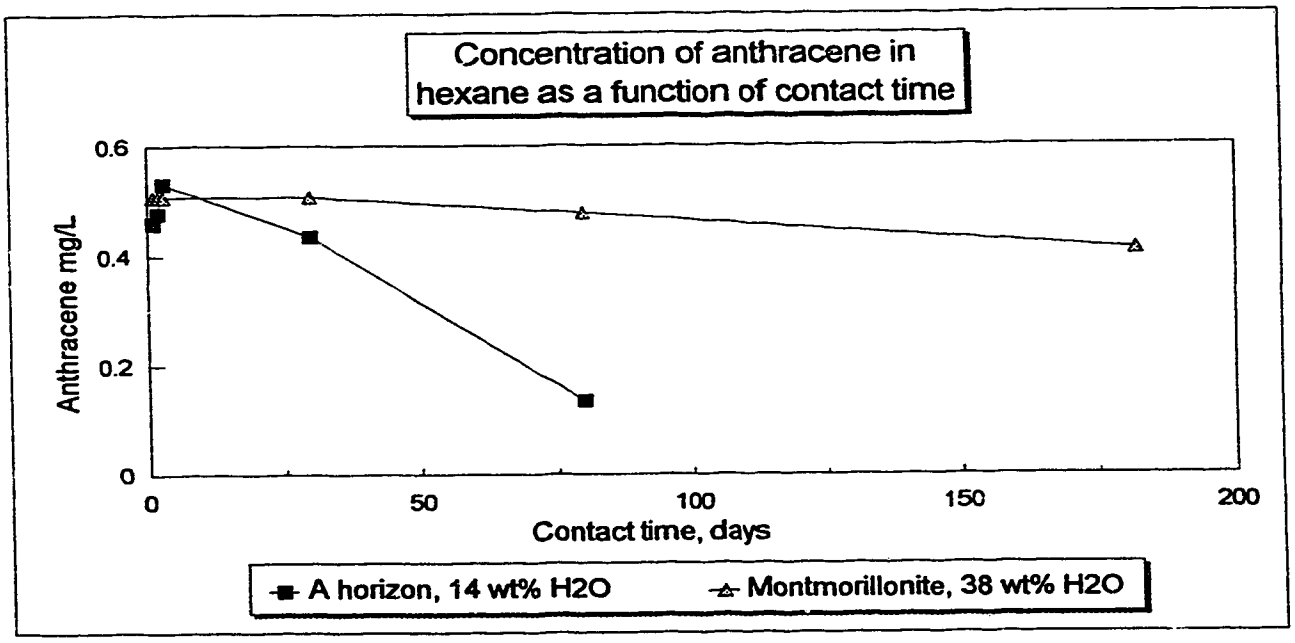

Figure 4.1 Concentration of anthracene in hexane as a function of contact time with A horizon soil or Ca-Montmorillonite. Initial concentration 0.4 mg/L.

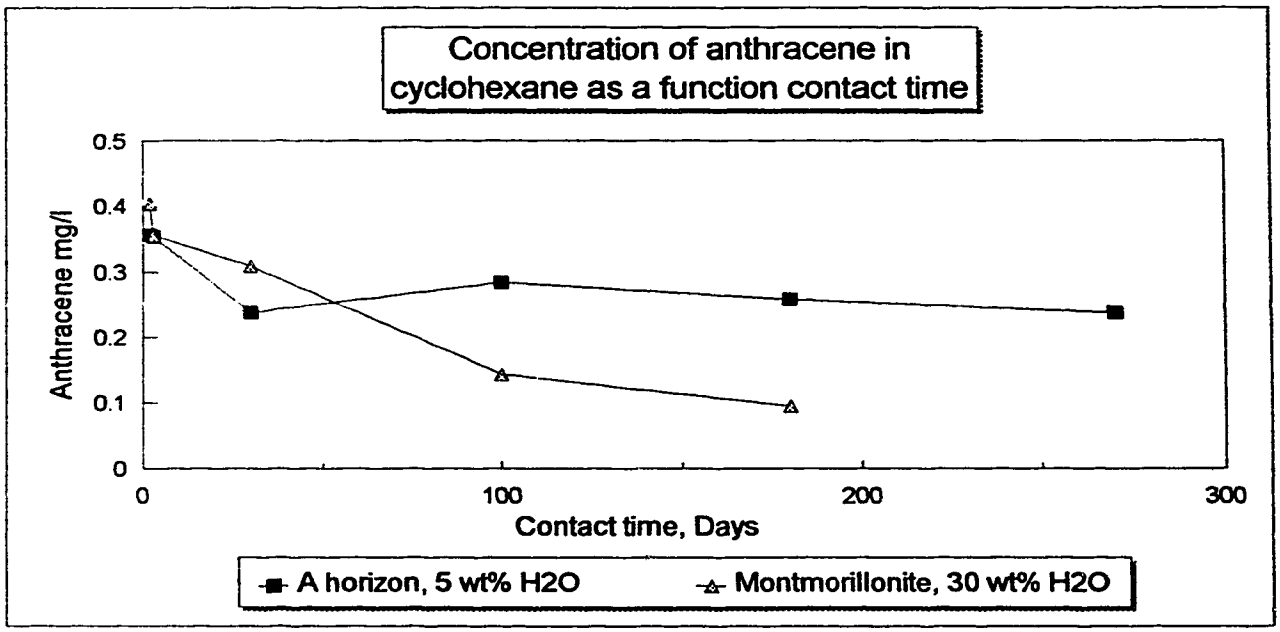

Figure 4.2 Concentration of anthracene in cyclohexane as a function of contact time with A horizon soil or Ca-Montmorillonite. Initial concentration 0.4 mg/L.

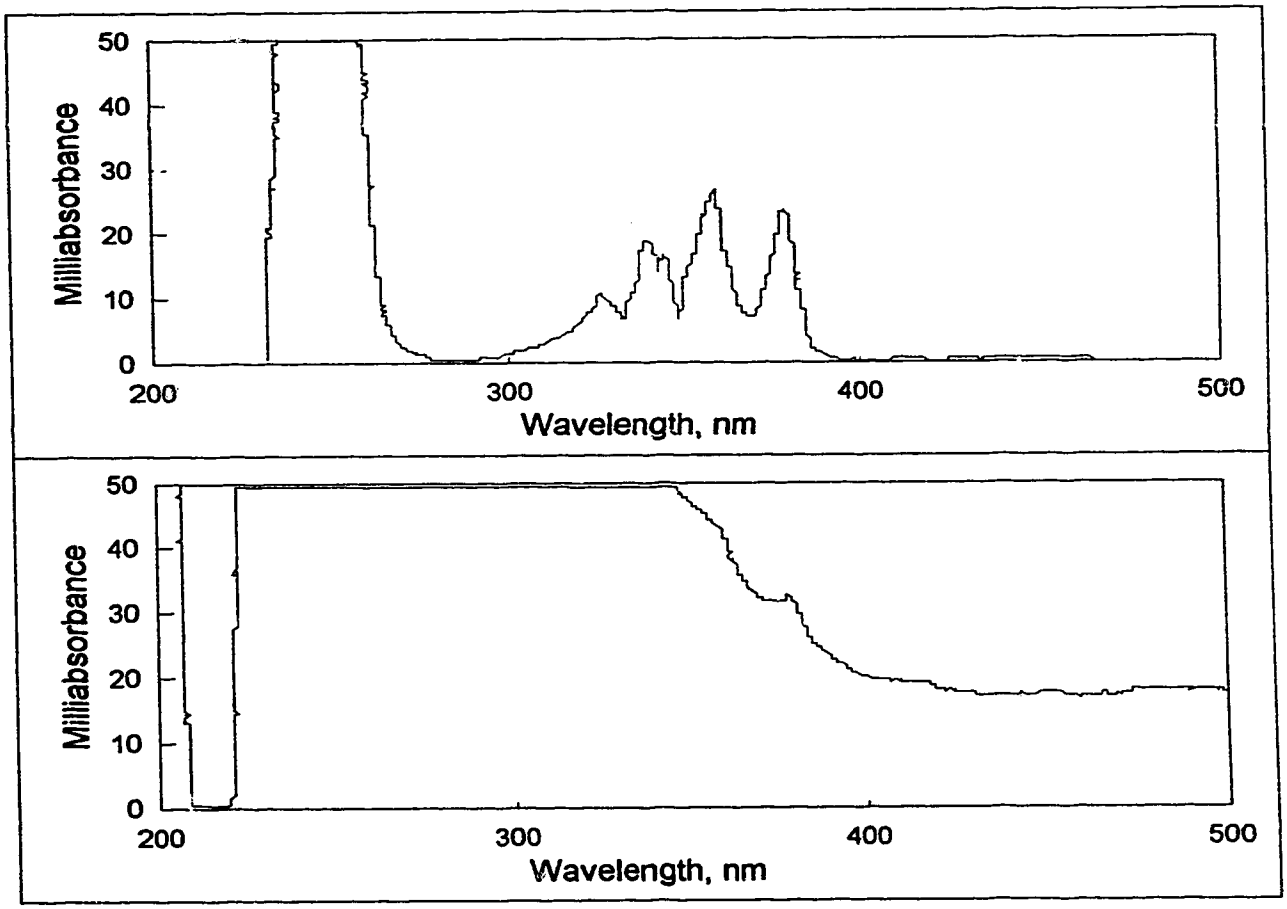

Figure 4.3 UV-spectra of anthracene 0.6 mg/L (top) and extract from montmorillonite after 180 days (bottom).

absorption of aromatic compounds are found.

4.1.2 Experiments with labelled anthracene

Experiments using ^{14}C-labelled anthracene were conducted with the A horizon soil at the low moisture content using hexanes and methanol as carrier solvents for anthracene. Liquid samples were measured by scintillation counting after 4 and 15 days and the uptake by the soil was calculated from the difference between added and measured activity.

A higher uptake was found for samples with methanol compared to samples with hexanes, and uptake was still occurring between day 4 and day 15 as shown in Figure 4.4 and 4.5. The difference between day 4 and day 15 was more pronounced for samples with methanol than for samples with hexanes. The isotherms for the samples with hexanes were close to linear.

4.1.3 Discussion

The results from the spectrophotometric scanning (Figure 4.3) showed a wide peak where aromatic compounds absorb and no distinct peaks like the ones for anthracene. This observation suggested that a chemical reaction, transforming anthracene into other organic compounds, had taken place. Extracted soil organic matter could not have contributed to aromatic absorbance since the extract was from a clay sample. This means that the anthracene must have reacted and therefore it

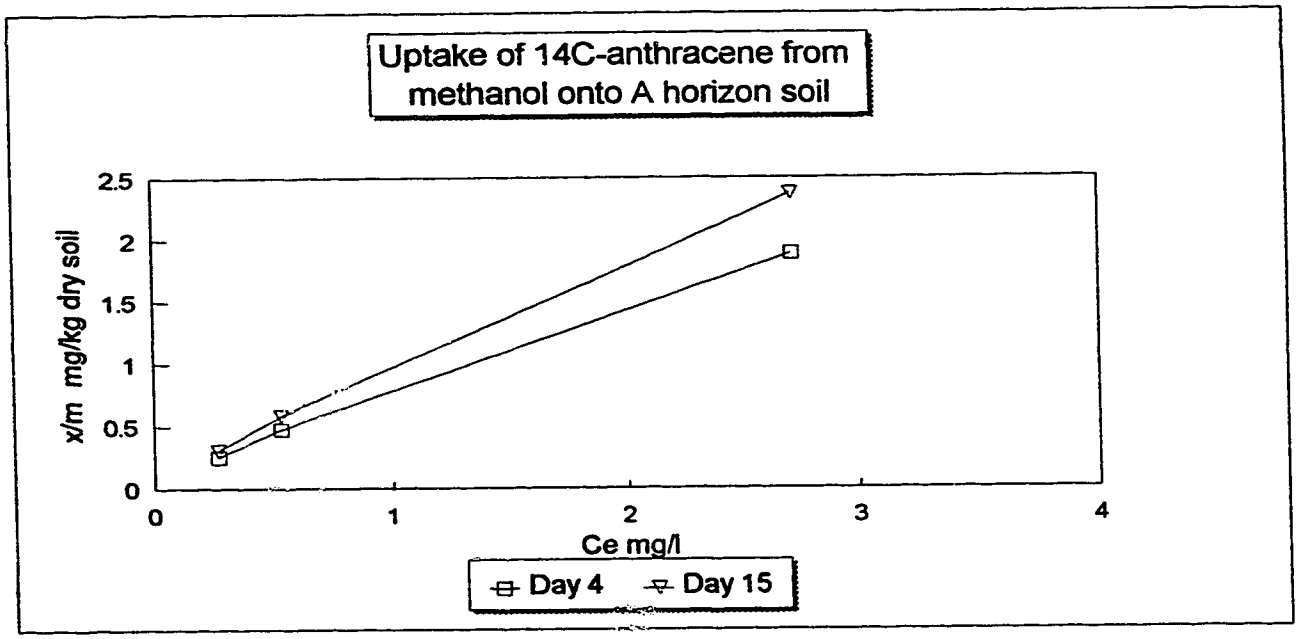

Figure 4.4 Uptake of labelled anthracene from methanol.

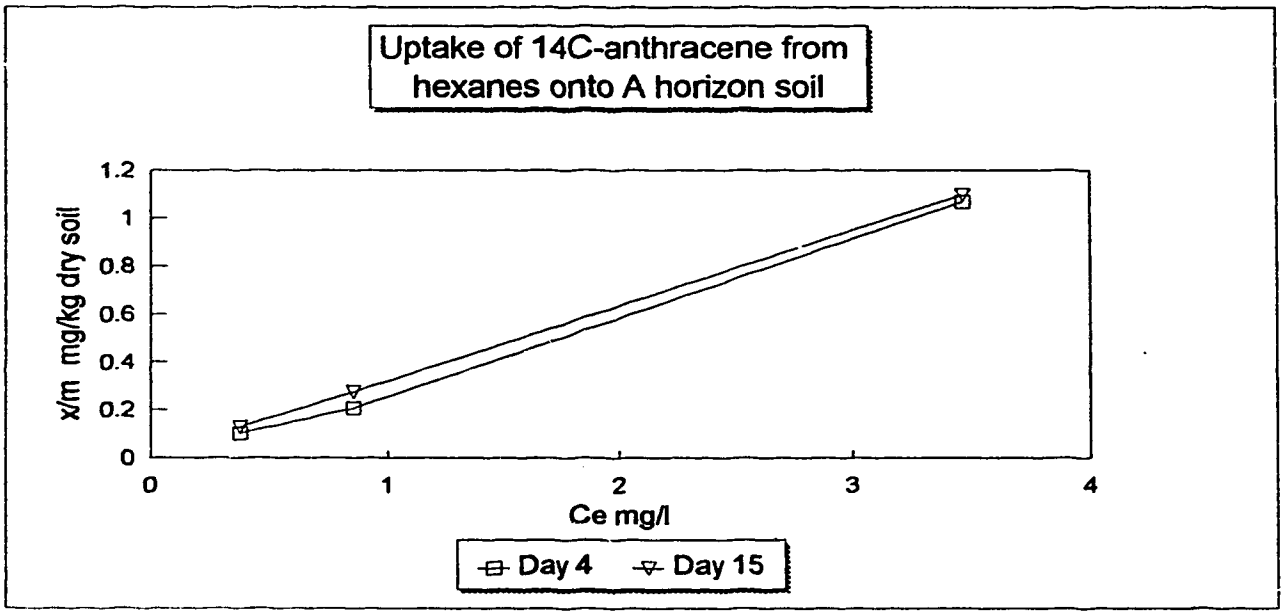

Figure 4.5 Uptake of labelled anthracene from hexanes.

could not be established if equilibrium had actually been reached.

These results are in agreement with the findings of Lotfabad (1994), whose results showed a polymerization of anthracene catalyzed by trace metals associated with the minerals.

Based on the few measurements for the cyclohexane-clay system a logarithmic plot (Figure 4.6) reveals that it could be a first order reaction, since linearity was obtained.

The finding of nearly linear isotherms for the uptake of ^{14}C-labelled anthracene from hexanes was in agreement with the findings of Chiou et al. (1985), who ascribed the linearity of uptake of nonionic organic compounds for fully hydrated soil to partitioning into soil organic matter. They did not consider the possibility of a reaction taking place.

Knowing that reaction was taking place, it would not make sense to use the results from the ^{14}C-labelled experiments to obtain a non-aqueous partition coefficient, since this would include unknown contributions from reaction products.

To obtain a non-aqueous partition coefficient at least one more analytical method should be included to identify the compounds causing the activity in the solution, so that the chemical reaction can be accounted for. For example, an extraction of the solids could be used to obtain the sorbed concentration directly, with a correction for the amount of compound left behind by the solvent in the pores of the solid.

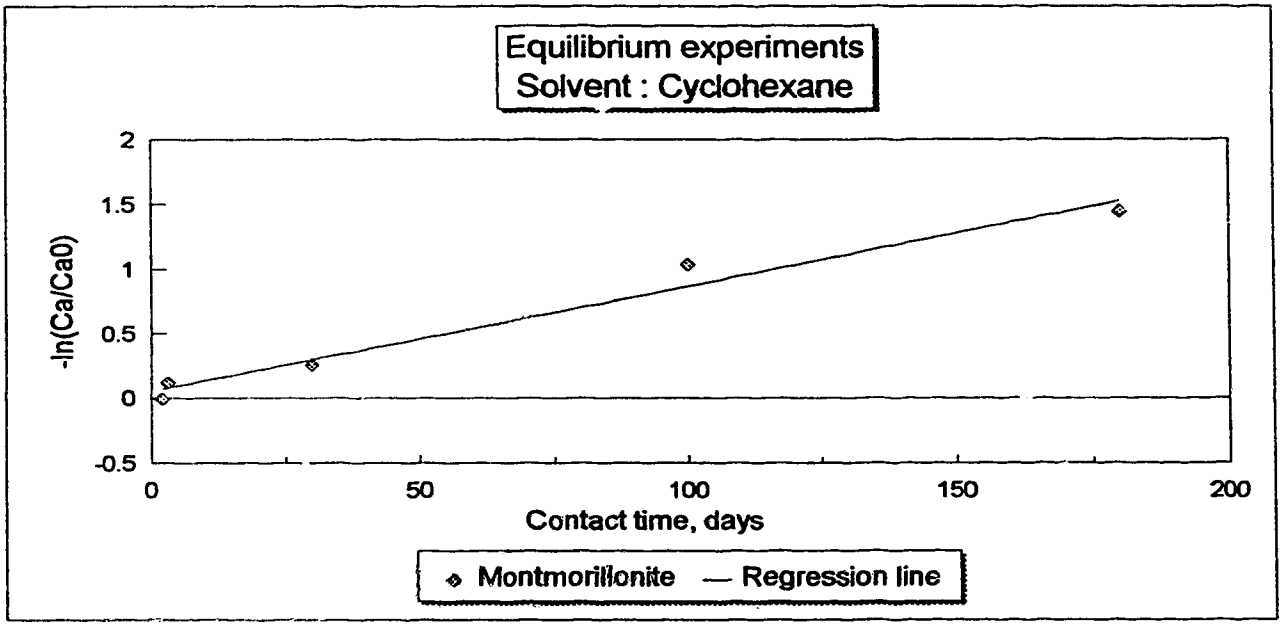

Figure 4.6 Negative logarithm of the ratio of measured anthracene in cyclohexane to initial concentration as a function of contact time. Ca is the measured concentration and Ca0 is the initial concentration of anthracene in solution.

Direct measurement of both liquid and solid phase concentrations would allow determination of the partition coefficient (or sorption isotherm) even in the presence of reaction products.

The finding of chemical reaction for the montmorillonite sample at monolayer coverage of the surface by water indicated that the montmorillonite fraction of the soils would also be active for chemical reaction.

4.2 Chemical degradation of anthracene

The two soils and the clay were loaded under different moisture conditions as described in chapter 3 using methanol and hexane as the carrier solvents for anthracene. The lower moisture level was chosen as a monolayer coverage of the surface, based on surface area measurements, to avoid the reaction of anthracene as found by Lotfabad (1994). The upper moisture level was chosen based on a soil moisture tension of 15 bars to keep the water activity (no free water) below the level required for bacterial activity.

Aggregation was observed in all of the samples after addition of water to obtain the required moisture level. Both soils had been sieved previously through a 1.7 mm sieve and the clay was used a received (powder). Under visual inspection the clay showed the highest degree of aggregation with lumps in the size range of approximately 1-7 mm. The C horizon soil, with lumps in the size range of approximately 1-4 mm, showed

a higher extent of aggregation than the A horizon soil, which contained lumps in the size range of approximately 1-2 mm. The subsequent contact with solvent had no effect at the mm scale, based on visual observation.

Crystals of anthracene with a size of approximately 50 μm or less was observed visually on the solid for samples in contact with anthracene in hexane for 180 days. The crystallization was due to slow unintended loss (50-90% of the added weight of hexane) of hexane by evaporation during the contact time.

Anthracene was extracted after the required contact time between the solvent solution and the soil had elapsed and the solvent had been removed. The concentration of anthracene was measured by GC. One batch of loaded sample was prepared for each of the loading conditions.

The standard error (95% confidence level) on the measurement of recovery of anthracene was determined to be in the range of 1-7 % recovery, with a median of 3 % recovery, except for samples where anthracene had crystallized due to loss of solvent. The standard error on these samples were from 14 to 47 % recovery due to difficulties in subsampling. Results for determination of standard error are given in appendix E. The results for percentage recovery of added anthracene from the A horizon soil are given in Figure 4.7 and results for recovery from both soils and the clay are all given in appendix D.

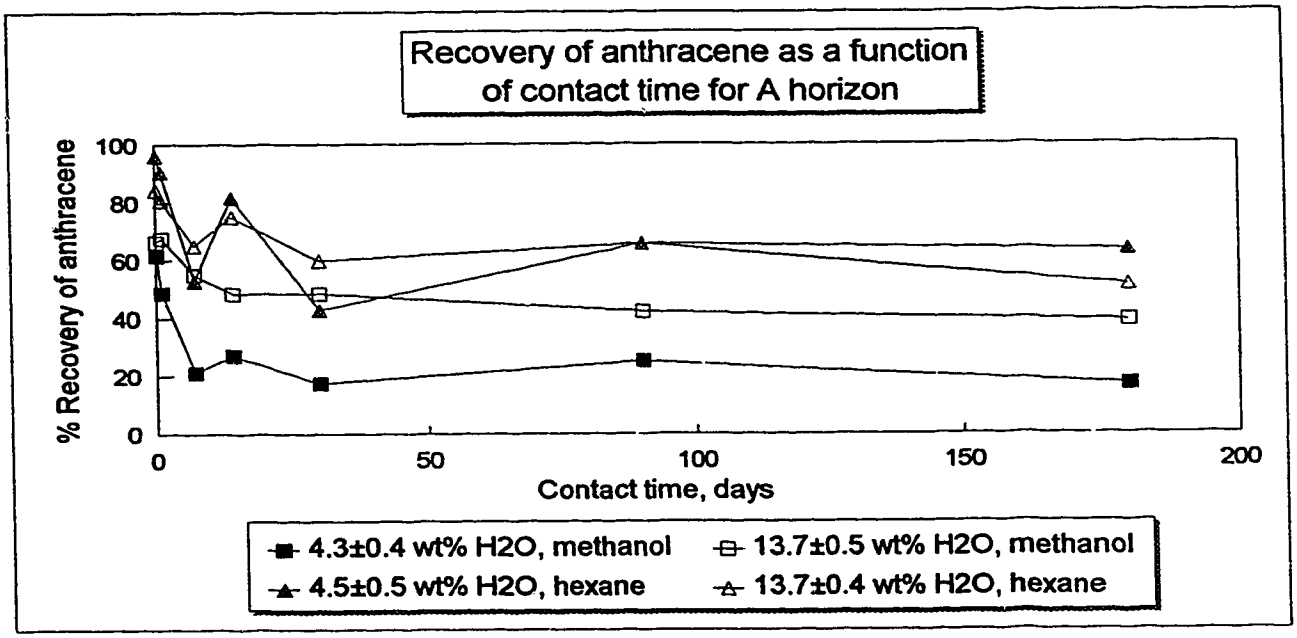

Figure 4.7 Recovery of anthracene from A horizon soil as a function of contact time. Points for day 180 represent the mean of 5 measurements all other are single point measurements.

4.2.1 Recovery of anthracene

The data in Figure 4.7 for the A horizon soil showed an initial decrease in recovery of added anthracene within the first 1-2 weeks and thereafter the recovery of anthracene stabilized at a low level. The results for hexane treated samples at different moisture levels showed no significant difference by two-sided F- and t-tests at the 95% confidence level when comparing from day 7 to day 180. The recovery of added anthracene for the hexane treated samples over this period was 62±8 % recovery (n=10). The methanol treated samples showed a significant difference in recovery of anthracene between the two moisture levels and a lower recovery compared to the hexane treated samples. The recovery for the methanol treated samples over the day 7 to day 180 period was 22±5 % recovery (n=5) and 47±7 % recovery (n=5) for the low and high moisture levels respectively.

No trend with time was found for the C horizon soil and the clay for any of the moisture levels or solvent treatments by two-sided F- and t-tests at the 95% confidence level. The recovery for all samples over a 180 day period was 87±3 % recovery (n=28) and 90±3 % recovery (n=28) for the C horizon and the clay respectively.

No significant amount of anthraquinone was detected by GC in any of the samples.

4.2.2 Discussion

A recovery of 100% of the added anthracene was not obtained for any of the soils or the pure clay, so that all showed some sign of a loss of anthracene with time. The loss in recovery could not be due to photooxidation of anthracene to anthraquinone, since no significant amount of anthraquinone was detected in any of the samples. The result implies an interaction with mineral sites not covered by water, since a loss was also seen for the high moisture content clay, which contained no organic matter. The loss in recovery is in agreement with the evidence of reaction as found for montmorillonite in the sorption study. This result indicated that the montmorillonite fraction present in the two soils would also be active for chemical reaction.

The loss of recoverable anthracene for the A horizon soil was a quick process, since the recoverable anthracene stabilized after the first week of contact at a level that depended on solvent polarity and moisture content. The first drop in recovery of anthracene from the A horizon soil cannot be attributed to microbial activity, since the experiment was designed to keep the water activity below the level needed for bacterial activity (i.e. no free water). The heating of the soil at 105°C for 48 hours was also expected to inhibit the microbial population and thereby decrease their activity. The storage of the soil in closed containers with the solvent present would not create a favourable environment for

microbial growth either, especially in the case of methanol, which is highly toxic to bacteria at high concentration. Bacteria and fungi could still be present, though, as spores since none of the treatments would sterilize the samples. The storage conditions, however, would not support active microorganisms.

The initial decrease in recovery of anthracene from the A horizon soil with contact time was in agreement with the results obtained by Lotfabad (1994) using the same A horizon soil, but at lower moisture contents. Lotfabad found the recovery of anthracene to decrease with increasing contact time and to be dependent on the moisture content. The highest recovery was for the highest moisture content when anthracene was applied to the soil in a methylene chloride solution. After a contact time of 9 days, Lotfabad found a decrease of 211 ppm from an initial concentration of 600 ppm (or a recovery of 389 ppm corresponding to 65% recovery) for a moisture content of 1.5% based on dry soil.

Comparing the soil organic matter for the two soils and the clay, the A horizon, which showed the lowest recovery of anthracene, had the highest amount of organic carbon (4.88%), the C horizon had 0.36% organic carbon and the clay, which showed the highest recovery, had no organic carbon. This observation suggests an interaction of anthracene with moist soil that depends on the soil organic matter.

The fast decrease in recoverable anthracene from the A horizon is not in agreement with the data of Hatzinger and Alexander (1995), who found that the recovery of phenanthrene from a soil with a high organic content (19.3% organic matter) decreased even after a period of 315 days. Their recovery of phenanthrene was much higher (87% after 315 days) than the recovery found for anthracene in this study. The higher recovery of phenanthrene in Hatzinger and Alexander's study was in agreement with the findings of Lotfabad (1994) that found the recovery of unreacted phenanthrene was higher than the recovery of anthracene from the same C horizon soil as used for this study. Lotfabad's work at much lower moisture contents showed that the loss in recovery was due to chemical reactions of both phenanthrene and anthracene.

Hatzinger and Alexander (1995) did their measurements with labelled phenanthrene, therefore, there could have been reactions occurring that they did not take into account. They only used their initial samples at very short contact times to determine that reaction was not taking place by comparing results from activity measurements with results from HPLC measurements.

Weissenfels et al. (1992) found a similar fast drop, within the first few hours, to approximately 60% recovery of anthracene oil added to a decontaminated soil in a mineral salts medium (aqueous solution) and thereafter a slow decrease in recovery as the contact time increased to 28 days. They

argued that the decrease could not be due to microbial activity since $HgCl_2$ was added to inhibit growth, and since microbial activity would not have caused a fast decrease in recovery within the first few hours. They measured the recovery by extraction and GC analysis, but they did not mention any considerations of a possibility of reaction taking place.

The low recovery of anthracene from the A horizon soil cannot be attributed to different solubility of anthracene in the solvent (methanol or hexane) used for loading the soil compared to the solvent used for extraction (methylene chloride), since anthracene has a higher solubility in methylene chloride than in the other two solvents and since close to 100% recovery was obtained for the other two solids at short times.

The difference in recovery for methanol treated A horizon samples compared to hexane treated samples must be attributed to the miscibility of methanol and water, whereas hexane and water are not miscible. The effect of methanol being miscible with water was seen most clearly for the low moisture content samples where the water activity was more influenced by the presence of methanol than for the high moisture content, thereby lowering the recovery of anthracene.

Methanol, being polar, could be competing with water for active adsorption sites on the minerals. This could create a more favourable environment for anthracene to interact with

the minerals as the methanol is removed, since anthracene is more soluble in methanol compared to water. The fact that the low organic carbon soil and the clay showed high recovery of anthracene even after 180 days of contact implies that the interaction between anthracene and the minerals is limited to a small fraction of the mineral sites or it is a slow process.

Soil organic matter may prevent water wetting of some mineral sites by covering them. The soil organic matter occupying mineral sites may be extracted by methanol, and thereby give anthracene better access to the mineral sites where chemical reaction can take place. Such a mechanism would result in a lower recovery of anthracene from a soil with a high organic matter content compared to one with low organic matter content.

High density non polar organic liquids are used for immiscible displacement of soil water by centrifugation (Elkhatib et al., 1986). Immiscible displacement of water by hexane could not have taken place in the samples for several reasons. First, there was no free water to displace and hexane is a low density liquid. Second, a displacement would have had the highest influence on the clay (i.e. lowest recovery of anthracene for the pure clay) compared to the soils, since displacement would cause anthracene to intercalate in the interlayer space in the montmorillonite, and thus be less extractable. Third, the unintended weight loss due to evaporation of hexane, and the crystallization of anthracene

as observed for samples with a contact time of 180 days, would not have occurred if displacement was taking place.

4.3 Bioavailability of anthracene

A portion of the samples prepared for studying chemical degradation of anthracene were used for studying biodegradation of anthracene in roller bottles to see if the bioavailability was dependent on how anthracene was deposited in the soil.

Subsamples were taken from the roller bottles at different times, extracted and analyzed by GC. The key results are given in Table 4.1, 4.2 and 4.3 (all results are listed in appendix F). No significant amount of anthraquinone was detected in any of the samples.

4.3.1 Role of contact time

It can be seen from Table 4.1, 4.2 and 4.3 that there was no effect on bioavailability for a contact time between the soil and the solvent solution of up to 90 days; all anthracene was degraded after 4 weeks in roller bottles. The one sample with a contact time of 7 days that showed a residual concentration in Table 4.2 was likely an outlier, since similar samples with a contact time of 1 and 30 days showed no residual anthracene. The data for a contact time of 180 days showed a significantly higher residual concentration of anthracene for both the hexane and methanol treated samples

for the C horizon soil and for the clay. For the A horizon soil, a significant residual concentration was only found for the hexane treated samples.

Table 4.1 Anthracene concentration in roller bottle experiments with A horizon soil. All concentrations are mg anthracene/kg dry soil (ppm).

Loading conditions	Inoculum	Time 0	1 week	4 weeks
Contact time 1 day				
Methanol, 4.7 wt% H_2O	Yes	61	5	1
Methanol, 13.9 wt% H_2O		68	6	1
Hexane, 4.3 wt% H_2O		227	8	<1
Hexane, 14.1 wt% H_2O		121	6	<1
Contact time 7 days				
Methanol, 3.9 wt% H_2O	Yes	27	19	2
Methanol, 6.7 wt% H_2O		24	21	2
Hexane, 4.2 wt% H_2O		132	54	1
Hexane, 13.4 wt% H_2O		98	9	2
Contact time 30 days				
Methanol, 3.8 wt% H_2O	Yes	22	11	2
Methanol, 14.1 wt% H_2O		48	5	1
Hexane, 4.7 wt% H_2O		107	2	1
Hexane, 14.4 wt% H_2O		90	4	1
Contact time 90 days				
Methanol, 4.7 wt% H_2O	Yes	31	2	1
Methanol, 14.0 wt% H_2O		42	3	1
Hexane, 4.9 wt% H_2O		164	4	1
Hexane, 13.9 wt% H_2O		99	3	1
Contact time 180 days				
Methanol, 3.6 wt% H_2O	Yes	21	6	1
Methanol, 12.4 wt% H_2O		39	6	2
Hexane, 3.3 wt% H_2O		159	238	22
Hexane, 13.7 wt% H_2O		77	31	7
Contact time 1 day				
Methanol, 4.7 wt% H_2O	No	61	15	2
Methanol, 13.9 wt% H_2O		68	10	1
Contact time 7 days				
Methanol, 3.9 wt% H_2O	No	27	9	10
Methanol, 6.7 wt% H_2O		24	10	3
Contact time 1 day	No			
Methanol, 13.9 wt% H_2O	+ $HgCl_2$	68	7	7

Table 4.2 Anthracene concentration in roller bottle experiments with C horizon soil. All concentrations are mg anthracene/kg dry soil (ppm).

Loading conditions	Inoculum	Time 0	1 week	4 weeks
Contact time 1 day				
Methanol, 2.9 wt% H_2O		91	3	<1
Methanol, 6.7 wt% H_2O	Yes	97	2	<1
Hexane, 2.4 wt% H_2O		223	9	1
Hexane, 6.3 wt% H_2O		152	8	<1
Contact time 7 days				
Methanol, 2.3 wt% H_2O	Yes	71	3	1
Methanol, 6.4 wt% H_2O		89	9	6*
Contact time 30 days				
Methanol, 3.5 wt% H_2O		86	2	1
Methanol, 5.8 wt% H_2O	Yes	93	1	<1
Hexane, 2.7 wt% H_2O		184	17	2
Hexane, 5.4 wt% H_2O		126	9	1
Contact time 90 days				
Methanol, 2.6 wt% H_2O		88	3	1
Methanol, 6.4 wt% H_2O	Yes	83	1	<1
Hexane, 2.7 wt% H_2O		214	6	<1
Hexane, 6.5 wt% H_2O		113	14	1
Contact time 180 days				
Methanol, 2.1 wt% H_2O		94	20	10*
Methanol, 5.6 wt% H_2O	Yes	80	7	5*
Hexane, 2.2 wt% H_2O		221	282	88
Hexane, 6.5 wt% H_2O		125	110	26
Contact time 30 days				
Hexane, 2.7 wt% H_2O	No	184	168	100
Hexane, 5.4 wt% H_2O		126	98	1

* n=2

Table 4.3 Anthracene concentration in roller bottle experiments with Ca-Montmorillonite. All concentrations are mg anthracene/kg dry soil (ppm).

Loading conditions	Inoculum	Time 0	1 week	4 weeks
Contact time 30 days				
Methanol, 30.5 wt% H_2O	Yes	126	2	1
Methanol, 37.1 wt% H_2O		93	3	<1
Hexane, 29.3 wt% H_2O		182	11	<1
Hexane, 37.7 wt% H_2O		187	6	1
Contact time 90 days				
Methanol, 30.6 wt% H_2O	Yes	107	2	<1
Methanol, 38.8 wt% H_2O		80	4	1
Hexane, 29.0 wt% H_2O		180	16	4
Hexane, 37.2 wt% H_2O		170	2	2
Contact time 180 days				
Methanol, 29.5 wt% H_2O	Yes	111	5	3*
Methanol, 39.1 wt% H_2O		87	20	8*
Hexane, 28.7 wt% H_2O		147	161	100
Hexane, 36.9 wt% H_2O		160	85	47
Contact time 30 days				
Hexane, 29.3 wt% H_2O	No	182	75	77
Hexane, 37.7 wt% H_2O		187	64	74

* n=2

4.3.2 Role of carrier solvent

No effect of the carrier solvent was found when the contact time between soil and solvent solution was 90 days or less. For the A horizon soil (Table 4.1), a residual concentration of anthracene was found for hexane treated samples at a contact time of 180 days, but not for the methanol treated samples. The C horizon soil (Table 4.2) and the clay (Table 4.3) both showed a residual concentration of anthracene for both solvents at a contact time of 180 days, with a higher residual for hexane samples than for methanol samples.

4.3.3 Role of soil moisture content

No effect was found when comparing samples with different soil moisture co ent for a contact time of 90 days or less. For a contact time of 180 days, the residual concentration of anthracene was higher for the low moisture content sample treated with hexane compared to the similar high moisture content sample for both soils and the clay. No difference was found for the methanol treated samples with a contact time of 180 days for the A horizon soil (Table 4.1). The methanol treated C horizon samples (Table 4.2) showed a higher residual at low moisture content than at high moisture content, whereas the results for the clay (Table 4.3) were opposite; a higher residual was found for the high moisture content sample compared to the low moisture sample.

Lumps (1-7 mm) were observed in the clay samples through the contact time and subsequent roller bottle treatment with some decrease due to attrition in the roller bottles. The same observation was found for clay samples treated in a similar way, but without contact with anthracene in solvent solution.

4.3.4 Discussion

The bioavailability of anthracene was dependent on the contact time consistent with the hypothesis. All anthracene was degraded when the contact time between soil and solvent solution was 90 days or less. In contrast 180 days of contact gave measurable concentrations of anthracene that were not

bioavailable. The conditions at 180 days gave a link between deposition history and bioavailability.

One explanation for the increase in unavailable anthracene after 180 days would be mass transfer limitations, which could be due to the aggregation of the samples. With a contact time of 180 days, the anthracene could have diffused slowly into pores too small for the microorganisms to enter. The anthracene could have crystallized in these pores when the solvent was removed, which could create a limitation due to slow solubilization and diffusion out of the pores. This would especially be important for the clay samples that stayed aggregated during the degradation process. A similar process could also occur in the C horizon soil even though aggregation was not observed visually after 4 weeks in roller bottles as with the clay. The difference in aggregation after moisturizing the samples of A horizon soil and the C horizon soil could explain the difference in the results for the two soils.

Hatzinger and Alexander (1995) found an increased mineralization rate of both freshly added and aged (300 days) phenanthrene after dispersion of the soil, which shows that the presence of aggregates could cause slow diffusion which would slow down the degradation. Steinberg et el. (1987) found an increased release of 1,2-dibromoethane after pulverization of the sample, after the chemical had been present in the soil for a minimum of 19 years, thus suggesting that the chemical

was trapped in micropores in aggregates.

The residual concentration found for samples that had been in contact with hexane solution for 180 days, could also be due to the crystallization of the anthracene on the soil as observed in these samples. Anthracene present as larger crystals or as crystals within soil aggregates could take longer to solubilize in the aqueous phase, so that it would be less available for the microorganisms as an energy source at the beginning of the degradation process. This reduction in substrate supply would mean a decrease in the number of microorganisms, unless there was another energy source present, and thereby a slower biological degradation of anthracene as it dissolved. This mechanism cannot explain the elevated concentrations in the case of methanol for the C horizon soil and the clay, since a much smaller fraction (1-10% loss of added weight of methanol) of the methanol evaporated during the contact time for these samples and no crystals were observed visually.

The decrease in bioavailability with contact time as found in this study was in agreement with the findings of Hatzinger and Alexander (1995). They found that mineralization of phenanthrene, as determined by evolving $^{14}CO_2$, to decrease with increasing contact time. In their work, a portion of the decrease could be caused by transformation of phenanthrene into compounds less available for biological degradation as found by Lotfabad (1994) for transformed anthracene. Hatzinger

and Alexander did not consider this possibility.

The disappearance of anthracene in roller bottles in this study was not only due to the added inoculum, since a decrease in concentration was also found for samples that were not inoculated (Table 4.1, 4.2 and 4.3). The role of inoculum for the A horizon soil is ambiguous, since low concentrations were found for both inoculated and non-inoculated samples. In all but one case, addition of inoculum to the C horizon soil and the clay gave much lower residual concentrations of anthracene showing the importance of inoculum for these samples. The one non-inoculated C horizon sample that showed a final concentration of 1 ppm (Table 4.2) could have been due to an error during sampling of the roller bottles. It was not possible to determine whether the value was real or due to a mistake during the sample handling, since all of the sample had been used. The concentration of anthracene for this non-inoculated sample was significantly higher than for the similar inoculated sample after one week in roller bottles, which showed the importance of the inoculum for this sample.

All three solids showed a rapid drop in concentration over the first 24 hours in roller bottles (appendix F), which is consistent with abiotic conversion. The decrease for the C horizon soil and the clay was in the range of 6 to 130 ppm (n=25) with a median of 63 ppm. The decrease for the A horizon soil was in the range of 2 to 86 ppm (n=20) with a median of 46 ppm. Subsequent removal of anthracene required inoculum for

the C horizon soil and the clay, whereas the influence of inoculum for the A horizon is questionable. The A horizon samples started at lower concentrations (due to lower recovery of anthracene), 22-226 ppm (typically 22-98 ppm) compared to the other two solids with a starting concentration of 71-223 ppm, which means that abiotic conversion of similar amounts in all samples would leave a very low residual concentration of anthracene in the A horizon samples. Subsequent biological activity, therefore, would have an insignificant effect on anthracene concentration.

A study of an A horizon soil sample was performed to determine whether the decrease in concentration of anthracene was caused by native microorganisms. The microbial activity in the sample was inhibited by addition of $HgCl_2$ to give a concentration of 10^{-4} M in the liquid phase in the roller bottle. This sample showed the same disappearance of anthracene as for the similar sample without $HgCl_2$ (Table 4.1). The fact that there is some dispute in the literature as to which concentration of free Hg^{2+} is needed to inhibit bacteria and since the concentration of free Hg^{2+} is unknown for this experiment means that bacterial activity cannot be ruled out.

The decrease in concentration of anthracene in the A horizon soil in roller bottles could not be due to anthracene binding to the hydrophobic interior of dissolved soil organic matter as suggested by several researchers (Liu and Amy, 1993,

McCarthy and Jimenez, 1985, Schlautman and Morgan, 1993, Wershaw, 1993) for two reasons. First, the entire slurry was dried with magnesium sulphate then extracted, which would remove such sorbed material. Second a decrease in concentration of anthracene was also found for the non-inoculated clay. Since the clay contained no organic matter, such a mechanism cannot account for the disappearance of anthracene.

The decrease in concentration of anthracene in the roller bottles cannot be due to photooxidation of anthracene to anthraquinone, since no significant amount of anthraquinone was found in any of the samples.

CHAPTER 5

CONCLUSIONS

A slow loss of anthracene was found to take place regardless of the moisture content. The UV spectrum of an extract from Ca-Montmorillonite did not show the specific peaks of anthracene. Instead it showed a wide peak in the range where aromatic compounds absorb, implying that anthracene was transformed into other aromatic compounds. The loss of anthracene in all the solids, therefore, was consistent with chemical reaction.

Equilibrium sorption measurements were suspect because of continued loss of anthracene. It could not be determined if equilibrium was reached due to interference from the probable reactions.

The recovery of anthracene was found to depend on soil organic matter content. The higher the organic content the lower the recovery. For a high content of soil organic matter, a dependency of carrier solvent and soil moisture content was found. The lowest recovery was found using methanol as a carrier solvent in a low moisture content soil, therefore, the polarity of the carrier solvent was important in determining the interaction between anthracene and soil.

Anthracene was highly available for biological degradation under the roller bottle conditions for samples with a contact time between the soil and anthracene in solvent solution of 90 days or less. Samples with a contact time of 180 days showed a residual concentration of anthracene after biodegradation over a period of 4 weeks, with the highest residual for samples where anthracene had crystallized during the contact time due to loss of solvent by evaporation.

REFERENCES

Abder-Ruhman M.; 1980. "Mineralogical Characteristics of Soils from East Central Alberta". M. Sc. dissertation, University of Alberta.

Ball W. P., Roberts P. V.; 1991. "Long-Term Sorption of Halogenated Organic Chemicals by Aquifer Material. 2. Intraparticle Diffusion". Environ. Sci. Technol., 25, 1237-1249.

Banerjee D. K., Fedorak P. M., Hashimoto A., Masliyah J. H., Pickard M. A., Gray M. R.; 1995. "Monitoring the Biological Treatment of Anthracene-Contaminated Soil in Rotating Drum Bioreactor". Appl. Microbiol. Biotechnol., 43, 521-528.

Block R., Stroo H., Sweet G. H.; 1993. "Bioremediation - Why Doesn't It Work Sometimes?". Chem. Eng. Progress., 89, 44-50.

Bollag J.-M.; 1992. "Decontaminating Soil With Enzymes An In Situ Method Using Phenolic and Anilinic Compounds". Environ. Sci. Technol., 26, 1876-1881.

Brady N. C.; 1984. "The Nature and Properties of Soils". 9th edition. Macmillan, New York.

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

Carter D. L., Mortland M. M., Kemper W. D.; 1986. "Specific Surface" pp 413-423 in "Methods of Soil Analysis. Part 1-Physical and Mineralogical Methods". 2nd edition. Klute A., Ed.; American Society of Agronomy Inc., Soil Science Society of America Inc., Madison, Wisconsin, USA.

Chiou C., Lee J.-F., Boyd S. A.; 1990. "The surface area of soil organic matter". Environ. Sci. Technol., 24, 1164-1166.

Chiou C., 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.

Chung G.-Y., McCoy B. J., Scow K. M.; 1993. "Criteria to Assess When Biodegradation Is Kinetically Limited by Intraparticle Diffusion and Sorption". Biotechnol. Bioengin., 41, 625-632.

Connaughton D. F., Stedinger J. R., Liou L. W., Shuler M. L.; 1993. "Description of Time-Varying Desorption Kinetics: Release of Naphthalene from Contaminated Soils". Environ. Sci. Technol., 27, 2397-2403.

Dragun J.; 1988. "The Fate of Hazardous Materials in Soil (What Every Geologist and Hydrologist Should Know): Part 3". Hazardous Materials Control, 1, No 5, 24-43.

Elkhatib E. A., Bennett O. L., Baligar V. C., Wright R. J.; 1986. "A Centrifuge Method for Obtaining Soil Solution Using an Immiscible Liquid". Soil Sci. Soc. Am. J., 50, 297-299.

Fedorak P. M., Grbić-Galić D.; 1991. "Aerobic Microbial Cometabolism of Benzothiophene and 3-Methylbenzo-thiophene". Appl. Environ. Microbiol., 57, 932-940.

Gamble D. S., Khan S. U.; 1990. "Atrazine in Organic Soil: Chemical Speciation during Heterogeneous Catalysis". J. Agric. Food Chem., 38, 297-308.

Gerstl Z., Yaron B.; 1978. "Adsorption and Desorption of Parathion by Attapulgite as Affected by the Mineral Structure". J. Agric. Food Chem., 26, 569-573.

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". Appl. Microbiol. Biotechnol., 40, 933-940.

Hance R. J.; 1965. "Observations on the relationship between the adsorption of diuron and the nature of the adsorbent". Weed Research, 5, 108-114.

Hatzinger P. B., Alexander M.; 1995. "Effect of Aging of Chemicals in Soil on Their Biodegradability and Extractability". Environ. Sci. Technol., 29, 537-345.

Inscoe M. N.; 1964. "Photochemical Changes in Thin Layer Chromatograms of Polycyclic, Aromatic Hydrocarbons". Anal. Chem., 36, 2505-2506.

Kile D. E., Chiou C. T., Zhou H., Xu O.; 1995. "Partition of Nonpolar Organic Pollutants from Water to Soil and Sediment Organic Matters". Environ. Sci. Technol., 29, 1401-1406.

Lavkulich L. M., Wiens J. H.; 1970. "Comparison of Organic Matter Destruction by Hydrogen Peroxide and Sodium Hypochlorite and Its Effects on Selected Mineral Constituents". Soil Sci. Soc. Amer. Proc., 34, 755-758.

Liu H., Amy G.; 1993. "Modeling Partitioning and Transport Interactions between Natural Organic Matter and Polynuclear Aromatic Hydrocarbons in Groundwater". Environ. Sci. Technol., 27, 1553-1562.

Lotfabad S. K.; 1994. "Interactions of Polynuclear Aromatic Hydrocarbons with Soil". M. Sc. Dissertation, University of Alberta.

Mackay D., Powers B.; 1987. "Sorption of Hydrophobic Chemicals from Water: A Hypothesis for the Mechanism of the Particle Concentration Effect". Chemosphere, 16, 745-757.

Manilal V. B., Alexander M.; 1991. "Factors affecting the microbial degradation of phenanthrene in soil". Appl. Microbiol. Biotechnol., 35, 401-405.

McCarthy J. F., Jimenez B. D.; 1985. "Interactions between Polycyclic Aromatic Hydrocarbons and Dissolved Humic Material : Binding and Dissociation". Environ. Sci. Technol., 19, 1072-1076.

Means J. C., Wood S. G., Hassett J. J., Banwart W. L.; 1980. "Sorption of Polynuclear Aromatic Hydrocarbons by Sediments and Soils". Environ. Sci. Technol., 14, 1524-1528.

Means J. C., Wood S. G., Hassett J. J., Banwart W. L.; 1982. "Sorption of Amino- and Carboxy-Substituted Polynuclear Aromatic Hydrocarbons by Sediments and Soils". Environ. Sci. Technol., 16, 93-98.

Mills A. C., Biggar J. W.; 1969. "Solubility-Temperature Effect on the Adsorption of Gamma- and Beta-BHC from Aqueous and Hexane Solutions by Soil Materials". Soil Sci. Soc. Amer. Proc., 33, 210-216.

Miller M. E., Alexander M.; 1991. "Kinetics of Bacterial Degradation of Benzylamine in a Montmorillonite Suspension". Environ. Sci. Technol., 25, 240-245.

Mittelstaedt W., Führ F.; 1984. "Degradation of Dinocap in three German Soils". J. Agric. Food Chem., 32, 1151-1155.

Paul E. A., Clark F. E.; 1989. "Carbon Cycling and Soil Organic Matter" pp 91-114 in "Soil Microbiology and Biochemistry". Academic Press, Inc., San Diego, California.

Pavlostathis S. G., Jaglal K.; 1991. "Desorptive Behavior of Trichloroethylene in Contaminated Soil". Environ. Sci. Technol., 25, 274-279.

Pavlostathis S. G., Mathavan G. N.; 1992. "Desorption Kinetics of Selected Volatile Organic Compounds from Field Contaminated Soils". Environ. Sci. Technol., 26, 532-538.

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

Piotrowski M. R.; "Integrated biological treatment of contaminated soil and aquifer at a superfund site". Paper No. 245d, AIChE Annual Meeting, Los Angeles, CA, Nov. 17-22 1991.

Qualizza C. V.; 1994. "The Influence of the Nature of Soil Organic Matter on the Sorption of Anthracene, Naphthalene and α-Naphthol". M. Sc. Dissertation, University of Alberta.

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.

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.

Schlautman M. A., Morgan J. J.; 1993. "Effects of Aqueous Chemistry on the Binding of Polycyclic Aromatic Hydrocarbons by Dissolved Humic Materials". Environ. Sci. Technol., 27, 961-969.

Sheldrick B. H., Wang C.; 1993. "Particle Size Distribution" pp 499-511 in "Soil Sampling and Methods of Analysis". Carter M. R., Ed.; Canadian Society of Soil Science, Lewis Publishers, CRC Press Inc., Boca Raton, Florida, USA.

Steinberg S. M., Kreamer D. K.; 1993. "Evaluation of the Sorption of Volatile Organic Compounds by Unsaturated Calcareous Soil from Southern Nevada Using Inverse Gas Chromatography". Environ. Sci. Technol., 27, 883-888.

Steinberg S. M., Pignatello J. J., Sawhney B. L.; 1987. "Persistence of 1,2-Dibromoethane in Soils : Entrapment in Intraparticle Micropores". Environ. Sci. Technol., 21, 1201-1208.

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.

Tiessen H., Moir J. O.; 1993. " Total and Organic Carbon" pp 187-199 in "Soil Sampling and Methods of Analysis". Carter M. R., Ed.; Canadian Society of Soil Science, Lewis Publishers, CRC Press Inc., Boca Raton, Florida, USA.

Topp G. C., Galganov Y. T., Ball B. C., Carter M. R.; 1993. "Soil Water Desorption Curves" pp 569-579 in "Soil Sampling and Methods of Analysis". Carter M. R., Ed.; Canadian Society of Soil Science, Lewis Publishers, CRC Press Inc., Boca Raton, Florida, USA.

Weissenfels W. D., Klewer H.-J., Langhoff J.; 1992. "Adsorption of polycyclic aromatic hydrocarbons (PAHs) by soil particles: influence on biodegradability and biotoxicity". Appl. Microbiol. Biotechnol., 36, 689-696.

Wershaw R. L.; 1993. "Model for Humus in Soils and Sediments". Environ. Sci. Technol., 27, 814-816.

Wu S., Gschwend P. M.; 1986. "Sorption Kinetics of Hydrophobic Organic Compounds to Natural Sediments and Soils". Environ. Sci. Technol. 20, 717-725.

Xing B., McGill W. B., Dudas M. J.; 1994. "Cross-Correlation of Polarity Curves to Predict Partition Coefficients of Nonionic Organic Contaminants". Environ. Sci. Technol., 28, 1929-1933.

Yaron B., Saltzman S.; 1972. "Influence of Water and Temperature on Adsorption of Parathion by Soils". Soil Sci. Soc. Amer. Proc., 36, 583-586.

APPENDIX A

Standard curves for spectrophotometer

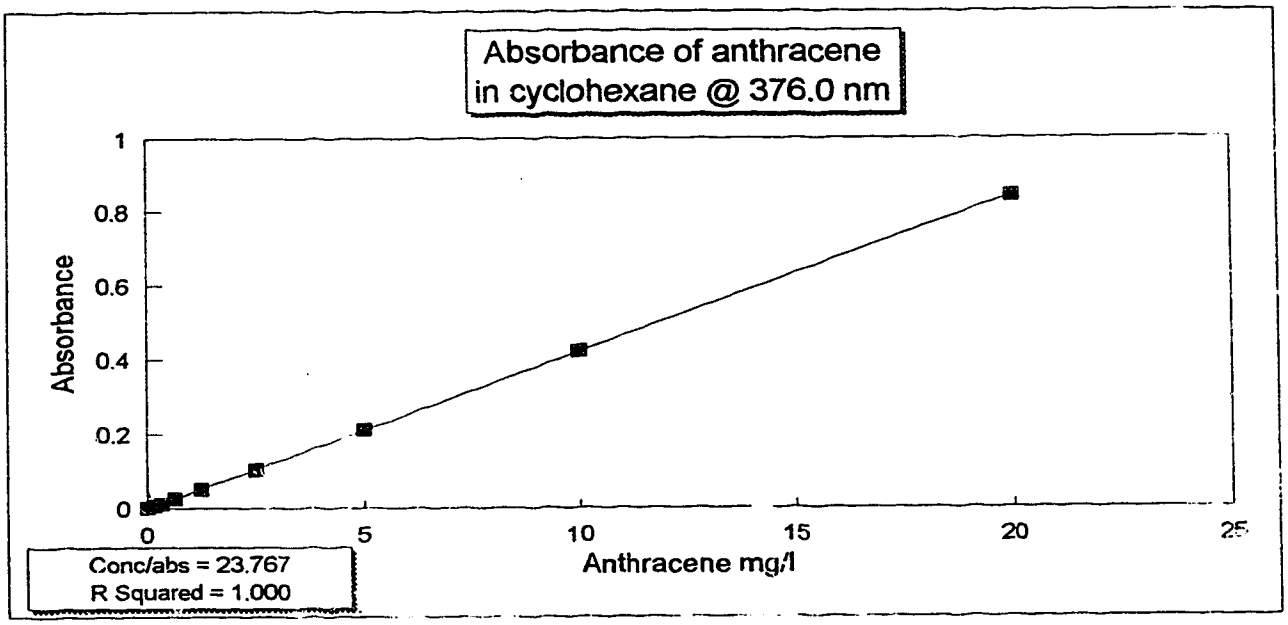

Figure A.1 Standard curve for anthracene in cyclohexane.

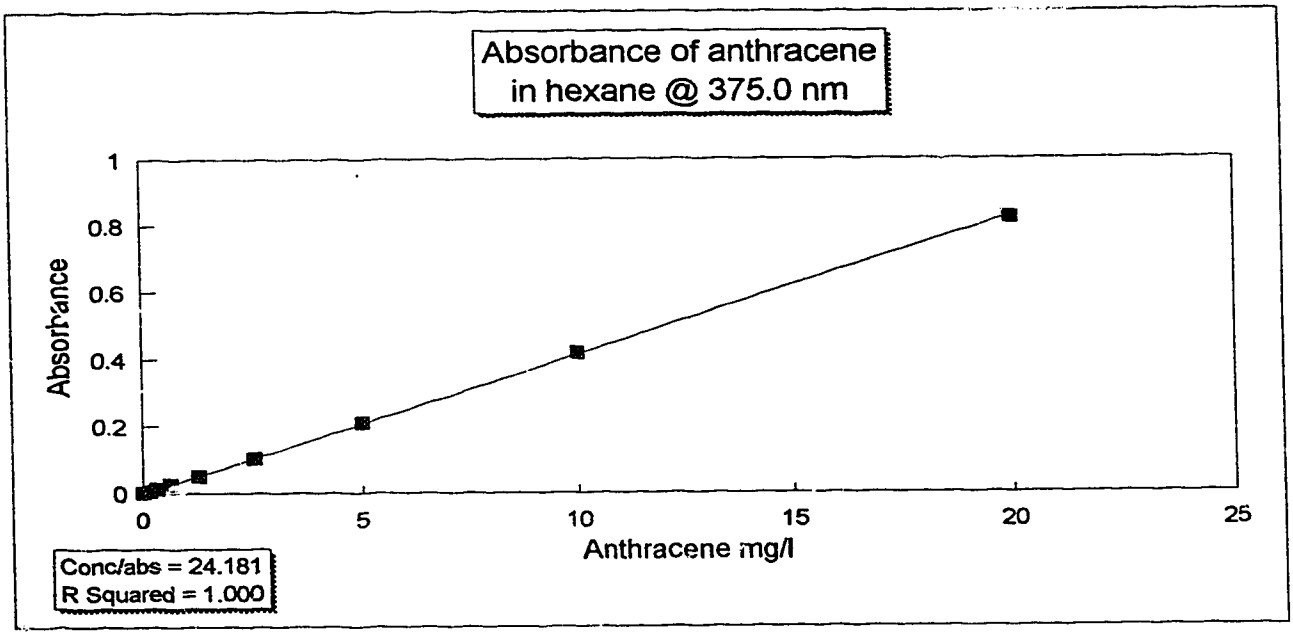

Figure A.2 Standard curve for anthracene in hexane.

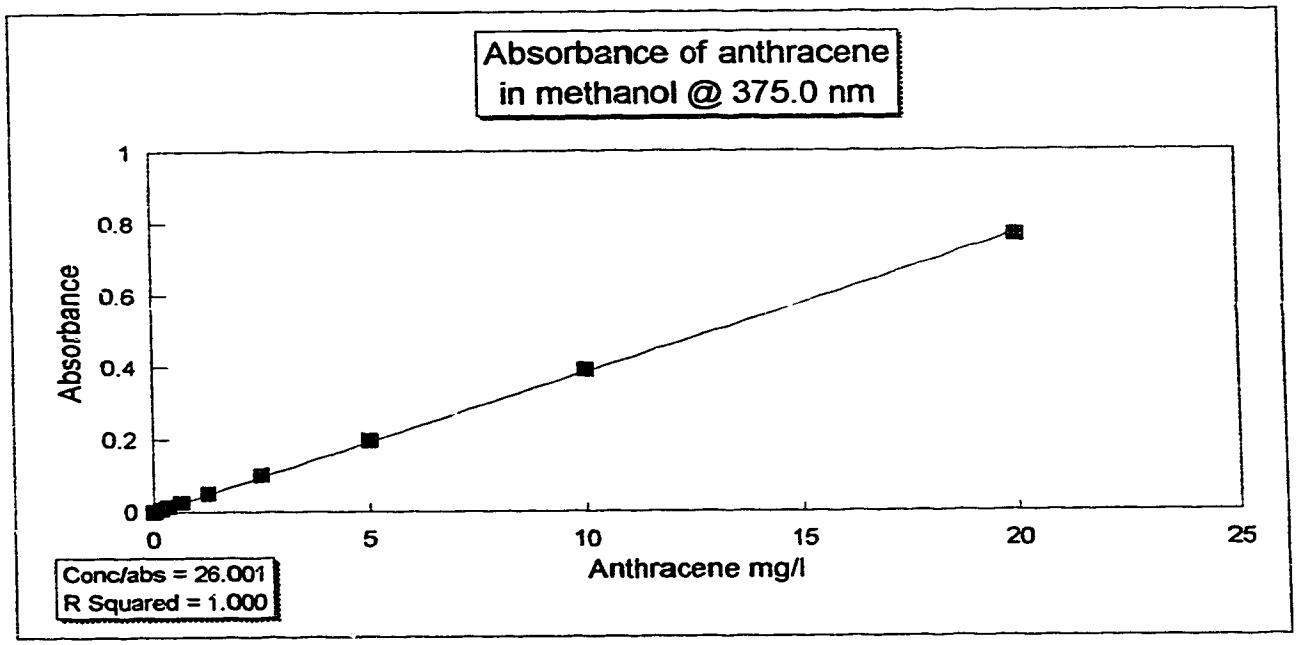

Figure A.3 Standard curve for anthracene in methanol.

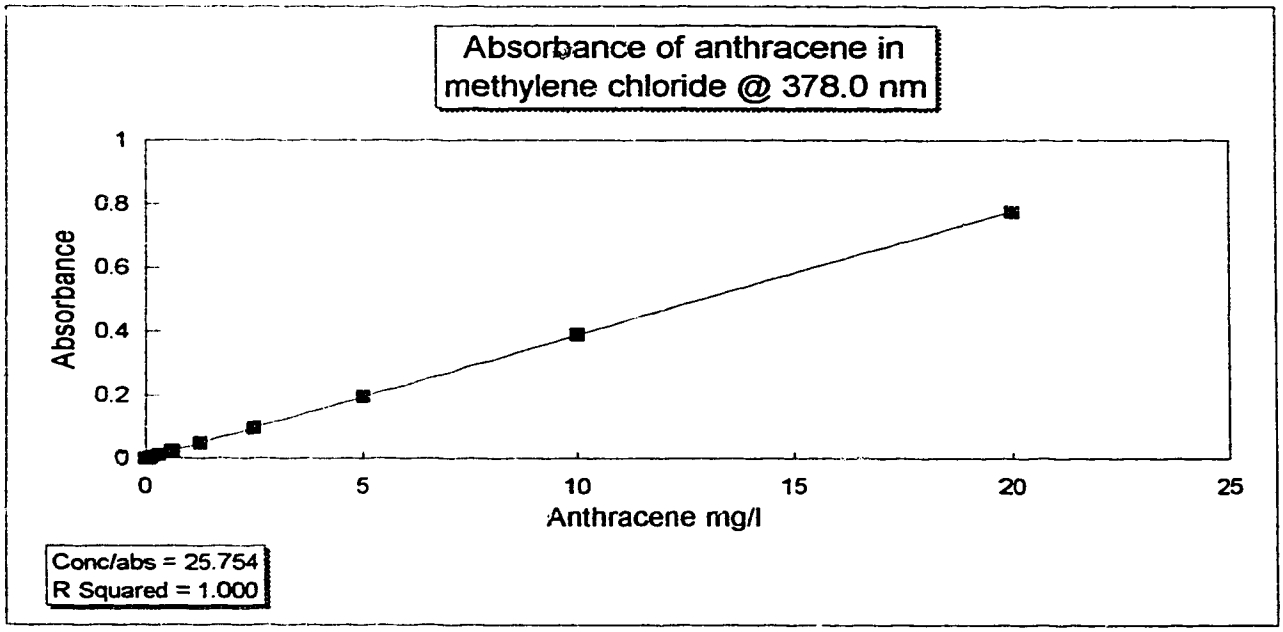

Figure A.4 Standard curve for anthracene in methylene chloride.

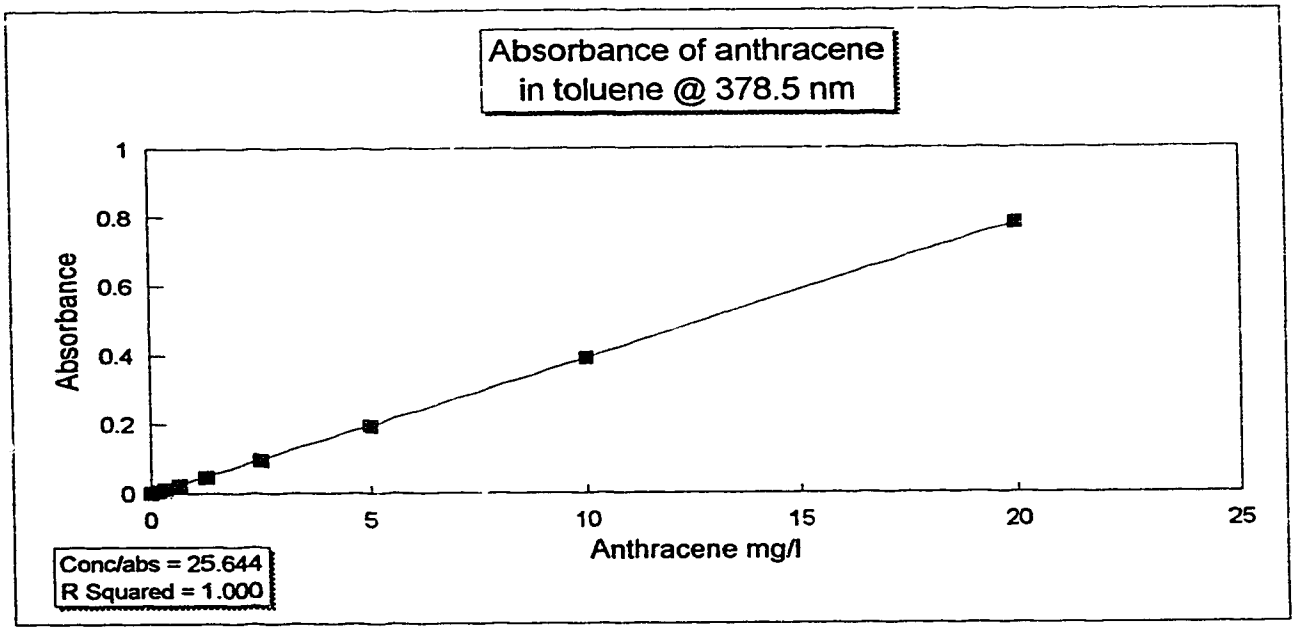

Figure A.5 Standard curve for anthracene in toluene.

APPENDIX B

Example of a standard curve for gas chromatograph

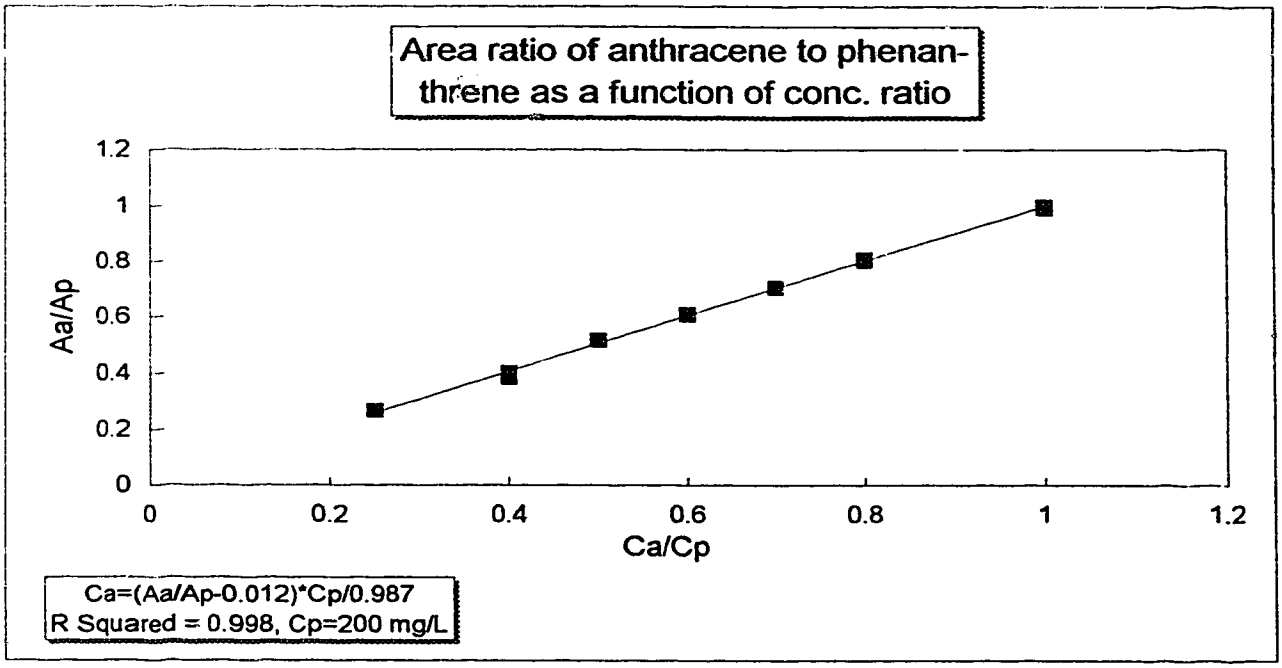

Figure B.1 Standard curve for addition of internal standard at a concentration of 200 mg/L (Cp).

Table B.1 GC settings.

Injection temperature	180°C
Detector temperature	300°C
Range	4
Attenuation	1
Purge B off	0.00 min
Purge B on	1.00 min
Zero	11.0

Table B.2 Temperature program.

Initial temperature	40°C
Initial time	2.00 min
Rate	30°C/min
Final temperature	180°C
Final time	21.34 min

Table B.3 Integrator settings.

Zero	10,0.08
Attenuation	4
Chart speed	0.5 cm/min
Peak with	0.16
Threshold	0
Area rejection	0

Injection liner : splitless (HP 18740-80200).

Column head pressure : 68 kPa.

Gas flowrates : Air 400 mL/min

H_2 37 mL/min.

APPENDIX C

Table C.1 % recovery of anthracene as a function of extraction time and pretreatment of the sample

		Extraction time , hours					
Sample #	wt% moist.	4	4 + MgSO4	8	8 + MgSO4	12	12 + MgSO4
1-7	3.9	21.3 19.2	16.2				
2-7b	13.7	54.4 55.1	25.9	56.3			32.3
3-7	4.2	52.9 45.3	42.3				
4-7	13.4	65.0 56.5	26.9				
5-7	2.3	69.6 70.8	78.1	74.5		72.5	
9-30	30.5	83.5	94.6 100.8		96.6		98.4

Table C.2 Loading conditions

Sample #	Soil	ontact. time,	Solvent	Load ppm
1-7	A horizon	7	methanol	125
2-7b	A horizon	7	methanol	100
3-7	A horizon	7	hexane	250
4-7	A horizon	7	hexane	150
5-7	C horizon	7	methanol	100
9-30	clay	30	methanol	125

APPENDIX D

Table D.1 % Recovery of anthracene as a function of contact time.

Contact time, days	Sample # 1	2	3	4	5	6	7	8	9	10	11	12
0.17	61.5	66.4	96.3	84.4	81.8	80.5	101.8	95.7	92.0	103.2	91.9	76.1
1	48.8	67.8	90.6	80.9	90.5	97.0	89.2	101.0	83.8	89.4	95.6	92.3
7	21.3	55.1	52.9	65.0	70.8	88.9	76.4	94.9	101.2	81.6	87.8	94.0
14	27.1	48.4	81.7	75.1	87.6	92.2	90.5	99.4	100.0	100.9	101.4	81.9
30	17.4	48.4	42.9	60.0	85.8	92.7	73.7	84.0	100.8	93.0	91.1	93.4
90	25.0	42.1	65.7	65.7	88.3	82.5	85.7	75.0	85.2	79.9	90.2	85.2
180	17.1	39.1	63.5	51.4	75.5	80.0	68.3	83.0	89.2	86.6	73.5	80.0
mean	21.6	46.6	61.4	63.4	82.9	87.7	86.5	90.4	93.2	90.7	90.2	86.1
std	4.0	5.6	13.0	7.8	6.8	6.2	8.7	9.1	6.9	8.3	7.9	6.6
n	5	5	5	5	7	7	7	7	7	7	7	7
stand. error	4.9	6.9	16.2	9.6	6.3	5.8	8.0	8.4	6.4	7.7	7.4	6.1

Day 180 measurements are an average of 5 extractions, all other are single measurements.
Mean and Std for sample 1 to 4 are for day 7 to 180, all others are for day 0.17 to 180.

Table D.2 Loading conditions.

Sample #	Soil	Moist., wt%	Solvent	Load ppm
1	A horizon	4.3±0.4	Methanol	125
2	A horizon	13.7±0.5	Methanol	100
3	A horizon	4.5±0.5	Hexane	250
4	A horizon	13.7±0.4	Hexane	150
5	C horizon	2.7±0.4	Methanol	100
6	C horizon	6.2±0.3	Methanol	100
7	C horizon	2.4±0.3	Hexane	250
8	C horizon	6.0±0.5	Hexane	150
9	Clay	30.5±0.5	Methanol	125
10	Clay	38.7±0.7	Methanol	100
11	Clay	29.8±0.9	Hexane	200
12	Clay	37.7±1.1	Hexane	200

Moist., wt% is given as mean ± standard error, n=7.

APPENDIX E

Table E.1 % Recovery of anthracene for repeated measurements of samples with a contact time of 180 days.

Extraction #	Sample #											
	3-180	4-180	7-180	8-180	11-180	12-180	15-180	16-180	17-180	18-180	19-180	20-180
1	18.3	40.3	41.5	48.2	76.6	80.5	100.9	81.8	89.0	86.6	61.5	67.4
2	17.2	39.7	90.1	50.5	78.3	79.5	70.7	83.3	85.6	77.0	49.6	81.8
3	16.9	38.7	78.3	52.6	73.2	80.5	156.4	100.7	88.4	92.0	95.6	84.5
4	17.0	38.1	45.6	51.9	71.5	85.4	53.5	64.7	91.5	90.5	77.7	97.7
5	16.1	38.4	62.1	53.6	77.7	74.1	60.1	84.5	91.5	86.8	83.2	68.6
mean	17.1	39.1	63.5	51.4	75.5	80.0	88.3	83.0	89.2	86.6	73.5	80.0
std	0.7	0.8	18.6	1.9	2.6	3.6	37.7	11.4	2.2	5.2	16.2	11.2
stand. error	0.9	1.0	23.1	2.3	3.3	4.4	46.9	14.2	2.7	6.5	20.2	13.9

Table E.2 Loading conditions.

Sample #	Corr.spond. Sample # in other series	Soil	Moist., wt%	Solvent	Load ppm
3	1	A horizon	3.6	Methanol	125
4	2	A horizon	12.4	Methanol	100
7	3	A horizon	3.3	Hexane	250
8	4	A horizon	13.7	Hexane	150
11	5	C horizon	2.1	Methanol	125
12	6	C horizon	5.6	Methanol	100
15	7	C horizon	2.2	Hexane	250
16	8	C horizon	6.5	Hexane	150
17	9	Clay	29.5	Methanol	125
18	10	Clay	39.1	Methanol	100
19	11	Clay	28.7	Hexane	200
20	12	Clay	36.9	Hexane	200

APPENDIX F

Table F.1 ppm anthracene as a function of time in roller bottles for samples with a contact time of 1 day.

Day #	Sample #										
	1-1 no in	1-1	2-1 no in	2-1no in Hg	2-1	3-1	4-1	5-1	6-1	7-1	8-1
0	61	61	68	68	68	226	121	90	97	223	152
1	19	20	8	8	36	79	52	52	65	134	110
2	14	18	9	8	48	83	49	54	54	123	102
3	14	14	11	9	26	69	47	22	25	66	44
4	17	11	9	7	16	64	34	11	7	20	26
7	15	5	10	7	6	7	6	3	2	9	8
28				7							
30	1	1	1		1	<1	<1	<1	<1		
31										1	<1

no in = not inoculated
Hg = 1 mL 1.85g HgCl2/L added to the roller bottle

Table F.2 Loading conditions.

Sample #	Soil	Moist., wt%	Solvent	Load ppm
1	A horizon	4.7	Methanol	125
2	A horizon	13.9	Methanol	100
3	A horizon	4.3	Hexane	250
4	A horizon	14.1	Hexane	150
5	C horizon	2.9	Methanol	100
6	C horizon	6.7	Methanol	100
7	C horizon	2.4	Hexane	250
8	C horizon	6.3	Hexane	150

Table F.3 ppm anthracene as a function of time in roller bottles for samples with a contact time of 7 days

Day #	Sample #								
	1-7 no in	1-7	2-7 no in	2-7	2-7b no in	3-7	4-7	5-7	6-7
0	27	27	24	24	55	132	98	71	89
0.02	62	27	11	28					
0.17	19	29	14	26					
1	14	25	14	31	9	69	45	47	83
2	15	26	11	23		63		16	27
3	12	22	12	31		60	20	4	14
4	7	18	9	23		60	15		
6								3	9
7	9	19	10	21		55	9		
11						37	5		
29								1	7
29 frozen									5
30						1	2		
32					1				
35	10	2	3	2					

no in = not inoculated
frozen = frozen sample used for measurement.

Table F.4 Loading conditions.

Sample #	Soil	Moist., wt%	Solvent	Load ppm
1	A horizon	3.9	Methanol	125
2	A horizon	6.7	Methanol	100
2b	A horizon	13.7	Methanol	100
3	A horizon	4.2	Hexane	250
4	A horizon	13.4	Hexane	150
5	C horizon	2.3	Methanol	100
6	C horizon	6.4	Methanol	100

Table F.5 ppm anthracene as a function of time in roller bottles for samples with a contact time of 30 days.

Day #	Sample #															
	1-30	2-30	3-30	4-30	5-30	6-30	7-30 no in	7-30	8-30 no in	8-30	9-30	10-30	11-30 no in	11-30	12-30 no in	12-30
0	22	48	107	90	86	93	184	184	126	126	126	93	182	182	187	187
1	12	11	38	44	62	59	121	132	119	98	44	36	53	52	76	68
2	12	10	31	13	28	23	121	121	108	94	32	125	58	56	84	79
3	11	8	5	5	12	5	150	57	94	52	32	16	82	76	86	57
4	10	9					170	33	97	27			75	48	71	41
6			2	4	2	1					2	3				
7	11	5					168	18	98	9			76	10	65	6
8																
11	7	3														
29					1	<1										
30	2	1	1	1			100	2	1	1	1	<1	77	<1	74	1

no in = not inoculated

Table F.6 Loading conditions.

Sample #	Soil	Moist., wt%	Solvent	Load ppm
1	A horizon	3.8	Methanol	125
2	A horizon	14.1	Methanol	100
3	A horizon	4.7	Hexane	250
4	A horizon	14.4	Hexane	150
5	C horizon	3.5	Methanol	100
6	C horizon	5.8	Methanol	100
7	C horizon	2.7	Hexane	250
8	C horizon	5.4	Hexane	150
9	Clay	30.5	Methanol	125
10	Clay	37.1	Methanol	100
11	Clay	29.3	Hexane	200
12	Clay	37.7	Hexane	200

Table F.7 ppm anthracene as a function of time in roller bottles for samples with a contact time of 90 days.

Day #	Sample #											
	1-90	2-90	3-90	4-90	5-90	6-90	7-90	8-90	9-90	10-90	11-90	12-90
0	31	42	164	99	88	82	214	112	106	80	180	170
1	8	12	78	34	49	63	132	178	62	49	85	97
2	4	10	64	12	25	33	89	117	39	17	49	51
3	3	5	19	5	7	4	23	29	16	11	30	25
6	2	3	4	3	3	1	6	14				
7									2	4	16	2
30	1	1	1	1	1	<1	<1	1	<1	1	4	2

Table F.8 Loading conditions.

Sample #	Soil	Moist., wt%	Solvent	Load ppm
1	A horizon	4.7	Methanol	125
2	A horizon	14.0	Methanol	100
3	A horizon	4.9	Hexane	250
4	A horizon	13.9	Hexane	150
5	C horizon	2.6	Methanol	100
6	C horizon	6.4	Methanol	100
7	C horizon	2.7	Hexane	250
8	C horizon	6.5	Hexane	150
9	Clay	30.6	Methanol	125
10	Clay	38.8	Methanol	100
11	Clay	29.0	Hexane	200
12	Clay	37.2	Hexane	200

Table F.9 ppm anthracene as a function of time in roller bottles for samples with a contact time of 180 days.

Day #	Sample #											
	3-180	4-180	7-180	8-180	11-180	12-180	15-180	16-180	17-180	18-180	19-180	20-180
0	21	39	159	77	94	80	221	125	111	87	147	160
6	6	6	238	31	20	7	282	110	5	20	161	85
30	1	2	22	7	9	4	88	26	2	8	100	47
30 frozen					10	5			4	8		

frozen = frozen sample used for measurement.

Table F.10 Loading conditions.

Sample #	Corr. spond sample # in other series	Soil	Moist., wt%	Solvent	Load ppm
3	1	A horizon	3.6	Methanol	125
4	2	A horizon	12.4	Methanol	100
7	3	A horizon	3.3	Hexane	250
8	4	A horizon	13.7	Hexane	150
11	5	C horizon	2.1	Methanol	100
12	6	C horizon	5.6	Methanol	100
15	7	C horizon	2.2	Hexane	250
16	8	C horizon	6.5	Hexane	150
17	9	Clay	29.5	Methanol	125
18	10	Clay	39.1	Methanol	100
19	11	Clay	28.7	Hexane	200
20	12	Clay	36.9	Hexane	200