Effect of Kaolinite and Cadmium on the Biodegradation of Naphthalene and Substituted Naphthalenes

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Abstract

Groundwater contamination by leachates from landfill sites, aquatic and terrestrial petroleum spillage and pollutant deposition from air, are problems that plague countries worldwide. Contaminants in wastes can be a mixture of any number of chemical compounds including heavy metals and polycyclic aromatic hydrocarbons. Biodegradation and adsorption-desorption studies together can provide insight into the behaviour of a contaminated soil system by providing information on the potential bioavailability of contaminants.

The objectives of this study were to look at the effect of kaolinite and cadmium on the biodegradation of naphthalene and substituted naphthalenes. The kaolinite mineral is present in many Quebec soils and is a primary constituent of tropical soils. Since kaolinite is also a well defined mineral, experiments were performed to examine the effect kaolinite might play on the degradation of naphthalene, 2-methyl naphthalene, and 2-naphthol in the presence and absence of cadmium. Furthermore, the results from these analyses were used to interpret the behaviour in the mineralization experiments of each PAH, using the Gram-negative species *Pseudomonas putida* (ATCC #17484).

It was found that the mineralization rate of naphthalene, 2-methyl naphthalene, and 2-naphthol were reduced in the presence of kaolinite. The presence of cadmium did not inhibit microbial activity, and in fact showed a slight increase in the total percent mineralized in the mineralization experiments. From the adsorption-desorption experiments, the removal of each PAH onto kaolinite was observed to correlate well with their respective octanol/water partition coefficients. 2-Methyl naphthalene showed the greatest affinity for kaolinite and consequently, in the mineralization experiments, gave the lowest mineralization rates.

The results of this study indicate that the presence of cadmium did not significantly alter the mineralization capacity of *P. putida* for naphthalene, 2-methyl naphthalene, and 2-naphthol. The presence of kaolinite however, did affect microbial activity by significantly reducing the rates of PAH mineralization. These results illustrate the need to identify the general mechanisms of interactions to develop effective bioremediation programs for contaminated sites.

Résumé

La contamination des eaux souterraines, qu'elle provienne de la lixiviation de lieux d'enfouissement sanitaire, de déversements accidentels de pétrole ou de la déposition de matières polluantes de l'air constitue un réel fléau pour les populations du monde entier. Les contaminants présents dans ces résidus regroupent de nombreux composés chimiques incluant les métaux lourds ainsi que les hydrocarbures aromatiques polycycliques (HAP). Les études de biodégradation couplées à celles d'adsorption-désorption peuvent améliorer la compréhension du comportement de systèmes de sols contaminés en apportant de l'information sur la biodisponibilité potentielle de ces contaminants.

Les objectifs de cette recherche sont de vérifier l'effet de la présence de kaolinite et de cadmium sur la biodégradation du naphtalène et de napthalènes substitués. La kaolinite est présente dans plusieurs sols du Québec et est un constituant important des sols tropicaux. Puisque la kaolinite est une argile bien définie, les expériences ont été conduites dans le but de vérifier l'effet que peut avoir la kaolinite sur l'atténuation du naphtalène, du 2-méthyl-naphtalène et du 2-naphtol en présence ou absence de cadmium. De plus, les résultats de ces analyses ont été utilisés afin d'interpréter le comportement de chaque HAP dans les expériences de minéralisation. Ces expériences ont été réalisées à l'aide de la souche gram-négative *Pseudomonas putida* (ATCC #17484).

Il a été démontré que les taux de minéralisation du naphtalène, du 2-méthyl-naphtalène et du 2-naphtol sont diminués en présence de kaolinite. La présence de cadmium n'a pas inhibé l'activité bactérienne, et en fait une légère augmentation du pourcentage total de minéralisation a été observée dans les expériences de minéralisation. Lors des expériences d'adsorption-désorption, l'enlèvement de chaque HAP de la surface de la kaolinite a été corrélé à leur coefficient de partage octanol/eau respectif. Le 2-méthyl-naphtalène a démontré la plus grande affinité pour la kaolinite et conséquemment, a produit les plus faibles taux de minéralisation.

Les résultats de cette étude indiquent que la présence de cadmium n'influence pas de façon significative la capacité de minéralisation de *P. putida* pour le naphtalène, le 2-méthyl-naphtalène et le 2-naphtol. Cependant, la présence de kaolinite a affecté l'activité bactérienne en réduisant de façon significative les taux de minéralisation des HAP. Ces résultats démontrent le besoin d'identifier les mécanismes généraux d'interactions afin de développer des programmes efficaces de biodégradation de sites contaminés.

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List of Symbols

ACS:	Aqueous Counting Scintillant
Al:	Aluminum
Al^{3+}:	Aluminum cation
AgCl:	Silver chloride
$AgNO_3$:	Silver nitrate
ATCC:	American Type Culture Collection
ATP:	Adenosine Triphosphate
^{14}C:	Radiolabelled carbon
Ca;	Calcium
Ca^{2+}:	Calcium cation
Cd;	Cadmium
Cd^{2+}:	Cadmium cation
CEC	Cation exchange capacity
Cl^-:	Chlorine anion
Cu:	Copper
Cu^{2+}:	Copper cation
H^+:	Hydrogen cation
$HClO_4$:	Hydrochoric acid
HF:	Hydrofluoric acid
Hg:	Mercury
Hg^{2+}:	Mercury cation
hr:	hour
IEP:	Isoelectric point
K_a:	Acidity constan
KOH:	Potassium hydroxide
Mo:	Molybdenum

Mg:	Magnesium
Mg^{2+}:	Magnesium cation
Mn:	Manganese
Mn^{2+}:	Manganese cation
Na^{+}:	Sodium cation
NaCl:	Sodium chloride
NO_3^-:	Nitrate anion
OMC:	Organic matter content
Pb:	Lead
Pb^{2+}:	Lead cation
pK_a:	$-LogK_a$
ppm:	parts per million
P.putida:	*Pseudomonas putida*
rpm:	revolutions per minute
Si^{4+}:	Silica cation
ug:	micrograms
Zn:	Zinc
Zn^{2+}:	Zinc cation

Glossary

Abiotic: processes occurring in the absence of biological intervention

Aerobic: an environment containing oxygen at a partial pressure similar to air.

Anaerobic: an environment in which oxygen is absent.

Anthropogenic: of, relating to, or resulting from the influence of human beings on nature.

Aromatic: an unsaturated cyclic organic compound based on a six-carbon (benzene) ring.

ASTM: American Standard and Testing Manual.

Autochthonous: indigenous or naturally occurring organisms in a given environment.

Biosurfactant: a biologically produced surface active organic compound with an amphipathic (having both hydrophilic and hydrophobic regions) regions.

Biotic: processes under the control of biological influence

Catabolism: the metabolic reactions by which organic compounds are degraded to simpler organic or inorganic compounds.

Chemotaxis: the movement of an organism in response to a chemical stimulus.

Colloid: particles with a diameter smaller than 0.001nm but larger than 10^{-6} mm. Clay particles fall within this range.

Cytoplasmic membrane: the unit lipid bilayer (membrane) surrounding and enclosing the contents of a cell.

czc: designation for cadmium, zinc, and cobalt resistance efflux gene isolated from *Alcaligenes eutrophus*.

DLVO: Derjaguin, Landau, Verwey, and Overbeck theory on energies of interaction between soil particles and ions.

DNA: deoxyribonucleic acid, the repository of genetic information in all cells. It is a polymer composed of a determined sequence of nucleotide sub-units.

Heterotroph: an organism which used organic compounds for most or all of its carbon requirements.

Hydrometer: an instrument that is used to measure the density of a liquid.

Hydrophobic: a molecule/compound that is virturally insoluble or immiscible in water.

Metal Speciation: refers to the formation of complexes between heavy metals and ligands in the aqueous phase, resulting thereby in competition between the ligands and the soil solids for "adsorption" of the heavy metals.

Microcosm: a small scale experimental set-up that is used in the laboratory to mimic the natural environment under controlled conditions and is used to study the mineralization of hydrocarbons in the soil.

Mineralization: the process in which organic materials are broken down into inorganic materials essential in the cycling of matter.

PAHs: polycyclic aromatic hydrocarbons-present in petroleum and are formed by the incomplete combustion of almost any organic matter.

Plasmid: a linear or covalently closed circular molecule of DNA, distinct from the chromosome, can replicate autonomously and is often dispensible to the cell.

Sorption: general term used to describe processes of both adsorption and absorption onto a solid matrix.

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Chapter 1
Introduction

1.1 Introduction

Contaminants such as PAHs and heavy metals enter the environment from a number of natural phenomena, however the excess concentrations found and the widespread distribution of these contaminants is mostly due to anthropogenic forces [Evans, 1989]. PAHs enter the environment by: petroleum spillage, land disposal, fossil fuel combustion [Cerniglia, 1992; Bossert and Bartha, 1984; Gibson and Subramanian, 1984]; and heavy metals enter the environment as constituents of pesticides and fertilizers, sewage sludge; and as waste from smelters, refuse incineration, mining and metal-plating industries [Fergusson, 1990; Barkay *et al*, 1992; John, 1971].

There is concern with regard to the fate of heavy metals and PAHs as these contaminants have been shown to transport through soils and contaminate groundwater systems. The risks to human health and safety are significant since PAHs are known to be mutagenic and carcinogenic [Cerniglia, 1992], as are some heavy metals such as cadmium [Ron, et al. 1992]. The build-up of cadmium in humans can cause acute and chronic effects such as, anemia, lung insufficiency, renal disturbances, and anosmia [Webb, 1979; Namasivayam, 1995; Ciavatta *et al*, 1993; Brown and Lester, 1979].

Sites are frequently contaminated with complex mixtures of organic and inorganic compounds [Francis, 1990; Baker and Herson, 1994]. The disappearance and transport of these contaminants are affected by both abiotic and biotic factors in soils [Leahy and Colwell, 1990; Farago and Mehra, 1993]. The ability of subsurface bacteria to remediate PAH contaminated soils has been demonstrated both *in-situ* and in

laboratory simulations with single bacterial species [Fredrickson *et al*, 1991] and by microbial consortia [Al-Bashir *et al*, 1990, 1994; Knaebel *et al*, 1994; Dibble and Bartha, 1979]. The ability to optimize the bioremediation of contaminated soils with mixtures of organics and metals requires a thorough understanding of the behaviour and speciation of the metal in soil and its interaction with the organic contaminants, soil constituents, and indigenous microbial consortium.

The abiotic factors contributing to the transport of contaminants in soils are mostly controlled by the clay fraction. Its high surface area and cation exchange capacity (CEC), interact strongly with metals and organic contaminants alike making clay the ideal soil constituent to study independently [Hutzinger, 1985; Stotzky and Burns, 1980; Hermosin *et al*, 1993].

A better understanding is required of the processes and mechanisms which control the fate of hazardous pollutants in soil. Such knowledge can help in the assessment and development of efficient biological treatment systems. Biodegradation and adsorption-desorption studies provide insight into understanding some of the fundamental processes occurring in a contaminated system which can help in the implementation of effective bioremediation programs.

1.2 Problem

Microorganisms are ubiquitous throughout nature, and have long been recognized for their ability to bring about transformation of organic and inorganic contaminants in soils [Francis, 1990]. A better understanding of these biological processes has led to significant developments in providing alternative cost effective technologies that can be used in the remediation of contaminated sites [Cookson, 1995].

One of the largest problems facing the environment is the volume of contaminants that are disposed of either intentionally, in accidents or as by-products of industrial processes. For example, crude petroleum, can consist of up to 40% PAHs where annual

spills are estimated around 5 X 10^7 tons [Betts, 1993; Cookson, 1995]. Also, heavy metals such as Pb has a yearly production of 4.1 X 10^6 tons and Cd has a yearly production of 1.4 X 10^4 tons. Much of the yearly production of Pb and Cd enters the environment as waste since both metals are used in battery production which constitutes 60 and 37%, respectively, of their consumption [Fergusson, 1990]. Both organic an metal contaminants present in excess concentrations in soils have been shown to severely affect the behaviour of a natural soil consortia by altering the physical-chemical environment of the microorganisms and by affecting microbial metabolism [Walker and Colwell, 1974; Capone *et al*, 1983; Mountfort and Asher, 1981; Barkay *et al*, 1986]. Studies looking at low concentrations of heavy metal contamination in soils, have also shown significant affects on microbial acitivity [Doyle *et al*, 1975]

The study of bioremediation processes is further complicated by the multiphasic, heterogenous environments of soils, which provide numerous surfaces for interactions with microorganisms and contaminants, resulting in a complex system [Francis, 1990]. Contaminants such as metal ions in soils, do not necessary remain in their original form and are usually distributed in various species, where only one of these species may be responsible for inhibiting microbial activity, rather than the total metal soil concentration [Hughes and Poole, 1991].

It is the intent of this research to study the phenomenon of a mixed system. *In-situ* contaminated sites rarely consist of a single contaminant, therefore to effectively evaluate the behaviour of a contaminated system, a mixed system must be investigated. The results from this research can contribute to improving bioremediation of contaminated soils with specific emphasis on processes of biostimulation or bioaugmentation.

1.3 Objectives

The objective of this research was to study the effect of kaolinite and cadmium on the biodegradation of naphthalene and substituted naphthalenes. The polycyclic aromatic hydrocarbons under investigation were naphthalene, 2-methyl naphthalene, and 2-naphthol as seen in Fig. 1.1. Table 1.1 lists some of the physical properties of the three PAH compounds.

To obviate the interactions in an *in-situ* contaminated system, it was reasoned that microcosms could provide the most effective means of simulating the stress effect of cadmium and kaolinite on mineralization activity. To provide information depicting the chemical activity within the mineralization microcosms, adsorption and desorption experiments were performed. Furthermore, to assess the speciation of cadmium in the mineral salts medium (MSM) used in this study, chemical equilibrium calculations were performed estimating the most likely complexes to form using known formation constants (outlined in Appendix E). The likely cadmium species to form given the constituents of the MSM, were $CdOH^+$, $Cd(OH)_2$, $CdNO_3^+$, and $CdHPO_4$. The formation of these complexes are described by the following equations [Lindsay, 1979]:

$$Cd^{2+} + H_2O \rightleftharpoons CdOH^+ + H^+$$

$$Cd^{2+} + NO_3^- \rightleftharpoons CdNO_3^+$$

$$Cd^{2+} + 2H_2O \rightleftharpoons Cd(OH)_2 + 2H^+$$

$$Cd^{2+} + H_2PO_4^- \rightleftharpoons CdHPO_4 + H^+$$

To facilitate the interpretation of experimental results, a single soil constituent was chosen; kaolinite clay. Several physicochemical properties of kaolinite were characterized in order to estimate the factors responsible for PAH and cadmium

sorption. The system was in suspension since aqueous systems tend to have greater homogeneity than non-aqueous systems [Bruemmer *et al*, 1988]. Single soil constituents are often studied separate from a natural soil since natural soils are too complex and since the presence of clay, its type and quantity tend to be the factors determining the behaviour and characteristics of the soil [John, 1971]. A clay was also chosen on the basis that most microbial activity is associated with the clay mineral fraction of soils [Stotzky, 1972]. Clays make a suitable habitat for microorganisms because they have the ability to maintain the pH of the ambient solution suitable for bacterial metabolism [Stotzky, 1972].

The experiments in this study used kaolinite in solution with a single heavy metal and a single bacterial species to eliminate the interactive effects between microbial species, competition between metals, and the significant effect organic matter plays on the availability and distribution of organics and metals to microorganisms [Gamble *et al*, 1983]. All of these factors can separately or jointly affect experimental results [Weissenfels *et al*, 1992; Bååth, 1989; Hattori and Hattori, 1976; Lyman *et al*, 1982].

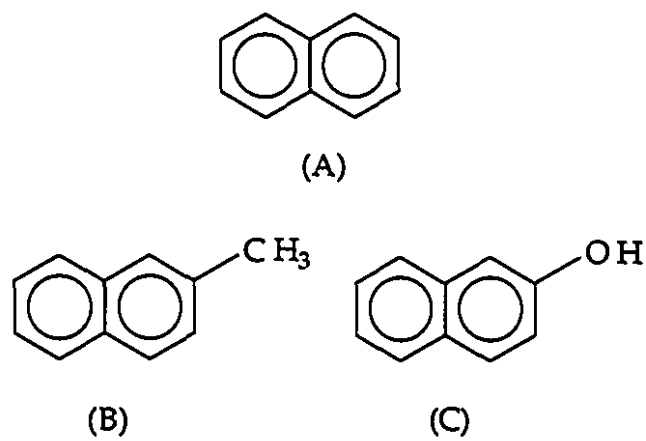
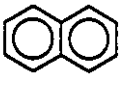
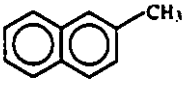
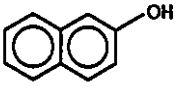


Figure 1.1. Polycyclic aromatic hydrocarbons used in this study. (A) Naphthalene, (B) 2-Methyl Naphthalene, and (C) 2-Naphthol.

All three PAHs chosen are representative environmental contaminants where naphthalene and 2-methyl naphthalene are components of petroleum and creosote and 2-naphthol is a major component of pesticides and dyes [Verschueren, 1983]. Cadmium was chosen because it is a significant environmental contaminant with no known biological function [Webb, 1979]. The PAHs and heavy metals selected were chosen to represent model compounds one may find in a contaminated site.

In routine testing for biodegradation of organic contaminants, the concentrations investigated are usually in the range of 2 to 100 mg L^{-1} (ppm) [Stucki and Alexander, 1987]. For this reason, 25 and 130 mg L^{-1} were the concentrations studied to cover a concentration above and below the solubility limits of two of the three compounds studied (A and B in Fig. 1.1).

Table 1.1. Structure and Physical/Chemical Properties of Carbon Compounds used in this Study.

Compound	*Structure*	*Molecular Formula*	*Molecular Weight*	*Solubility* mgL^{-1}	*Log* K_{ow}*
Naphthalene		$C_{10}H_8$	128.12	31.7†	3.36†
2-Methyl Naphthalene		$C_{11}H_{10}$	142.13	25‡	4.11†
2-Naphthol		$C_{10}H_8O$	144.12	750°	2.84°

*Values obtained from the Handbook of Chemical Property and Estimation Methods[Lyman *et al*, 1982], †Karickhoff and Brown (1979), ‡Mackay and Shiu (1977), °stands for the log of the octanol/water partition coefficient

The background cadmium concentration found in soils is in the range of 0.01 to 1.8 mg Kg^{-1} [Trevors *et al*, 1986]. Soil pollution with cadmium has been found at concentrations ranging from 0.2 to 10 000 mg Kg^{-1} [Babich and Stotzky, 1978; Chlopecka *et al*, 1996; Flyhammer, 1995; Keller and Védy, 1994]. The concentrations examined in this study were 5 and 238mg Kg^{-1} (0.044 and 2.12mM cadmium). Cadmium was chosen

since it is generally found in only two oxidation states: Cd(0) and Cd(II), whereby the latter is the most common [Lindsay,1979]. Cadmium is a relatively mobile element in the environment, because the Cd^{2+} cation persists over a wide range of pH values making species estimations in soil more predictable than other transition metals [Fergusson, 1990].

In the thesis following, it should be noted that when the abbreviation Cd or the term cadmium are used, it refers to whatever form of cadmium may be present; otherwise if cadmium is in its cationic form (Cd^{2+}) or of some other species, it is so designated.

1.4 Contributions

Based on the outline presented in the problem section (1.2), the contributions of this work consist of the following:

- Through experimental and basic theoretical means the ability to estimate and predict cadmium removal onto kaolinite.
- Use of *Pseudomonas putida* as an indicator organism for predicting degradation of PAH compounds in soil.
- Studying the impact kaolinite and cadmium can exert on the mineralization activity of PAH contaminants.

1.5 Thesis Organization

This thesis consists of five chapters and five appendices summarized as follows: Chapter 1. Introduction of the general issues along with the problem and objectives addressed in this study and a summary of the contributions.

Chapter 2. The literature review addresses the topics that are involved with the objectives outlined in section 1.3.

Chapter 3. Description of the materials and methodology used in this study.

Chapter 4. Results and discussions of the experiments performed.

Chapter 5. Conclusions and future research suggestions for the topic outlined in this thesis.

Appendix A. Provides a summary of Tables listing the mineralization data obtained to create the control mineral salts medium and 2% kaolinite with mineral salts medium microcosm curves.

Appendix B. Provides all the adsorption-desorption data for naphthalene, 2-methyl naphthalene, and 2-naphthol in the presence and absence of cadmium in kaolinite; and adsorption-desorption data for cadmium in kaolinite.

Appendix C. The data used to determine the equilibrium time for all PAHs and cadmium in kaolinite.

Appendix D. Optical density readings used to create the biomass growth curves.

Appendix E. Sample speciation calculation for cadmium.

Chapter 2
Literature Review

The purpose of this chapter is to give a brief overview on subjects that are related to the research objective addressed in this thesis.

2.1 Kaolinite Characteristics and Features

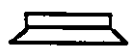
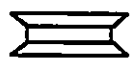
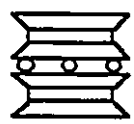
Kaolinte is a common constituent of tropical lateritic and saprolitic soils being an end product of intense weathering common to tropical soils [ISSMFE, 1985]. Kaolinte is also present in many natural soils in Quebec [Duquette and Hendershot, 1987].

The kaolinite mineral is composed of alternating silica tetrahedral and alumina octahedral sheets. The successive layers are stacked and bonding between layers occurs via hydrogen bonding and van der Waals forces which together are strong enough to prevent interlayer swelling. Due to the non-swelling nature of kaolinites, kaolinite in suspension provides mostly external surfaces so adsorbed molecules are exposed to the aqueous environment [Miller and Alexander, 1991; Knaebel *et al*, 1994; Huang, 1990].

Kaolinite, unlike montmorillonite and illite clays, has a variable charge or pH-dependent surface and is amphoteric in nature [Reed and Nonavirakere, 1992]. The variable charge comes from the broken bonds on the edges of the sheets which constitute 10-20% of the total crystal surface and are associated with reactions of protons [Evans, 1989; Yong *et al*, 1992]. The areas of permanent negative charge on the kaolinite surfaces comes from isomorphous substitution. The negative charge is compensated by inorganic hydrated cations which are exchangeable. [Evans, 1989; Yong *et al*, 1992; Bolland *et al*, 1976; Hermosin *et al*, 1993]. Cation exchange capacities for kaolinites in general, are in

the range of 3 to 15 meq/100g with specific surface areas in the order of 10 to 20 $m^2 g^{-1}$ of dry clay [Mitchell, 1976]. Compared to montmorillonite and illite clays, kaolinite provides a comparatively lower reactive surface area. Table 2.1 summarizes some of the basic properties of kaolinite, montmorillonite and illite clays.

Table 2.1. Mineral Characteristics of Kaolinite, Montmorillonite and Illite.

Mineral	*Basic Structure*	*Basil Spacing (Å)*	*Interlayer Bonding*	*Cation Exchange Capacity (meq $100g^{-1}$)*	*Specific Surface Area ($m^2 g^{-1}$)*
Kaolinite		7.2	O-OH (strong)	3-15	10-20
Montmorillonite		9.6	O-O (very weak)	80-150	50-120 Primary 700-840 Secondary
Illite		10	K ions (strong)	10-40	65-100

alumina sheet, silica sheet, potassium molecules. Source: Mitchell (1976)

2.2 Adsorption Mechanisms for Heavy Metals onto Kaolinite

Adsorption is a physicochemical process whereby ionic and nonionic solutes become concentrated from solution at the solid-liquid interfaces [Sposito, 1989; Yong *et al*, 1992]. Adsorption is usually fully or partially reversible (desorption), and is of primary importance in transport considerations, in the sense that it largely affects the mobility of heavy metals and organic contaminants.

The factors affecting adsorption and desorption processes include: temperature, ionic strength, surface concentration of sites occupied by the adsorbate, pH of the soil solution, mineralogy of the soil, ligands and the soil:solution ratio [Yong *et al*, 1992; Garcia-Miragaya and Page, 1976; Sposito, 1989].

In the case of kaolinite as discussed in the previous section, the edge surfaces are the most reactive area on the mineral where the reactive functional group is the hydroxide species (-OH). Kaolinite edges consist of two types of hydroxyl groups, those that are singly coordinated to Si^{4+} and those that are singly coordinated to Al^{3+}. Both types of edges function as Lewis acid sites as do hydrated metals which generally function as a slightly stronger acid when their oxidation state is high [Brown and Lemay, 1985; Huang, 1990]. The alumina hydroxide groups are able to undergo a two-step ligand exchange reaction seen in the equations below. The lower oxidation value of Al^{3+} compared to Si^{4+} , allows a greater negative charge around the oxygen atom [Sposito, 1989; Huang, 1990].

$$-MOH_{(s)} + H^{+}_{(aq)} \rightleftharpoons -MOH_2^{+}{}_{(s)}$$

$$-MOH_2^{+} + S\text{-}COO^{-} \rightleftharpoons -MOOC\text{-}S_{(s)} + H_2O_{(l)}$$

Where M is the metal of the edge surface of the octahedral sheet, and S is the contaminant with the attached functional group.

Kaolinite surfaces have been shown to function as nucleation centers for heavy metals. In the studies by Garcia-Miragaya and Davalos (1986), and Farrah and Pickering (1976), the CEC was insufficient for accurate evaluation of metal removal since the kaolinite surface was shown to exhibit surface nucleation potential for both Zn^{2+} and Cu^{2+}, resulting in greater metal removal than predicted. Both studies observed that cation adsorption at concentrations below the CEC resulted in an adsorption mechanism where the cations were easily desorbed and therefore were considered non-specifically adsorbed. The cations adsorbed at concentrations above the CEC of kaolinite, could only be desorbed using HF + $HClO_4$ indicating that the mechanisms of interaction were stronger than ion exchange.

Studies by Yong and Sheremata (1991) and Haas and Horowitz (1986), examined how the presence of complexation ligands significantly affected the adsorption of Cd onto a Na^+-saturated kaolinite surface. It was observed that as the concentration of free Cl^- increased, the concentration of the Cd-Cl complexes increased reducing Cd adsorption onto the kaolinite surface.

The study by Holm *et al* (1995), observed that the presence of high concentrations of monovalent cations such as Na^+ and K^+ had no effect on the distribution of Cd^{2+} and Zn^{2+} onto Amberlite, however Garcia-Miragaya and Page (1976), found that the presence of macroconcentrations of competing cations such as Na^+ decreased the ability of Cd^{2+} to adsorb onto the surface of a Na^+-saturated montmorillonite.

2.3 Heavy Metal Contamination and Microorganisms

Heavy metals added to soils in industrial and municipal wastes are generally several orders of magnitude higher than their concentrations in nature, therefore the metals often precipitate as secondary minerals [Chlopecka *et al*, 1996]. For example, cadmium has a background concentration in soil averaging around 3.6×10^{-6} M (0.4ppm) and contamination of soil by cadmium can be as high as 0.3M which is a loading high enough to cause surface precipitation.

Due to the variability in the soil environment, metals are capable of being present in various chemical forms. The transition of metals between different forms in soils is called speciation. The definition of metal speciation is described best as the distribution and transformation of metal species in the media or soil under investigation [Förstner, 1993]. The factors affecting metal speciation most significantly in soil include: pH, type and amount of clay, organic matter and hydrous metal oxide, cation exchange capacity, specific surface area, and the nature and chemical properties of the heavy metal [Babich and Stotzky, 1983; Hattori, 1992]. The factors that dictate metal speciation, for

example with cadmium include the concentration of the ligand present and/or the pH of the system. For example Cl^- ion concentrations as low as 0.001M begins $CdCl^+$ formation; 0.3M Cl^- begins $CdCl_2$; and 1M begins $CdCl_3^-$ and $CdCl_4^-$ formation [Hahne and Kroontje, 1973]. At a pH value less than 8, cadmium exists completely in its cationic free form (Cd^{2+}). Cadmium only begins to hydrolyze at pH 9 where species such as $CdOH^+$ and $Cd(OH)_2$ begin to form [Babich and Stotzky, 1978].

Speciation of metal ions is one of the most difficult processes to follow in an abiotic or biotic system as the species of the metal may change during growth of the organism, for example by precipitation of metal ions as oxalates or phosphates, precipitation resulting from a change in pH, complexation by cell ligand synthesis, and metabolites may all complex with free metals present [Hughes, and Poole, 1991; Babich and Stotzky, 1978].

In soil or solution the species of the metal is extremely important since only one of the metal species, which may represent only a minor fraction of the total metal ion concentration, may be responsible for the effect exerted on the microorganisms present [Hughes and Poole, 1991; Lövgren and Sjoberg, 1989]. Although it is difficult to corroborate findings from different studies, it is generally agreed upon that the soluble forms of the heavy metals exhibit the greatest toxicity and hence the greatest inhibitory effect on microorganisms [Hattori, 1992; Tubbing *et al*, 1994; Shuttleworth and Unz, 1991; Babich *et al*, 1981]. However, the soluble or leachable fraction does not always correspond to the amount available to biota [Förstner, 1993; Hughes and Poole, 1991]. Microorganisms have been shown to utilize metals in their precipitated form which has been evidenced in studies looking at the corrosion of jet fuel tanks [Engel and Swatek, 1966, 1968; Engel and Owen, 1969; Hedrick *et al*, 1967, 1968; Reynolds *et al*, 1966].

Microorganisms have been exposed to heavy metals throughout time and so the interplay of metals and microorganisms is not a new phenomenon; the novelty in heavy

metal/microorganism interactions lies in the ability of the microorganism to tolerate elevated concentrations resulting from pollution.

Microorganisms require certain metallic elements for growth and function. These include, the bulk elements Na, K, Mg, and trace elements such as Mn, Fe, Cu, Zn and Mo [Hughes and Poole, 1991].

All heavy metals are capable of inhibiting microbial activity, and at elevated concentrations all metals are poisonous [Barkay *et al*, 1992; Bååth, 1989]. Metal ions are unique in that over a rather narrow concentration range, their status can change from an essential growth-promoting element to a toxin and so bacteria maintain a tight control over the level of metal to which they are exposed [Geesey and Jang, 1989].

It has been suggested that since heavy metal resistance is most often plasmid encoded, increased tolerance in a microbial community occurs through gene transfer via plasmids [Daubaras and Chakrabarty, 1992; Bååth, 1989]. Tolerance of heavy metal contamination may also occur through adaptation, but more likely results from proliferation of an indigenous metal-resistant organism. Selectivity for heavy metal tolerance can take years to occur as was seen in the study by Doelman and Haanstra (1979), where more Pb-tolerant strains were found three years later in a sandy soil than the same soil unamended with Pb.

Population shifts commonly observed in heavy metal contaminated sites, are increases in fungal populations [Frostegård *et al*, 1993]. These eukaryotic organisms tend to exhibit a greater tolerance towards heavy metals [Hattori, 1992], and are more tolerant to acidic conditions that often occur due to acidification of soils from the addition of metal salts [Farago and Mehra, 1993; Doelman and Haanstra, 1984]. The preponderance towards fungal presence under acidic or high metal conditions does not always occur and is significantly affected by such factors as soil type and metal type since some metals such as Ag^{+} and Hg^{2+} are known to inhibit fungal growth [Cornfield, 1977].

Several studies have shown that systems exposed to elevated heavy metal concentrations, show a preponderance towards Gram-negative bacteria [Barkay, *et al*, 1985; Doelman and Haanstra, 1979; Farago and Mehra, 1993; Frostegård *et al*, 1993; Bååth, 1989]. Gram-negative bacteria have been shown to be more metal-tolerant than Gram-positive bacteria in soils with low levels of metal contamination [Silver *et al*, 1982; Duxbury and Bicknell, 1983; Frostegård *et al*, 1993].

For several environmentally significant metals, severe metal pollution may substantially alter the flow of carbon and the final electron acceptor in a given system [Capone *et al*, 1983; Said and Lewis, 1991; Hughes and Poole, 1991]. In the study by Capone *et al* (1983), it was found that the chlorides of Hg, Pb, Ni, Cd, and Cu completely inhibited sulfate-reducing bacteria, and stimulated methanogenesis.

2.3.1 Heavy Metal Resistance Mechanisms

Heavy metal microbial resistance mechanisms range from physical/chemical interactions to genetically evolved defence mechanisms. Due to the evolution of microorganisms in the presence of metals, genetic mechanisms have evolved over thousands of years [Beveridge, 1989].

The living organism interacts with dissolved metals as a living, metabolizing body and as an biotic colloid [Francis, 1990]. The removal of dissolved metals by microorganisms results from three mechanisms: biosorption, bioaccumulation, and the removal by metabolic by-products. All three mechanisms can work together if cells are grown in the presence of inhibitory concentrations of metals [Remacle, 1988]. The inhibitory effect of heavy metals on microbial activity is mainly due to their interference with microbial metabolism or their altering of the physicochemical environment of cells [Barkay *et al*, 1986].

Microbial mechanisms implicated in the survival in the presence of potentially inhibitory concentrations of metal species include extracellular precipitation, intra and

extracellular complexation [Nies, 1992] and crystallization [Remacle, 1988]: transformations including oxidation, reduction, methylation [Silver and Misra, 1983], and dealkylation: biosorption to cell walls and extracelluar polysaccharide crystallization [Remacle, 1988], binding proteins [Mago and Srivastava, 1994]: impermeability: decreased transport: efflux [Mago and Srivastava, 1994; Silver and Misra, 1983]: intracellular compartmentation and/or sequestration [Mago and Srivastava, 1994]. A given organism often relies directly and/or indirectly on several survival strategies [Cooney and Gadd, 1995].

A significant portion of resistance mechanisms are plasmid-encoded, and the majority involve transport phenomena. With evolution, microorganisms developed two distinct mechanisms to counter the continual transport of ions across the membrane whether in a contaminated area or not: intracellular complexation and efflux [Nies, 1992]. Efflux pumps are the major currently-known group of such plasmid resistance systems which have been extensively studied and documented in the cadmium efflux system of *Staphylococcus aureus* [Ron *et al*, 1992; Silver and Misra, 1983]. For example, mercury resistance systems are highly homologous in all bacteria studied and tend to show enzymatic or chemical transformation [Trevors *et al*, 1986]; whereas cadmium resistance appears to follow a different evolutionary path between Gram-negative and Gram-positive bacteria. Gram-positive bacteria such as *Staphylococcus, Listeria,* and *Bacillus,* use an ATPase, but the energy for cadmium efflux in Gram-negative bacteria is chemiosmotic [Ji and Silver, 1995]. Systems such as the czc (cadmium, zinc, cobalt) efflux resistant systems have been characterized in *Alcaligenes eutrophus* , a Gram-negative bacteria. Bacteria possessing the czc system have been shown to decrease the net accumulation of the metal ions within the cell [Nies, 1992; Nies and Silver, 1989; Ji and Silver, 1995].

Resistance towards cadmium in *Pseudomonas putida* strains varies from efflux, sequestration to extracellular complexation mechanisms, [Ghosh and Bupp, 1992;

Higham, 1984; Trevors *et* al, 1986; Nies, 1992]. The sequence of these systems in *P. putida* have not been sufficiently characterized.

2.4 Abiotic Factors Affecting Metal Resistance

Abiotic factors play a significant role in affecting the speciation and hence potential inhibitory effect of a given metal in soil. Some of these abiotic factors include: clay content, organic-matter content, pH, temperature, Fe and Mn oxides, and the presence of ligands [Doelman and Haanstra, 1984; Bååth, 1989; Gadd and Griffiths, 1978; Babich and Stotzky, 1977a, b, c].

In the review of metal effects on microbes by Bååth (1989), it was discussed that the most significant abiotic factors affecting metal availability and toxicity are cation exchange capacity (CEC) and soil pH. Contradictory results are found between studies because of differences in pH for reasons which include: variety of ligand-metal complexes; effect on metabolic state of microorganisms; and rates of soil processes [Bååth, 1989].

Clays and sediments modify the metal effect when present, by adsorbing the metal species [Hughes and Poole, 1991]. In the study by Babich and Stotzky (1977c), it was generally observed that the inhibitory effects of cadmium to bacteria, fungi, and actinomycetes were reduced in the presence of montmorillonite and kaolinite. Montmorillonite provided greater protection which appeared to be related to its higher CEC. Similar results were found in the studies by Stotzky (1966), Stotzky and Rem (1966), and Babich and Stotzky (1977a).

2.5 Adsorption Mechanisms of Organic Molecules onto Clays

Clay minerals due to their high surface area and reactivity are effective sorbents of organic contaminants, especially those of cationic or polar character [Stotzky and Burns, 1980; Hermosin *et al*, 1993].

Acidic organic compounds ionize in aqueous solutions to form anion species. In their anionic forms, acidic organic compounds are expected to be repelled by negatively charged clay surfaces. For example, with a Na^+-saturated montmorillonite, it was found that adsorption of acidic compounds occurred when the pH of the bulk solution was about 1 to 1.5 units above their pK_a value [Bailey *et al*, 1968]. The primary mechanisms of interaction for acidic organics are: van der Waals adsorption, hydrogen-bonding, and increased adsorption due to high electrolyte concentration ("salting-out" effect), and ligand exchange [Baham *et al*, 1994, Yong *et al*, 1992; Huang, 1990].

Cationic organic compound mechanisms of interaction include: cation exchange, H-bonding, ion-dipole interactions, charge transfer cation exchange of inorganic cations, and van der Waals forces [Huang, 1990; Baham *et al*, 1994, Yong *et al*, 1992]. Large organic cations are adsorbed more strongly than inorganic cations by clays because of their length and high molecular weights [Morrill *et al*, 1982]. The important features of organic molecules that affect their adsorption are: shape, size, configuration, polarity, polarizability, water solubility, and functional groups [Greenland *et al*, 1981; Huang, 1990].

Hydrophobic organic compounds can exhibit "cooperative" adsorption where the molecules tend to adsorb in rows or clusters; most likely with the planar aromatic nucleus face to face and perpendicular to the soil particle surface exhibiting an S-curve adsorption isotherm [Giles *et al*, 1974]. The contaminants can also adsorb edge-on to the substrate surface, this is usually indicative of strong adsorbate-substrate attractive forces [Giles *et al*, 1974]. The predominant forms of interaction are via van der Waals

forces [Yong *et al*, 1992] and hydrophobic interactions [Hassett *et al*, 1981]. High molecular weight hydrocarbons with low solubilities tend to show a preponderance towards high association with clay particles. In the study by Yong and Rao (1991), results indicated that adsorption of hydrocarbons by clays occurs more favourably when the solubility of the hydrocarbon is exceeded. Van der Waals attractive forces, although weak, are additive and can result in large total forces. Hydrophobic sorption is the result of weak solute-solvent interactions, so as the compounds become less polar and as the number of carbons increases, hydrophobic reactions predominate [Hassett *et* al, 1981]. Other studies suggest that the removal of insoluble organic molecules from clays in adsorption studies is a result of the "umbrella effect" where the organic precipitates are carried into the pellet during centrifugation [Stotzky and Burns, 1980].

2.6 Microorganism-Clay Interaction Mechanisms

Research has shown that most microbial activity in soil occurs in thin films of water associated with the surfaces of the clay particles or in "necks" between clay particles [Bååth, 1989]. The types of clay minerals present in the soil exerts an influence on the activity and ecology of microbes in soil micro-environments [Babich and Stotzky, 1977].

Clays exert both direct and indirect effects on the biochemical activities of soil microorganisms. Direct effects include sorptive interactions, and indirect effects include interaction of the clays with nutrients or with products of cell metabolism [Filip, 1973]. A direct effect is experienced when clay particles coat bacterial cells which protects the cells physically by preventing protozoal grazing or viral attack [van Loosdrecht *et al*, 1990]. It is generally agreed that the indirect effect of clays play the most significant role since their presence essentially determines the behaviour of the soil as discussed earlier and therefore the clay exerts control over the physico-chemical environment of the microbes [van Loosdrecht *et al*, 1990; Stotzky and Burns, 1980]. The feature of clays

that has the most significant impact on microbial activity is the CEC of the clay and its buffering capacity [Babich and Stotzky, 1978]. In the study by Stotzky (1973) it was found that montmorillonite stimulated the respiration of bacteria by maintaining the pH of the environment suitable for sustained growth. It was also observed in the study by Babich and Stotzky (1978), that montmorillonite homoionic to cadmium, was more toxic to the microbes present than homoionic kaolinite. Montmorillonite has a higher CEC, and therefore exchanged more cadmium to the ambient environment. In a study by Gadd and Griffiths (1978) kaolinite and montmorillonite were shown to protect certain microorganisms from the inhibitory effects of Cd and that the enhancement of this ability was related to the cation exchange capacity of the mineral.

The physical presence of bacterial cells can also affect the chemical behaviour of the clay particle surface. In the study by Bellin and Rao (1993), it was found that the presence of microbial biomass added to a subsurface soil resulted in an alteration of the soil surface properties which reduced the sorption of quinoline and naphthalene enhancing their transport in the soil column. A similar finding was made by Jenkins and Lion (1993), with phenanthrene in a sand column study. Gannon *et al*, (1991) summarized the mechanisms of binding of microorganisms to solid surfaces as: cell hydrophobicity, net surface electrostatic charge of the cells, presence of capsular polysaccharides and cell size. Hattori and Hattori (1976), suggest that the mechanisms involved in bacteria reaching solid surfaces include: Brownian movement, flagella movement, tactic movement, hydrophobic effect, physical interactions between cells and surfaces, cell adhesion and detachment from surfaces.

2.7 Polcyclic Aromatic Hydrocarbons (PAHs)

A significant percentage of hazardous spills are petroleum based where PAHs make up over 40% of its composition [Cookson, 1995]. Creosote contamination is the second most prevalent hazardous waste problem, and PAHs, make up over 85% of the

chemical constituents of creosote [Cookson, 1995; U.S. EPA, 1991]. PAHs are a class of hazardous organic chemicals that consist of one or more fused benzene rings in linear, angular and cluster arrangements [Cerniglia, 1992; Solomon's, 1984]. PAHs are sparingly soluble, hydrophobic compounds that tend to sorb strongly to soil surfaces as discussed in detail in section 2.5 [Cerniglia, 1992; Falatko and Novak, 1992; Wallnöfer and Engelhardt, 1984]. PAHs can persist for a relatively long time in soils due to their lipophilic properties [Bulman *et al*, 1988; Gibson and Subramanian, 1984]. The higher the molecular weights of these compounds the greater their degree of recalcitrance and the more strongly they adsorb onto soil surfaces [Cerniglia, 1992; Leahy and Colwell, 1990]. The lower the molecular weight of these compounds and the greater their solubility, the greater their presence in the liquid fractions of soil systems and the greater their availability for microbial degradation [Cerniglia, 1992 Leahy and Colwell, 1990]. It has, however been shown in numerous studies that sorbed organic contaminants are capable of being degraded. The study by Guerin and Boyd (1992) gave evidence that *Pseudomonas putida* #17484 (the strain used in this thesis), has a rate of naphthalene degradation which exceeds expected rates if only dissolved naphthalene were used suggesting the bacteria use sorbed naphthalene.

Many PAHs are used as chemical solvents and as intermediates in the manufacturing of dyes [Windholz, 1983]. One of the PAHs that has been extensively studied is naphthalene. Its methylated derivatives are among the most toxic compounds found in the water-soluble fraction of petroleum [Heitkamp *et al*, 1987; Cerniglia *et al*, 1992]. The compounds naphthalene, 2-methyl naphthalene, and 2-naphthol are the compounds under investigation in this study as detailed in section 1.3. Naphthalene and 2-methyl naphthalene are significant constituents of petroleum and 2-naphthol is commonly used as a component in fungicides and insecticides [Verschueren, 1983; Cerniglia, 1992]. A measure of the hydrophobicity of a solute is its octanol-water

partition constant (K_{ow}) [Valsaraj and Thibodeaux, 1989], which has been found to relate to its water solubility [Lyman *et al*, 1982].

Because of misguided disposal and accidental spillage by industry, these compounds are commonly found contaminants in both terrestrial and aquatic systems. Several "PAHs" and their analogues have been found to be both carcinogenic and mutagenic [Cerniglia, 1992].

A number of factors are responsible for the fates of PAHs in the environment some of which are summarized in Fig. 2.1. The processes include: volatilization which is affected by the solubility, molecular weight, and vapour pressure of the compound; photooxidation, chemical oxidation, bioaccumulation, adsorption to soil particles, and leaching. The process having the greatest impact on the disappearance of PAHs in soils is microbial degradation [Cerniglia, 1992; Weissenfels *et al*, 1992].

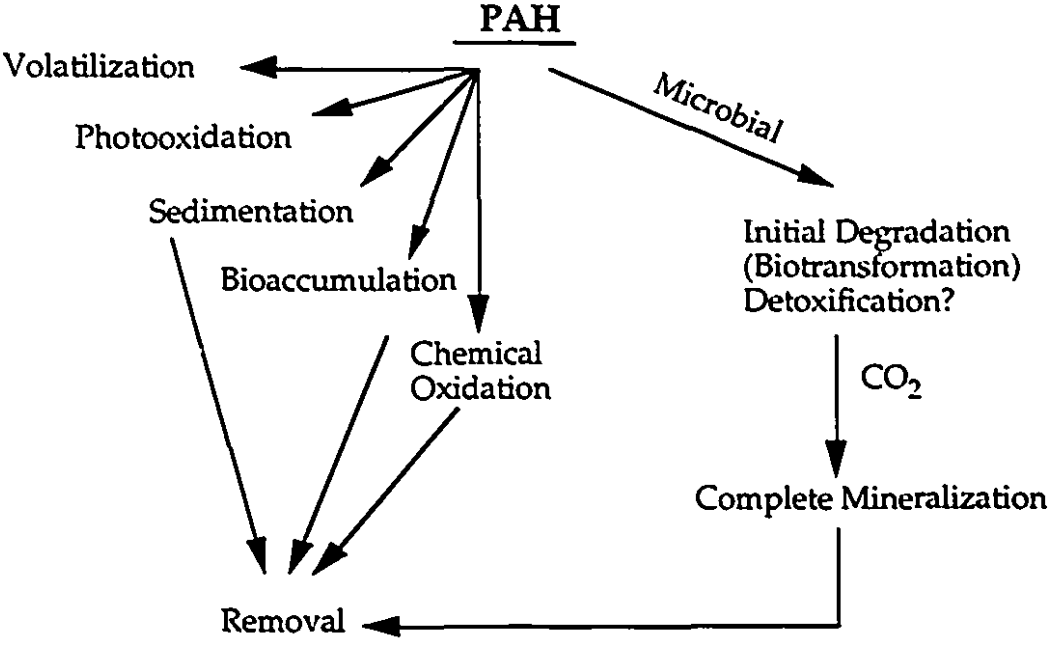


Figure 2.1. Schematic representation of the environmental fate of polycyclic aromatic hydrocarbons. Recreated from Cerniglia (1992).

2.7.1 Biodegradation of Polycyclic Aromatic Hydrocarbons (PAHs)

Hydrocarbons born of natural phenomena such as components of plant cells and bacterial cells, methane from swamps and sediments, and by-products of forest fires; have been in contact with living organisms throughout evolutionary periods [Hutzinger, 1985]. It is understandable how PAHs entering the environment from anthropogenic forces are capable of degradation due to their pyrolitic origins such as those of natural PAHS .

The study of bioremediation technologies is a relatively new field which is proving to be a cost-effective, safe and rapid approach for removal of synthetic and natural materials by indigenous microorganisms in *in-situ* soil remediation projects [Atlas, 1991; Betts, 1993]. Soil bacteria are important ecologically because they form a substantial part of the producing, consuming and transporting members of the soil ecosystem and therefore are involved in the flow of energy and in the cycling of chemical elements [Farago and Mehra, 1993]. Microbial activity is one of the primary mechanisms in the disappearance of PAHs in surface soils and sediments [Bossert and Bartha, 1984; Cerniglia, 1992]. PAHs can be totally mineralized or partially transformed by either a community of microorganisms [Al-Bashir *et al*, 1990, 1994; Knaebel *et al*, 1994; Dibble and Bartha, 1979; Cerniglia, 1992] or by a single species [Fredrickson *et al*, 1991]. A community can consist of bacteria and other species such as fungi and actinomycetes which have also been identified as PAH degraders [Babich and Stotzky, 1977].

The specific properties of polycyclic aromatic hydrocarbons affecting their degradability include: water solubility, volatility, molecular size, number and type and arrangement of functional groups [Koch, 1982; Berry *et al*, 1987].

The mineralization rates of PAHs are related to aqueous solubilities rather than to total substrate concentration [Leahy and Colwell, 1990]. The degradation rates depend on the mass transfer rates of the PAH from the solid phase to the water phase. Low water solubility, however does not necessarily mean slow biodegradation. In the

study by Thomas *et al* (1986), it was shown that a *Flavobacterium sp.* grew faster on the less soluble phenanthrene than on biphenyl. This may suggest that the soluble fraction is not the determining factor dictating bioavailability. This finding may also indicate a more effective dissimilation pathway for phenanthrene.

The growth of microorganisms on carbon substrates is generally seen as a process that combines the breakdown products of other organisms and so is a cooperative effect. For this reason it has been suggested that the mineralization of a single carbon substrate by an organism in the laboratory may be a laboratory artifact [Haigler *et al*, 1992]. The optimal contaminant concentration for biodegradation is species specific [Lyman *et al*, 1982].

The biodegradation of organic contaminants is strongly dependent on where the organic contaminant is located in the soil. In the study by Knaebel *et al* (1994), the mineralization of [^{14}C]radiolabelled surfactants adsorbed separately onto montmorillonite, kaolinite, illite, sand, and humic acids was measured and it was observed that the rates of biodegradation were significantly different between each matrix type.

Figure 2.2 gives the breakdown pathways for the three compounds investigated in this study. The biodegradation of naphthalene has been well documented in the literature [Dagley, 1971; Gibson, 1984]. The breakdown of 2-methyl naphthalene is based on the research of Cane and Williams (1982), and the breakdown products for 2-napthol are based on the degradation of phenol [Powlowski and Shingler, 1994]. The overall breakdown of 2-naphthol follows almost immediately the identical pathway as that for naphthalene. The breakdown studies for the compounds shown in Figure 2.2, require molecular oxygen for the cleavage of the aromatic ring which involves an initial hydroxylation reaction. The resulting dihydrodiols undergo further enzymatic reactions finally producing catechols which then undergo either *meta* or *ortho* cleavage depending on the organism in question.

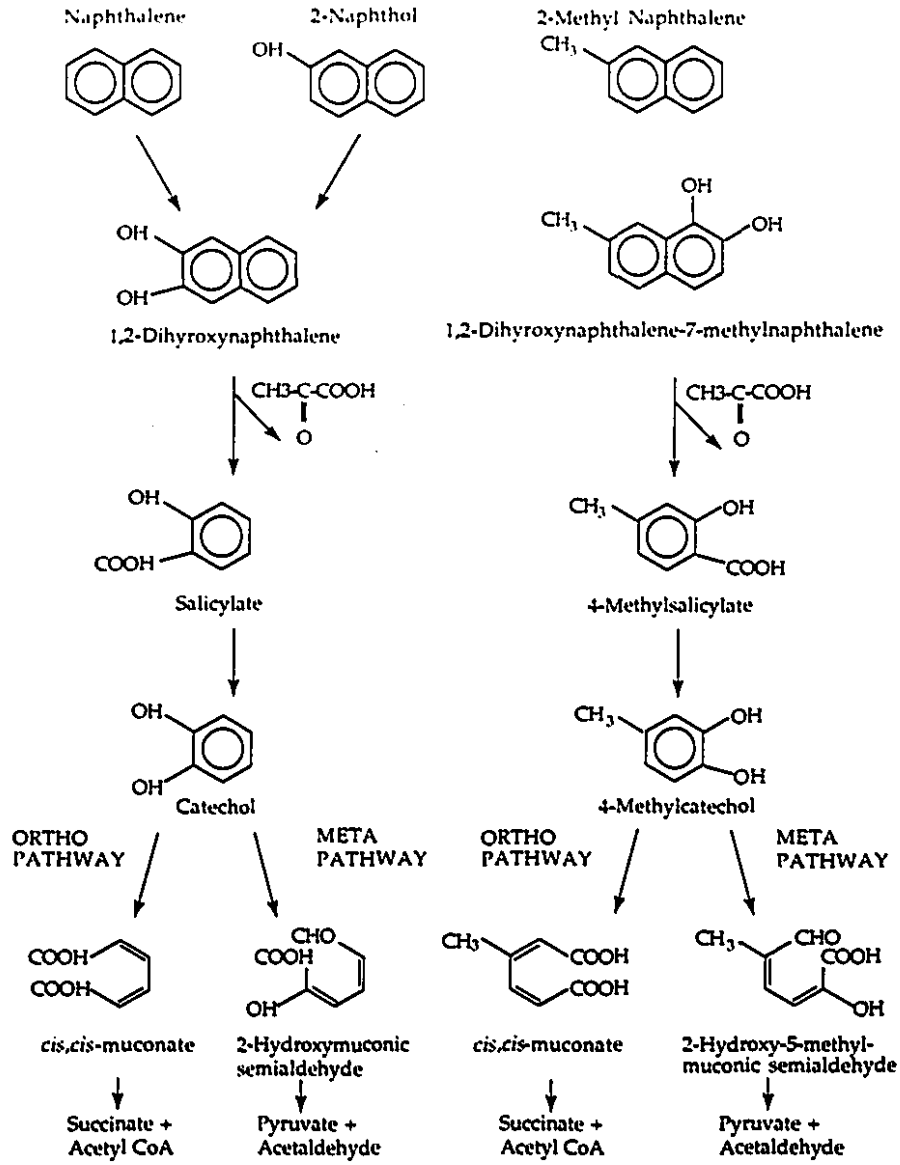


Figure 2.2. Degradation pathways for naphthalene, 2-methyl naphthalene, and 2-naphthol.

2.7.2 Mechanisms of Hydrocarbon Uptake

It is speculated that the membranes are the sites of initial hydrocarbon oxidation [Mihelcic *et al*, 1993]. With respect to *Pseudomonads*, these Gram-negative bacteria possess a semipermeable membrane that allows diffusion of small hydrophilic solutes and is almost impermeable to hydrophobic compounds [Trias and Nikaido, 1990]. The

hydrophobic compounds require more active uptake systems which include: a) solubilization, where the hydrocarbon is dissolved directly in the lipid bilayer membrane; emulsification, use of extracellular emulsifying agents [Foght *et al*, 1989; Falatko and Novak, 1992], and b) physical adhesion, where the presence of special structures or organelles aid in the transport and degradation of the carbon contaminant [Gannon, 1991].

2.7.3 Prior Exposure to Hydrocarbons

Prior exposure of a microbial community to a given hydrocarbon has been shown to greatly influence how rapidly the compound will be degraded [Atlas, 1991; Leahy and Colwell, 1990; Bauer and Capone, 1988; Fredrickson *et al*, 1991]. Cells, however grown in the presence of one contaminant does not guarantee its ability to degrade another contaminant of a similar form [Fredrickson *et al*, 1991]. In the study by Fredrickson (1991) it was shown that the isolate F199 grown in the presence of toluene could mineralize naphthalene, but that these cells grown on naphthalene did not mineralize toluene.

The delay observed in the adaptation towards a given compound can be a result of the presence of more easily degradable compounds [Bauer and Capone, 1988; Haigler *et al*, 1992].

Fortuitous metabolism is an event sometimes observed as a result of a lack of enzyme specificity brought about as a result of the tremendous metabolic energy needed to initiate the breakdown of aromatic compounds [Leisinger *et al*, 1981].

2.7.4 *Pseudomonas putida*

Naphthalene degradation by *Pseudomonad* species has been reported to be mainly plasmid encoded [Yen and Gunsalus, 1982; Yen and Serdar, 1988; Gibson and

Subramanian, 1984]; although *Pseudomonas stutzeri* degraders of naphthalene have a characteristic chromosomal location for catabolic pathway genes [Rossello-Mora *et al*, 1994].

Species of the genus *Pseudomonas* are metabolically versatile, and a large number of organic compounds, among them aromatic hydrocarbons, can be used as unique carbon and energy sources. This versatility allows them to be present in many environments as natural autochthonous microorganisms with a high potential for bioremediation of pollutants [Rossello-Mora *et al*, 1994].

The specific strain *of Pseudomonas putida* used in the present study, has received a great deal of attention in the literature and the following are some of the observations made:

- *P. putida* can degrade both sorbed and aqueous phase naphthalene as the initial naphthalene degradation rate exceeded that predicted by degradation of the aqueous phase localized naphthalene only [Mihelcic *et al*, 1993; Guerin and Boyd, 1992].
- *P. putida* is a motile, Gram-negative organism [Guerin and Boyd, 1991, 1992].
- The organism is chemotactic toward naphthalene and attaches reversibly to soils [Guerin and Boyd, 1992]
- *P. putida* establishes steep concentration gradients to promote desorptive diffusion and mineralization of nonlabile naphthalene partitioned onto organic matter [Guerin and Boyd, 1992].
- No surfactant production [Guerin and Boyd, 1991].
- *P. putida* culture age has no impact on mineralization kinetics of naphthalene [Guerin and Boyd, 1992].

2.8 Abiotic Factors Affecting PAH Biodegradation

The efficiency of the microbial degradation of PAHs and its analogues is dependent on a number of environmental factors including: temperature, pH, nutrients, oxygen and water availability, salinity, and soil type [Atlas, 1991; Connan, 1986; Betts, 1993].

The presence and availability of certain heavy metals in the environment has also been shown to affect an organisms ability to degrade or tolerate an organic contaminant. In the study by Inoue *et al* (1991), concentrations of Mg^{2+} > than 7.5mM or concentrations of Ca^{2+} > 3.0mM are found to be effective in enhancing toluene tolerance.

It is generally agreed that the organic carbon content of soil is the single most important factor determining the sorption of hydrophobic molecules such as PAHs [Weissenfels *et al*, 1992; Helmstetter and Aldenn, 1994].

The dissolution rate of organic contaminants has a significant effect on their degradability. The calculation of growth rate made by Stucki and Alexander (1987), suggest that the dissolution rate of phenanthrene may limit its rate of biodegradation. In the study by Thomas *et al* (1986), it was found that the rates of dissolution of 4-chlorobiphenyl and naphthalene were inversely related to particle size. Although the study by Wodzinski and Bertolini (1972), observed that growth rates on naphthalene were independent of the surface area of the solid substrate. These variations in experimental results between studies is very much system and/or species specific.

The sorption of contaminants onto solid surfaces is considered one of the strongest controlling variables affecting contaminant availability in soils [Weissenfels *et al*, 1992; Mihelcic *et al*, 1993; Xing *et al*, 1994; Lyman *et al*, 1982; Guerin and Boyd, 1992; Sittig, 1985]. The physical/chemical properties of the organic compound play a significant role in determining its adsorption/desorption behaviour in soils. In the study by Huang (1990), the presence of montmorillonite prevented enzymatic attack of

organics attached to the surface while the effect was less apparent in the presence of kaolinite which has a lower CEC.

Volatilization is a feature of the contaminant that also affects its availability and is affected by: soil properties, physicochemical properties and environmental conditions. The physicochemical properties include: vapour pressure, solubility in water, basic structural type and the number, nature and position of its functional groups [Yong *et al*, 1992]. In the study by Park *et al* (1990), under unsaturated conditions, volatilization accounted for 20 and 30% loss of 1-methyl naphthalene and naphthalene, respectively.

Chapter 3
Methodology

Fig. 3.1 provides the basic outline of the types of experiments performed and the general sequence in which they were followed to fulfill the objectives outlined in Chapter 1.

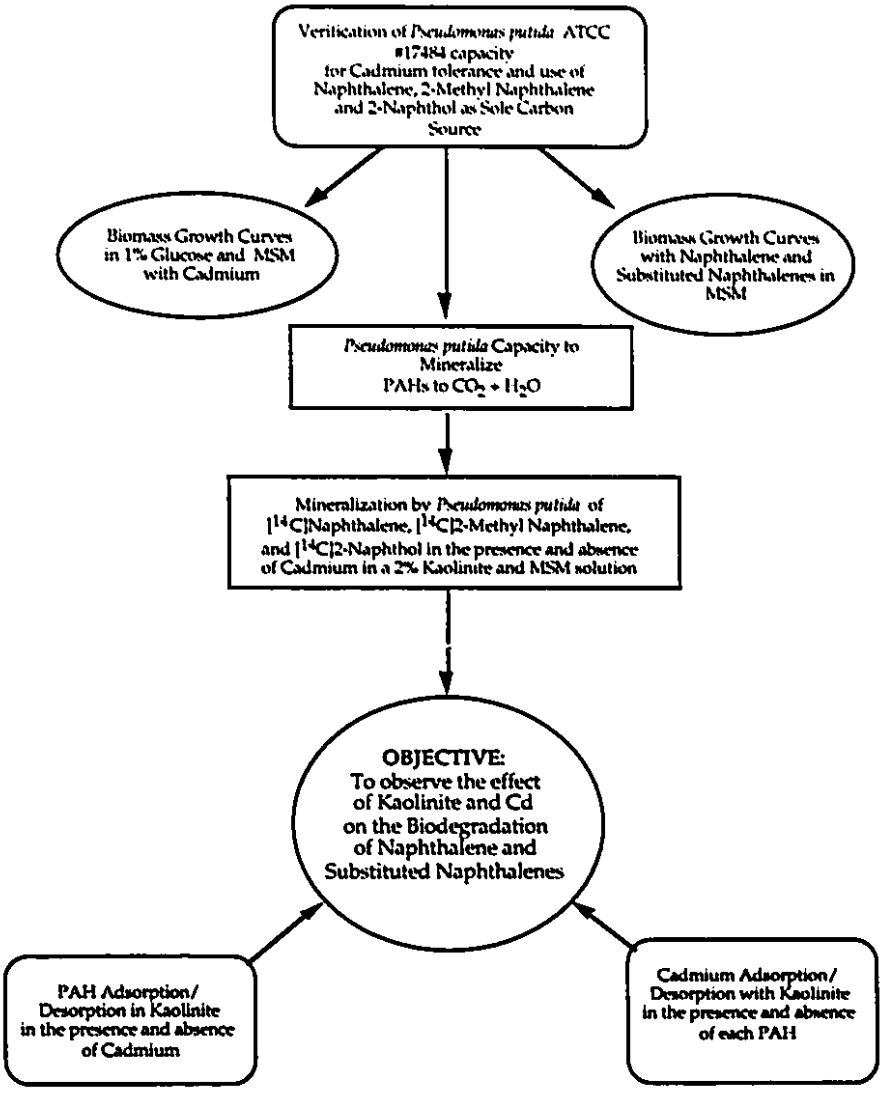


Figure 3.1. General overview of experiments performed to achieve the objectives outlined in section 1.3.

3.1 Clay Characterization

The clay mineral used in these experiments was kaolinite hydrite PX obtained from the Georgia Kaolin Company. To create a Na^+-saturated homoionic clay, the kaolinite was washed four times with a 1M NaCl solution at a pH<5 followed by six successive washings with deionized water to remove any excess Cl^- in solution. To ensure that the Cl^- anions had been removed, a few drops of $AgNO_3$ were added to an aliquot of supernatant from each successive washing to observe if any AgCl precipitate formed. The soil washing method used was done according to the method outlined by Yong and Ohtsubo (1986).

Table 3.1, lists the properties of kaolinite hydrite PX. The cation exchange capacity (CEC) had been determined at pH 7 using the silver-thiourea method outlined in Chhabra *et al*, (1975), where the decrease in Ag^+ concentration in solution was a measure of CEC. Surface area analysis was measured using the ethylene glycol monoethyl ether (EGME) method outlined by Jackson (1975), where the mass of the monomolecular layer of EGME on the clay surface, was used as a measure of surface area. The particle size analysis was done using the hydrometer method following the ASTM Test D421 and D422 and the results are shown in Figure 3.2. The mineral composition of the kaolinite was determined by X-Ray diffraction analysis using a Siemens D-500 X-Ray diffractometer the results of which are shown in Figure 3.3. The organic matter content analysis was done involving the wet combustion of organic matter with a mixture of potassium dichromate and sulfuric acid according to the method outlined by the US Department of Agriculture (1954).

The method of sterilization chosen for kaolinite used in the mineralization and adsorption-desorption experiments was ^{60}Co-irradiation since this technique unlike other sterilization methods has the least effect on changing the properties of the soil or clay [Wolf *et al*, 1989]. The irradiation strength was 2.5 megarads and was done at Nordion International, in Laval, Quebec. A sterile clay was used to prevent the death of

introduced bacteria from predators or parasites that may have been present in the nonsterile clay.

Table 3.1 Composition and Properties of Kaolinite Hydrite PX

Parameter	*Kaolinite*
Clay (% 2 μm)	86
Mineral Composition	Kaolinite (with no crystal impurities)
CEC (cmol Kg^{-1})	8
Surface Area ($m^2 Kg^{-1}$ X 10^{-3})	12
Organic Matter (% w/w)	0

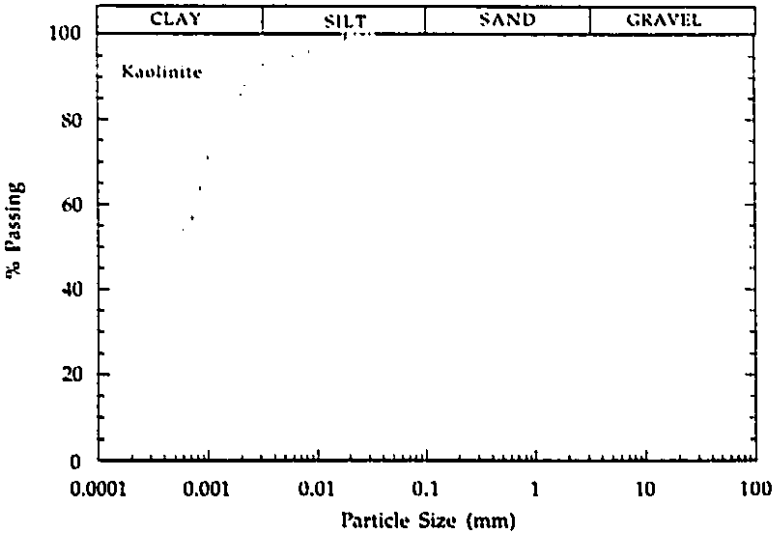


Figure 3.2 Particle size analysis for kaolinite hydrite PX

The combined isoelectric point of kaolinite used in this study (hydrite PX from the Georgia Company) was determined to be 4.2 [Yong *et al*, 1987], so as the pH increases above 4.2, the charge at the edges becomes increasingly negative due to the dissociation of H^+; and the edges are positively charged below a pH of 4.2 due to protonation with H^+ [Mitchell, 1976; Hughes and Poole, 1991].

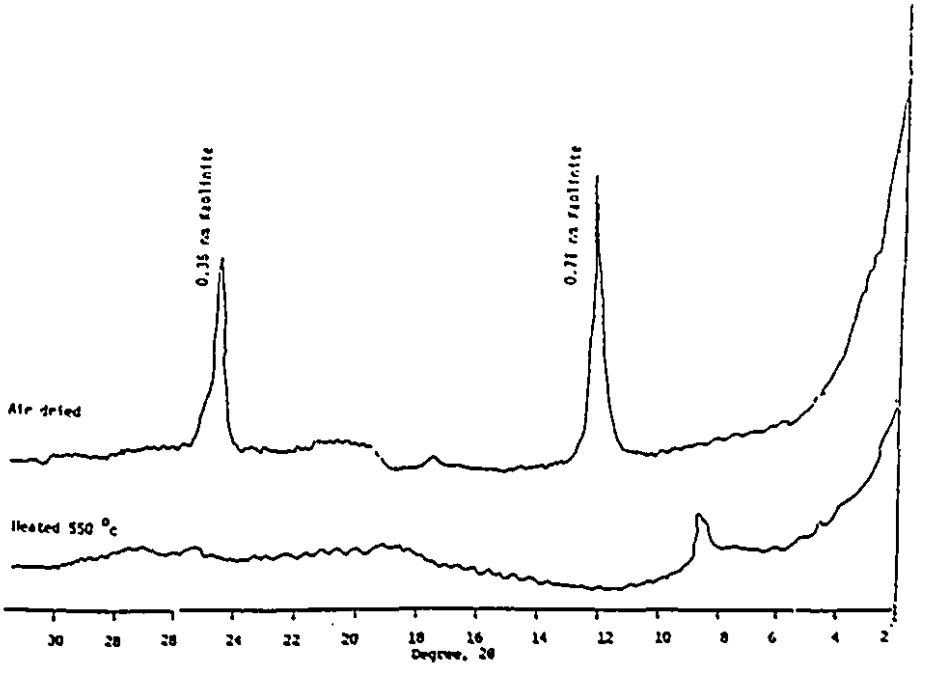


Figure 3.3 X-Ray diffractogram analysis for kaolinite hydrite PX

3.2 Bacterial Strain and Culture Conditions

A *Pseudomonas putida* strain was obtained from the American Type Culture Collection (ATCC #17484). This strain was chosen since much of its biochemistry and genetics have been studied [Barnsley, 1976; Nies *et al*, 1987; Schell, 1990; Connors and Barnsley, 1982]. *Pseudomonas putida* is a Gram-negative organism which is chemotactic towards naphthalene [Guerin and Boyd, 1992] and certain strains have shown resistance towards cadmium either through efflux mechanisms [Nies, 1992], sequestration [Trevors *et al*, 1986;], or extracellular complexation [Ghosh and Bupp, 1992; Higham, 1984].

The cells were stored at -80°C as a concentrated suspension in 20% glycerol. Before any experiment the strain was subcultured 2 times in nutrient broth before

inoculation to ensure culture viability. Harvesting of cells from the nutrient broth was done by centrifugation (Beckman Instruments, Palo Alto, California) at 6000 rpm for 10 minutes at 4°C, after which the supernatant was removed and replaced three time with fresh sterilized MSM (contents of MSM described in section 3.3). The biomass concentration determination of the resuspended cells was done by spectrophotometry at a wavelength of 600_{nm} (Perkin Elmer, Junior Model 35, Oak Brook, Illinois). All manipulations were performed using asceptic technique.

3.3 Mineral Salts Medium (MSM)

The following mineral salts medium was used for all experiments in this thesis. The provision of trace metals and other elements are essential for growth. The mineral salts medium provides a buffered system able to compensate for the hydrogen ion concentration released during the degradation of organic compounds.

The salt constituents were dissolved in deionized water and their final concentrations are listed in Table 3.2.

Table 3.2 Minimal Salts Medium Constituents and Their Concentrations

Salt	*Final Concentration (mmol L^{-1})*
$NaH_2PO_4 \cdot 4H_2O$	6.4
K_2HPO_4	12.99
$(NH_4)_2SO_4$	8.33
$MgSO_4 \cdot 7H_2O$	0.395
$Co(NO_3)_2 \cdot 6H_2O$	0.001
$AlK(SO_4)_2 \cdot 12H_2O$	0.001
$CuSO_4$	0.001
$ZnSO_4 \cdot 7H_2O$	0.01
$MnSO_4 \cdot H_2O$	0.01
$FeSO_4 \cdot 7H_2O$	0.01
$Na_2MoO_4 \cdot 2H_2O$	0.002
$Ca(NO_3)_2 \cdot 4H_2O$	0.01

The pH of the final solution was 7. The sterilization of MSM by autoclaving with phosphates present often leads to the formation of heat induced insoluble precipitates,

therefore the method used for sterilizing the mineral salts medium was via filter sterilization using 0.22µm cellulose acetate Corning filter systems.

3.4 Cadmium Nitrate Solution

The cadmium stock solutions were prepared according to the methodology outlined by Standard Methods (1985) [Greenberg *et al*, 1985], except 1 ml concentrated nitric acid was substituted for concentrated hydrochloric acid to avoid the introduction of Cl^- anions. Studies have shown that Cl^- anions interact strongly with cadmium [Babich and Stotzky, 1978; Yong and Sheremata, 1991]. Cadmium nitrate tetrahydrate ($Cd(NO_3)_2 \cdot 4H_2O$) was obtained from Aldrich Chemical Co. (Milwaukee, Wis., USA) with a 99.9% purity. The resulting cadmium stock solution was filter sterilized using a sterile Millex®-GS 0.22µm Filter Unit (Millipore Products Division, Bedford, MA., U.S.A.).

3.5 [^{14}C]Radiolabelled PAH Stock Solutions

Radiolabelled compounds were used in the experiments in this study since carbon-14 and the development of liquid scintillation counting techniques have proven to be a great asset in mechanistic studies [Butler, 1972a].

Naphthalene and 2-Naphthol were obtained from Aldrich Chemical Inc. (Milwaukee, Wisc., USA) and 2-Methyl Naphthalene was obtained from Fluka Chemika-BioChemika Inc. (distributed by Caledon Laboratories Ltd., St. Laurent, Quebec), as scintillation grade crystalline powders with 99% purity. The uniformly (all carbon) radiolabelled [^{14}C]naphthalene, [^{14}C]2-methyl naphthalene, and [^{14}C]2-naphthol were obtained from the Sigma Chemical Company (St. Louis, Mo., USA) with 98%+ purity and 4.7mCi mmol^{-1} specific activity. Solutions with specific activities of 70 000, 17000, 55 000 dpm mg^{-1} for naphthalene, 2-methyl naphthalene, and 2-naphthol

respectively were prepared by adding the radiolabeled carbon compound as a spike to cold stock solutions to obtain the final radioactivity indicated above. The final concentrations of the [^{14}C]radiolabeled stock solutions for naphthalene, 2-methyl naphthalene, and 2-naphthol were 50; 62.5; and 67.5 g L^{-1} methanol respectively.

3.6 Biomass Curves Determining PAH and Cd Tolerance

Biomass curves provided a fast qualitative method for observing the growth of *P. putida* using naphthalene, 2-methyl naphthalene, and 2-naphthol as the sole carbon source. The MSM constituents, pH, and temperature are all identical to the set-up of the mineralization experiments discussed in section 3.7.

All glassware used in the experiment was acid washed in a 10% nitric acid solution for 2 hours, followed by 5 times rinsing with deionized water. All glassware was autoclaved (Market Forge, Sterilmatic, USA) at 250^{0}F. 15ml glass centrifuge tubes were used with Teflon-lined black screw caps. Each sample was done in duplicate and filled with 5ml of MSM. The tubes were spiked with appropriate volumes from cold methanol stock solutions of each carbon contaminant possessing the same concentration as the [^{14}C]radiolabelled PAH stock solutions discussed in section 3.5, to obtain a final concentration for each of the three carbon compounds of 25 and 130ppm, respectively. The controls were identical as above but without the inoculant. The biomass was prepared according to the procedure described in section 3.2, and the total biomass added to the tubes resulted in an optical density (O.D) reading at a wavelength of 600nm, of approximately 0.2. The biomass change over time was measured on a spectrophotometer (Perkin-Elmer Junior Model 35, Oak Brook, Illinois).

To determine if the bacteria chosen could tolerate the presence of cadmium, a similar experiment as above was used except the sole carbon source was glucose. A 1% glucose with MSM stock solution was prepared and sterilized by autoclaving. The concentration range of cadmium was 0, 0.044; 2.12; and 4.23 mmol L^{-1} and the metal

was spiked from a cadmium nitrate stock solution described in section 3.4, to obtain the final concentrations given above. The controls were identical as the above, but without the inoculant.

3.7 Microcosm Mineralization Experiments

For a mineralization experiment to be successful the bacterium or consortium used must breakdown the target contaminants to CO_2 and H_2O. Mineralization experiments were designed to trap the released [^{14}C]CO_2.

Microcosm studies are examples of controlled environments that are good for providing presumptive information on hazards towards the function and potential integrity of an environment, but like all tests microcosm studies are most effective when performed in conjunction with other evaluative studies [Draggan and Giddings, 1978].

There are a number of analytical procedures used to evaluate the disappearance of target contaminants including: chromatography, spectrophotometry, and radio-labeling with carbon-14 which is the most accurate method [Lyman *et al*, 1982], and also the method chosen in this study.

The experimental set-up of the microcosms used in these experiments is shown in Fig. 3.4, which consisted of 20ml of a 2% kaolinite and MSM slurry added to 100ml autoclaved and acid washed microcosm vials. Each vial was equipped with a [^{14}C]CO_2-trap containing 1ml 0.5N KOH in a 5ml glass pyrex tube. The KOH trap was placed inside the 100 ml glass vial such that the clay slurry and the KOH trap shared the same headspace. The initial concentrations of naphthalene, 2-methyl naphthalene, and 2-naphthol each separately, were 25 and 130 ppm total slurry weight added as a methanol stock solution. The microcosms were concurrently spiked with cadmium to obtain final cadmium concentrations of 0, 5 and 238ppm (0, 0.044 and 2.12 mmol L^{-1}) for each of the two carbon concentrations. The microcosms were inoculated with a concentration of biomass totaling 5×10^8 to 10^9 cells ml^{-1} into each microcosm. The cells

were harvested according to the procedure outlined in section 3.2. Each vial was sealed by Teflon®-lined stoppers (Supelco Canada, Oakville, Ontario) and crimped with aluminum caps and placed in an incubator shaker (New Brunswick Scientific Co. Inc., Model G25, Edison, New Jersey) at 150rpm at 26°C. Teflon®-lined caps were used to seal the microcosm bottles as opposed to using polyurethane which is known to adsorb volatilized naphthalene. Each sample was in triplicate. The KOH solution with trapped $[^{14}C]CO_2$ was removed by piercing the Teflon®-coated rubber septum with a syringe. To compensate for the vacuum created, air was added through a 0.22µm membrane (Millex GS). The $[^{14}C]CO_2$ trap was rinsed with 1ml of 0.5M KOH, and refilled with 1ml of 0.5M KOH. The two fractions of KOH were combined and placed in a scintillation vial with 18ml scintillation cocktail (ACS, Amersham, Arlington Heights, Ill., USA) and tested for radioactivity using a Packard scintillation counter (Tri-carb model 4530; Packard, Downersgrove, Ill., USA).

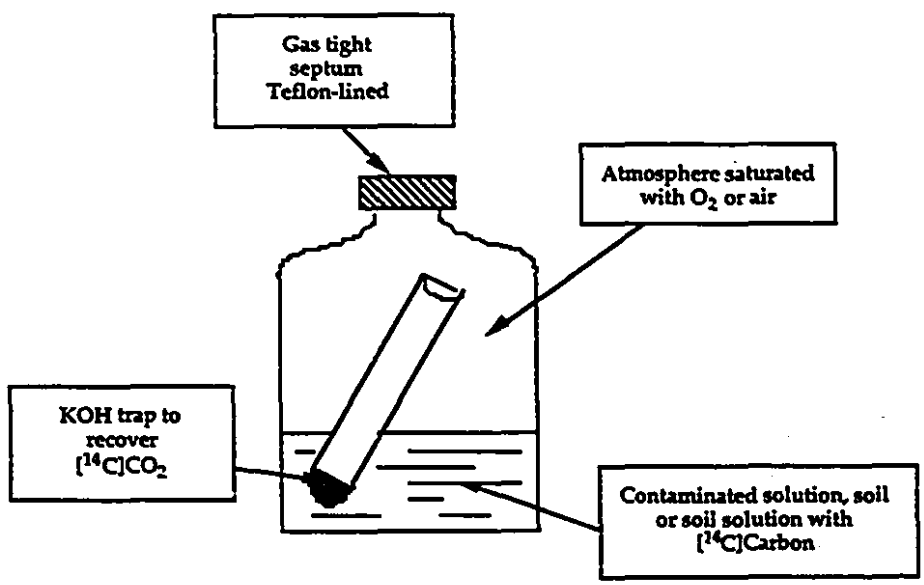


Figure 3.4. Diagram of Microcosm Set-up.

As a control for the kaolinite microcosms, identical microcosms were set-up as above but the 2% kaolinite solution was replaced with minimal salts solution. To ensure that the bacterial cells used in the experiments were viable, cells were allowed to grow in

minimal salts medium with yeast extract under the same conditions of pH, temperature, and shaking speed as the microcosms above.

3.8 PAH and Cd Equilibrium Determination

Before the adsorption-desorption experiments could be conducted, a preliminary experiment was performed to determine how long the samples would need to equilibrate with respect to the concentration in the liquid and kaolinite fractions, for both the PAH compounds and cadmium. The buffered medium (mineral salts medium discussed in section 3.3) maintained the soil suspension pH at 7. The soil solution ratio was constant throughout both the mineralization and adsorption-desorption experiments at 2% or 1:50 (w/v) ratio of kaolinite to mineral salts solution. Equilibrium determinations for both carbon [Lane and Loehr, 1992] and metals [Sposito, 1989], tends to be an instantaneous reaction and is generally governed by the pH of the system.

Equilibrium determination was done by making duplicate tubes of 10ml sterile 2% kaolinite suspensions in 15ml acid washed, sterilized glass centrifuge tubes with Teflon®-lined screw caps. The PAH concentrations were adjusted to 25ppm and 130ppm for [^{14}C]naphthalene, [^{14}C]2-methyl naphthalene, and [^{14}C]2-naphthol each separately. For cadmium equilibrium determination the tubes were set up identically as above except the tubes were only adjusted to obtain final cadmium concentrations of 0.044 and 2.12mM. All tubes were placed on a Wrist-Action® Shaker and at time intervals of 0, 24, 48, and 72hr, the designated tubes were sacrificed. For measurement of the radiolabelled carbon concentrations, 1 ml was removed and 18ml scintillation cocktail was added and the radioactivity was counted on a scintillation counter (Tri-Carb model 4530; Packard, Downersgrove, Ill., USA). For the cadmium concentration determination, the supernatant was removed, acidified with concentrated nitric acid and measured by atomic adsorption (Varian Atomic Adsorption Spectrophotometer, AA-975 Series, Ontario, Canada). The equilibrium figures are shown in Chapter 4.

3.9 Adsorption-Desorption Experiments

With a comprehensive understanding of the surface features of a soil constituent such as kaolinite, and of the chemistry and behaviour of the contaminant under investigation; adsorption-desorption experiments can be an effective tool in interpreting the possible mechanistic interactions between contaminants and soil constituents. Results obtained from laboratory simulations are of practical value in attempting to model field situations and help predict the transport of contaminants in soil [Greenland, 1981; John, 1971]. Variations in experimental parameters such as temperature, pH, soil:solution ratio, soil constituents and the manner in which they have been prepared all can have a significant effect on the results obtained [Bruemmer *et al*, 1988; Stotzky and Burns, 1980].

For analysis, 15 ml acid washed and sterilized Kimax® glass centrifuge tubes with Teflon®-lined screw caps were used and filled with 10ml 2% kaolinite and MSM slurry. To observe the effect of cadmium on the PAHs, the adsorption-desorption experiments were done using the [^{14}C]radiolabeled PAH stock solutions. To observe the effects the PAHs may have on the cadmium behaviour, cold PAH stock solutions were used.

The PAH concentrations were adjusted to 5, 25, 50, 130, and 200ppm. Each carbon concentration was observed in the presence of an initial cadmium concentration of 0, 0.044, and 2.12mmol L^{-1} in kaolinite. Only the samples possessing an initial PAH concentration of 25 and 130ppm were used for further desorption analysis. All tubes were placed on a Wrist-Action® Shaker, Model 75 (Burrell Co., Pittsburg, Pa., USA). After 24hr shaking, the vials were centrifuged for 30min at 3000g and 4°C (Centra 4, International Equipment Co., Needham Heights, Mass., USA). To determine the [^{14}C]PAH concentration, 1ml of the supernatant was added to 18ml scintillation cocktail (ACS, Amersham, Arlington Heights, Ill., USA) and tested in a scintillation counter

(Packard Tri-Carb model 4530; Packard, Downersgrove, Ill., USA). The amount of PAH content in the kaolinite fraction was calculated by difference.

The cadmium concentrations for the cadmium adsorption experiment were adjusted to 0, 0.044, 0.936, 2.12, and 3.51mmol L^{-1}. The adsorption of cadmium was observed in the presence of each PAH separately at initial concentrations of 25 and 130ppm. Only the samples possessing an initial cadmium concentrations of 0.044 and 2.12mmol L^{-1} were used for further desorption analysis. To measure the cadmium concentration in the supernatant in the cold samples, the entire supernatant was removed, acidified with concentrated nitric acid and analyzed on an atomic adsorption spectrophotometer (Varian Atomic Adsorption Spectrophotometer, AA-975 Series, Ontario, Canada). The amount of cadmium adsorbed onto kaolinite was calculated as the amount of cadmium in samples with MSM only minus the amount of cadmium present in the supernatant of the 2% kaolinite and MSM samples.

The desorption experiment followed the procedure outlined by DiToro and Horzempa (1982) for consecutive desorption protocol, where the initial adsorption point is extended by performing subsequent multiple desorptions. All radioactive and non-radioactive samples followed the same procedure. After each consecutive 24hr shaking procedure (three in total were performed), the supernatant was removed by decanting and replaced with fresh MSM. The procedure of introducing fresh amounts of MSM was such that each time a new equilibrium state was determined. The initial and final concentrations of [^{14}C]carbon in the solution phase was determined by liquid scintillation counting. The cadmium content of the cold samples was done following the same procedure outlined above.

Chapter 4
Results and Discussions

4.1 Introduction

Remediation of contaminated sites using biotechnologies such as bioaugmentation or biostimulation requires studies to evaluate the characteristics of the site and whether the target contaminants are amenable to mineralization. Soil contamination presents a challenge since both the soil and contaminants contain an array of constituents, where some or all mutally or each separately, can affect the ability of microorganisms to degrade organic contaminants.

As mentioned in Chapter 1, this thesis examined the effect of kaolinite and cadmium on the mineralization of naphthalene and substituted naphthalenes. In conducting this study, a series of experiments were designed to interpret the potential behaviour within the contaminated system. This study gave special emphasis to:

- evaluating the functional group effect of the substituted naphthalenes on mineralization and interactions with kaolinite,
- soil impact on mineralization of the PAHs by *P. putida*, and
- the effect cadmium may exert on the mineralization process.

To facilitate data interpretation, kaolinite, a pure clay mineral was chosen as the soil matrix. Interactions of heavy metals and polycyclic aromatic hydrocarbons with kaolinite can reflect the behaviour of contaminants in *in-situ* environmental systems [Garcia-Miragaya and Davalos, 1986]. Both adsorption-desorption and mineralization

experiments were performed in suspension to maintain consistency between experiments.

Where appropriate, results are presented in graphical format and the significance of these results is reviewed.

4.2 Preliminary Experiments

Subsequent to designing the adsorption-desorption and mineralization experiments, preliminary experiments were performed to determine the values of certain parameters. Some of these parameters included: time required for cadmium and PAH contaminants to achieve equilibrium in the kaolinite system; to determine if *P. putida* could use naphthalene, 2-methyl naphthalene, and 2-naphthol as the sole carbon source; and the ability of *P. putida* to tolerate the presence of cadmium.

4.2.1 Equilibrium Determination Experiments

The results from this experiment gave the time required to reach equilibrium in the soil suspensions for both PAHs and cadmium with respect to the concentration in the aqueous and kaolinite phases. Equilibrium was determined under the same conditions as the adsorption-desorption and mineralization experiments outlined in the **Methodology** section in Chapter 3. The concentrations for the carbon sources were 25 and 130ppm and the cadmium concentrations were 5 and 238ppm final concentration (0.044mmol L^{-1} and 2.12mmol L^{-1}, respectively). The clay:solution ratio was 1:50(w/v) i.e. 1g in 50ml mineral salts solution making a 2% kaolinite slurry.

The [^{14}C]PAH concentration for each contaminant left in the aqueous fraction, was measured after 24, 48 and 72 hours. The results of this experiment are presented in Figure 4.1. From this figure it can be seen that the concentration of [^{14}C]PAH in the aqueous fraction remained constant after 24 hours. Hydrophobic compounds such as

PAHs tend to sorb primarliy via hydrophobic interactions. The study by Hassett *et al* (1981), looked at the sorption of α-naphthol. It was concluded that the degree of sorption depended most on the percent organic carbon content and the water solubility of the compound. Based on this study and the results seen in Figure 4.1, it is reasonable to assume that the high solubility of naphthol dictated its negative interaction with the kaolinite surface, which resulted in its total concentration in the aqueous phase. Naphthalene and 2-methyl naphthalene, unlike 2-naphthol, have very low solubilities and likely experienced hydrophobic interactions with the kaolinite surface. As can be seen from Figure 4.1, both the high and low concentrations of these two PAHs partition with the same concentration in the aqueous phase, again illustrating the importance of solubility on PAH partitioning in soil. The data used to construct Figure 4.1 is found in Table C.2 in Appendix C.

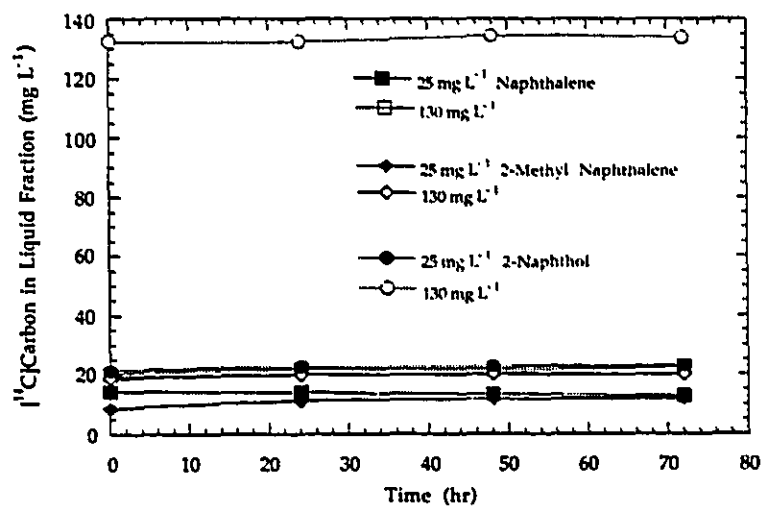


Figure 4.1. PAH equilibrium determination (with respect to concentration in the aqueous and kaolinte phase) for naphthalene, 2-methyl naphthalene and 2-naphthol at concentrations of 25 and 130ppm in a 2% kaolinite slurry.

In the cadmium equilibrium experiment, the cadmium concentration in the aqueous fraction was measured after 24 and 48 hours. The results are presented in Figure 4.2. From this figure it can be seen that the cadmium concentration in solution, remained constant from 0 hours onward. Heavy metals have been shown to equilibrate with clay surfaces within seconds [Sposito, 1989]. The data used to create Figure 4.2 is given in Table C.1 in Appendix C.

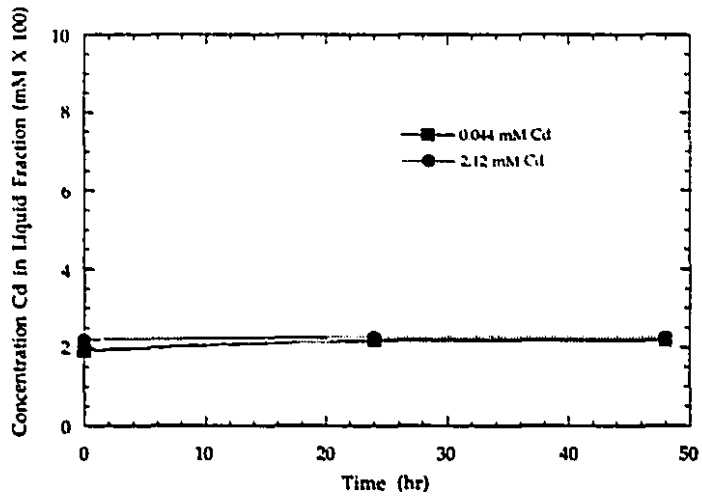


Figure 4.2. Cadmium equilibrium determination (with respect to concentration in the aqueous and kaolinite phase) at concentrations of 0.044mmol L^{-1} and 2.12mmol L^{-1} in a 2% kaolinite slurry.

4.2.2 Growth of *Pseudomonas putida*

The results in this section show the ability of *P. putida* to degrade the three PAH compounds investigated. The experiment performed measured an increase in biomass concentration while growing on each of the three PAHs in solution from two initial PAH concentrations of 25 and 130 ppm. The results are plotted in Figures 4.3 and 4.4 seperately for the two concentrations investigated. From both Figures 4.3 and 4.4 it can be seen that *P. putida* could grow using naphthalene, 2-methyl naphthalene, and 2-

naphthol as the sole carbon source. Studies by Guerin and Boyd (1991) and Cane and Williams (1982), have shown the ability of *P. putida* to degrade naphthalene and 2-methyl naphthalene respectivley, as the sole carbon source.

The concentration of 130ppm for naphthalene and 2-methyl naphthalene, was sufficiently above their respective solubilities of 31 and 26 mg L^{-1}, which resulted in their precipitation. The presence of solid contaminants clearly did not restrict rapid biomass increase seen in Figure 4.4. The precipitation of naphthalene and 2-methyl naphthalene in solution made optical density analysis difficult. To compensate for this contingency, the controls for these samples consisted of the same experimental design as the active samples but without biomass. All samples including the controls were allowed to equilibrate for approximately 4 hours before the designated biotic samples were inoculated. The concentration of 130ppm for 2-naphthol appeared to prevent growth and was assumed to be toxic to this organism at elevated concentrations.

In conjunction with the determination of PAH degradation capacity, a parallel experiment was performed to determine *P. putida*'s tolerance to cadmium. In this experiment the sole carbon source available was glucose (1% w/v). The initial cadmium concentrations examined were 0, 0.044, 2.12 and 4.23 mmol L^{-1}, added to 1% sterilized, glucose solution in MSM. The results from this experiment are plotted in Figure 4.5. This figure illustrates *P. putida*'s ability to tolerate the presence of cadmium at the concentrations used in the mineralization experiments.

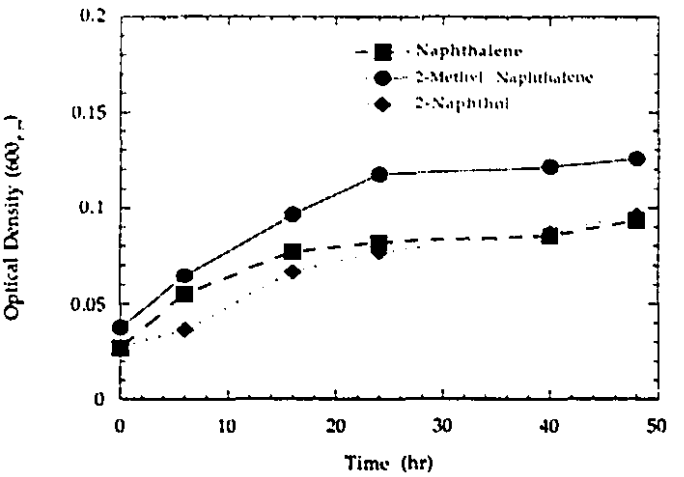


Figure 4.3. Growth of *Pseudomonas putida* in mineral salts medium amended with 25ppm naphthalene, 2-methyl naphthalene, and 2-naphthol separately. Measurements made at 600_{nm}.

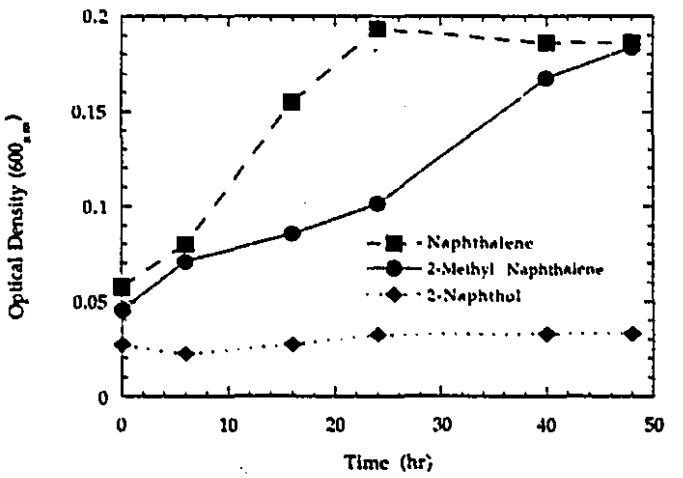


Figure 4.4. Growth of *Pseudomonas putida* in mineral salts medium amended with 130ppm naphthalene, 2-methyl naphthalene, and 2-naphthol separately. Measurements made at 600_{nm}.

Each of the controls used for each cadmium concentration examined, were amended with the same cadmium concentration but no biomass was added. It should be noted that the results obtained are only qualitative since the buffered medium used contains a significant concentration of phosphate which precipitates readily with cadmium [Hughes and Poole, 1991]. Precipitates have an impact on the optical readings and so the variation in the optical density readings observed in Figure 4.5 may not be due to the impact of the cadmium on microbial activity.

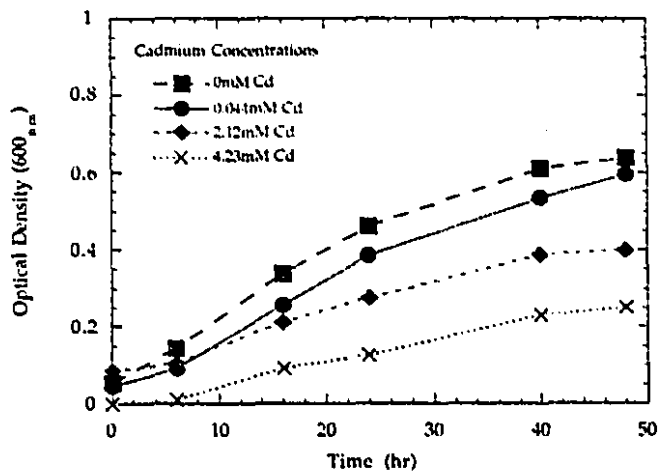


Figure 4.5. Effect of various concentrations of cadmium added to a 1% glucose and mineral salts medium on the growth of *Pseudomonas putida*.

Details concerning the possible interactions of cadmium and its speciation in this system and the effects on microbial activity will be discussed in detail in sections 4.1 to 4.5. The data used to plot Figures 4.3, 4.4, and 4.5, is tabulated in Tables D.1, D.2 and D.3 in Appendix D.

4.3 Cd Adsorption-Desorption Experiments

The MSM contains a high concentration of phosphate anions, therefore the effect of phosphate complexation is examined by comparing actual results of cadmium adsorption to the predicted amount of cadmium adsorption based on the theoretical speciation of cadmium outlined in Appendix E.

For the experiment of cadmium adsorption onto kaolinite, initial cadmium concentrations of 0.044, 0.936, 2.12, and 3.51 mmol L^{-1} were added to 2% solutions of irradiated kaolinite in MSM (discussed in section 3.5). The results of this adsorption experiment are plotted in Figure 4.6 as ***Adsorption from MSM***. The data are plotted in terms of the initial concentration of cadmium in mmol L^{-1} versus the amount of cadmium adsorbed onto kaolinite in mmol Kg^{-1}. From Figure 4.6, it can be seen that the total amount of cadmium actually adsorbed increased with higher initial cadmium loading and experienced linear adsorption. The complete data used to construct Figure 4.6 is tabulated in Table B.28 in Appendix B.

In addition to this experiment, a parallel experiment was performed involving the adsorption of cadmium onto kaolinite in the presence of naphthalene, 2-methyl naphthalene, and 2-naphthol at 25 and 130ppm. The results of this adsorption experiment are not included in this section since no effect on cadmium behaviour was observed. The data from this experiment are tabulated in Table B.1 to B42 in Appendix B. The lack in variation in the cadmium adsorption with PAHs indicates that these PAHs did not compete for the same adsorption sites on the kaolinite surface, as would be expected in the case of hydrophobic aromatic compounds. Nonionic hydrophobic PAHs would not be expected to compete for adsorption sites on the kaolinite, since the means of interaction are dissimilar. Naphthalene and 2-methyl naphthalene are nonionic and interact with the kaolinite surface via hydrophobic interactions. Under neutral conditons 2-naphthol is deionized and does not interact with the kaolinite surface and so does not interfere with cadmium adsorption.

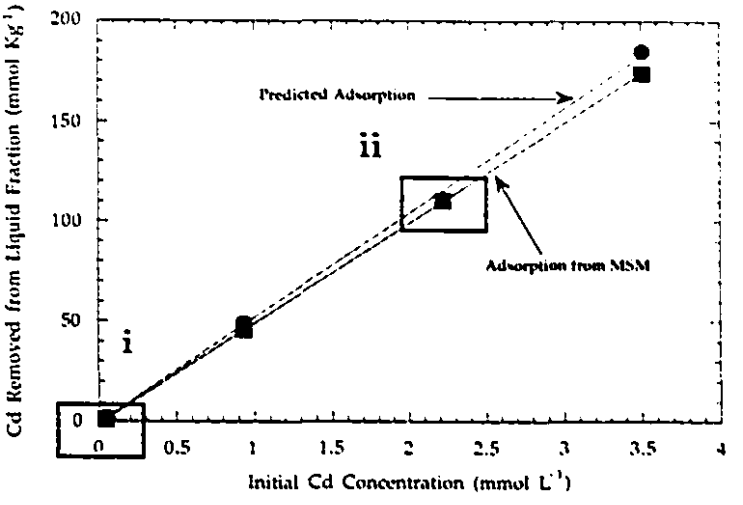


Figure 4.6 . Cadmium adsorption onto kaolinite. (at pH 7). The boxed-in areas are the points from which desorption analysis was continued.

The presence of multiple constituents in the MSM and the presence of kaolinite can effect the speciation of cadmium. In order to assess the potential degree of cadmium complexation, speciation was determined by chemical equilibrium calculations using documented equilibrium constants (outlined in Appendix E). Table 4.1 lists various species of cadmium that could form given the constituents found in the buffered minimal salts medium used in all experiments in this study. The results of these calculations did compare favourably with the results obtained from experimentally determined cadmium adsorption. The results from these calculations are plotted in Figure 4.6 as ***Predicted adsorption.*** The ***Predicted adsorption*** is based on the amount of cadmium calculated to adsorb onto kaolinite according to the outline discussed in Appendix E. The amount of cadmium adsorbed onto the negatively charged kaolinite is taken as the sum of the positively charged cadmium complexes such as Cd^{2+}, $CdOH^{+}$, and $CdNO_3^{+}$ and the total concentration of cadmium precipitated as $Cd(OH)_2$ and $CdHPO_4^{0}$. The predicted

adsorption of cadmium was higher than the experimental amount of cadmium adsorbed. This would be expected since these chemical equilibrium calculations are based on solution chemistry and accordingly, any phosphate is predicted to complex with all cadmium present. Cadmium hydrogen phosphate is highly insoluble having a solubility product (Ksp) of 2.53×10^{-33} [Lide, 1991]. It should be noted that the pH of all samples remained around neutral with the exception of those samples amended with 2.12 mmol L^{-1} cadmium, where the pH dropped by about 0.2 units.

Table 4.1. Possible Cadmium Speciation in MSM

Cadmium Complex	*Primary Factors Affecting Formation*	*Formation constants**
Cd^{2+}	pH <7-8	-
$CdOH^{+}$	pH >7.5	7.95×10^{-11}
$Cd(OH)_2$	pH ≥9	5.01×10^{-21}
$CdSO_4$	very soluble	-
$Cd(SO)_4 \cdot H_2O$	very soluble	-
$2CdSO_4 \cdot Cd(OH)_2$	very soluble	-
$CdHPO_4$	$[H_2PO_4^-] > 10^{-5}$	1.00×10^{-4}
$CdNO_3^+$	$[NO_3^{2-}] > 10^{-2}$	2.04

Sources: [*Lindsay, 1979; Fergusson, 1990; Babich and Stotzky, 1977,1978; Babich *et al*, 1981]

From the results in Figure 4.6, and the aqueous concentration of cadmium listed in Table B.28 in Appendix B, it is reasonable to assume that cadmium did experience competition with the trace metals present in the MSM for adsorption sites on the kaolinite surface, since for each initial cadmium loading, the same concentration remained in the aqueous fraction. In the study by Holm *et al* (1995), the distribution of Cd^{2+} and Zn^{2+} onto Amberlite was significantly reduced in the presence of divalent cations such as Ca^{2+} and Mg^{2+}. Furthermore, Figure 4.6 shows that the total amount of cadmium removed from solution increases with higher initial cadmium concentrations. Similar results have been observed in studies by Yong and Sheremata (1991) and Haas and Horowitz (1985), looking at Cd^{2+} adsorption onto a Na^{+}-saturated kaolinite. The high removal of cadmium at higher initial loadings may be accounted for by the potential precipitation of phosphate onto the kaolinite surface. In the study by Bolland *et al*

(1977), it was speculated that the high removal of zinc from solution was a result of Zn^{2+} complexation with the phosphate anions adsorbed onto the geothite surface.

To estimate how cadmium was adsorbed onto the kaolinite surface, desorption experiments were performed according to the consecutive desorption protocol outlined by DiToro and Horzempa (1982), where multiple desorptions extend an initial adsorption point. This protocol removes the metal that is loosely bound to the clay surface. Desorption analysis was performed on samples containing initial cadmium concentrations of 0.044 and 2.12 mmol L^{-1} indicated by the boxed in points in Figure 4.6. The desorption data is given in Table 4.2. The percent desorption was considerably lower at the higher rate of cadmium adsorption and suggests that some cadmium was more strongly held by the kaolinite surface. The small proportion of Cd desorbed from the surface of kaolinite suggests that a large proportion of the cadmium was irreversibly bound, indicating that hysteresis behaviour was observed.

Table 4.2 Experimental consecutive desorption results

Cd Supplied as [Solution] (mmol L^{-1})	*Cd Supplied as [Soil] (mmol Kg^{-1})*	*Actual Cd Adsorbed (mmol Kg^{-1})*	*Consecutive Desorption Analysis (% of Cd Adsorbed)*		
			1st sample	**2nd sample**	**3rd sample**
0.043	2.2	1.11	43	40	32
0.936	46.8	45.7			
2.22	111	110	1	<1	<1
3.51	175.5	174			

Similar desorption behaviour was seen in the study by Miller and Alexander (1991) using Na^{+}-saturated montmorillonite and a dilution desorption technique. The percent desorption of Cd was found to be much lower at a high rate of Cd adsorption. Similarly, in the study by Garcia-Miragaya and Davalos (1986) it was found that a Zn^{2+} concentration adsorbed up to the CEC of the Ca^{2+}-saturated kaolinite, was easily removed. A Zn^{2+} concentration adsorbed above the CEC, could only be desorbed using

0.5M KNO_3 indicating that the zinc was adsorbed by mechanisms stronger than ion exchange.

In addition to this desorption experiment, a parallel experiment involving the desorption of cadmium in the presence of naphthalene, 2-methyl naphthalene, and 2-naphthol each seperately at 25 and 130ppm, was performed. As with the adsorption of cadmium in the presence of these PAHs, no significant variation in desorption analysis was observed. The results from this analysis are given in Tables B.31 to B.42 in Appendix B. Based on the desorption results for cadmium in the presence of 2-naphthol at either initial concentration, no enhanced concentration was observed in the liquid fraction suggesting that cadmium did not interact significantly with the deprotonated hydroxide functional group of 2-naphthol under these conditions.

4.4 PAH Adsorption-Desorption Experiments

For the experiment involving the adsorption of each PAH separately onto kaolinite, initial concentrations of 5, 25, 50, 130, and 200 ppm (mg L^{-1}) were added to 2% kaolinite and MSM slurries. To observe the effect cadmium may have exerted on PAH adsorption, parallel experiments were conducted examining the adsorption of each PAH at initial cadmium concentrations of 0.044 and 2.12 mmol L^{-1}, separately.

Naphthalene

The results of the adsorption experiment of naphthalene are plotted in Figure 4.7. The data are plotted in terms of naphthalene aqueous concentration in mg L^{-1}, versus naphthalene adsorbed in mg Kg^{-1}. The curves seen in Figure 4.7 show a sharp rise in naphthalene concentration adsorbed added at concentrations higher than its solubility limit. The naphthalene present at concentrations above its solubility limit are likely present both sorbed to the kaolinite surface and in a precipitated form. Other studies

examining adsorption of hydrophobic PAHs have shown that the higher the initial concentration added, the greater the concentration of contaminant adsorbed onto the clay surface [Yong and Rao, 1991].

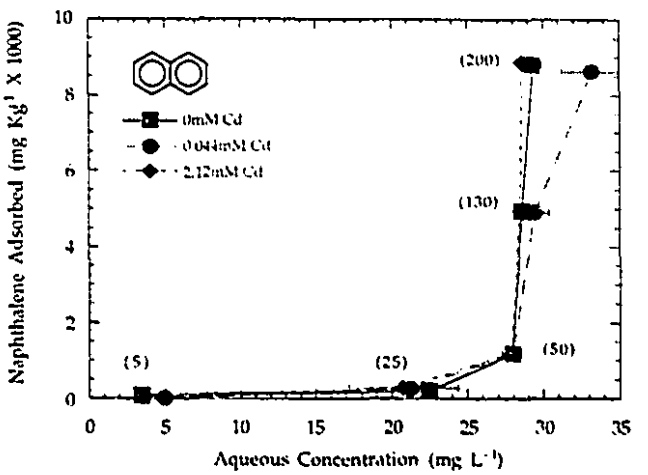


Figure 4.7. Naphthalene adsorption curves showing partitioning between the kaolinite and aqueous fraction. The values in parentheses indicate the concentration of naphthalene injected. Adsorption of naphthalene observed in the presence of 0, 0.044, and 2.12 mmol L^{-1} Cd.

The naphthalene adsorption curves in the presence of cadmium, seen in Figure 4.7, showed no significant variation from adsorption in the absence of cadmium. The presence of cadmium was not expected to have an impact on the adsorption-desorption of naphthalene since the interactions of naphthalene with kaolinite are governed by the surface area features of the solid matrix and hence on van der Waals and primarily hydrophobic interactions [Thomas *et al*, 1986; Hassett *et al*, 1981; Giles *et al*, 1974]. The curves are based on known initial concentrations indicated by numbers in parentheses.

To observe how strongly naphthalene was adsorbed onto the kaolinite surface, desorption experiments were perfomed according to the consecutive desorption protocol outlined by DiToro and Horzempa (1982). The adsorption points from initial

naphthalene concentrations of 25 and 130ppm were further analyzed for desorption behaviour. The results from this experiment are plotted in Figure 4.8.

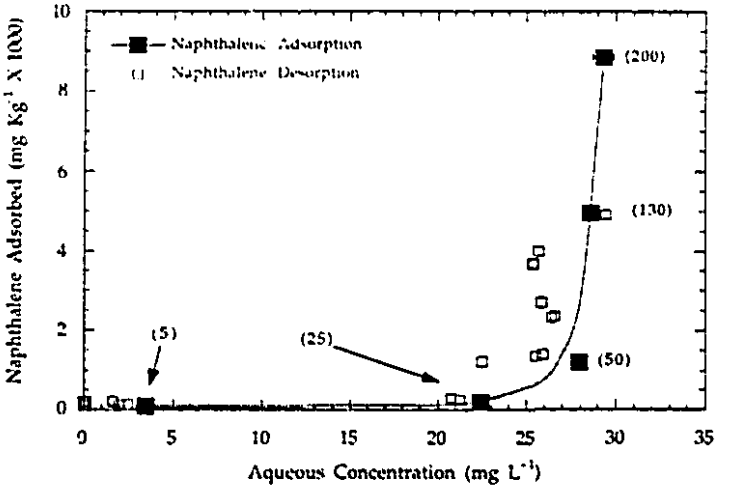


Figure 4.8. Naphthalene adsorption-desorption curves showing the adsorption isotherm and experimantal consecutive desorption points. Data are averages of two replicates. The initial naphthalene concentrations injected are indicated in parentheses as ppm.

The data are plotted in terms of aqueous concentration in mg L^{-1} versus amount of naphthalene adsorbed in mg Kg^{-1}. For clarity, only the naphthalene adsorption curve in the absence of cadmium, was plotted in Figure 4.8. Each desorption data point is representative of the new equilibrium obtained after 24hr of mixing with fresh mineral salts medium and is the average of duplicate samples. The 24hr equilibrium time was determined based on the preliminary experimental results discussed in section 4.1.1. The desorption points do not vary significantly from the adsorption curve indicating a minimal hysteresis effect which suggests naphthalene is reversibly bound to kaolinite. Therefore, naphthalene was not strongly adsorbed onto the kaolinite surface.

Tables B.1 to B.3, and B.10 to B.15 in Appendix B, contain the data used to create Figures 4.7 and 4.8.

2-Methyl Naphthalene

The results of the adsorption experiment with 2-methyl naphthalene are plotted in Figure 4.9. The data are plotted in terms of aqueous 2-methyl naphthalene in mg L^{-1},versus amount adsorbed in mg Kg^{-1}.

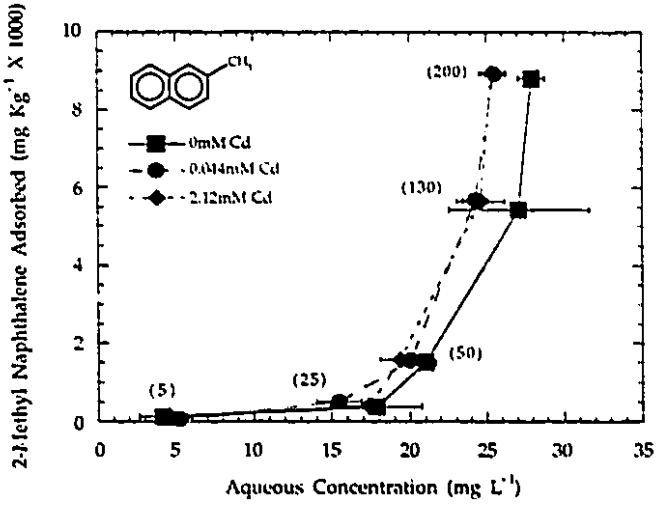


Figure 4.9. 2-Methyl Naphthalene adsorption curves showing partitioning between the kaolinite and aqueous fraction. The values in parentheses indicate the concentration of 2-methyl naphthalene injected. Adsorption of 2-methyl naphthalene observed in the presence of 0, 0.044, and 2.12 mmol L^{-1} Cd.

The curves seen in Figure 4.9 resemble those seen in Figure 4.7 for naphthalene adsorption The curves in Figure 4.9, also illustrate that the greater the initial 2-methyl naphthalene concentration added, the higher the concentration adsorbed onto kaolinite.

Unlike naphthalene, 2-methyl naphthalene appears to show a greater affinity towards kaolinite which is likely a result of the greater hydrophobicity of 2-methyl naphthalene attributed to the presence of the $-CH_3$ functional group. The uptake of naphthalene and 2-methyl naphthalene was observed with correlate to their respective octanol/water partition coefficients of 3.36 and 4.11 (Table 1.1). The methyl group

functions as a moderately activating group which via an inductive effect, increases the negative electron density towards the naphthalene structure [Solomons, 1984], which can result in an increase in the aromatic π-electron density. The resulting electron rich aromatic nucleus of 2-methyl naphthalene might thus interact with the cadmium component on the kaolinite surface forming coordination complexes or π-interactions. In the event of a π-complex, there would be a donation of electrons from the vacant π orbital of the aromatic ring to the populated *d* orbital of the metal making a strong association [Solomons, 1984].

The results of the experimental consecutive desorption study for 2-methyl naphthalene are shown in Figure 4.10. The desorption experiments for 2-methyl naphthalene were performed according to the same protocol as that for naphthalene. The higher partitioning and lower solubility of 2-methyl naphthalene relative to that of naphthalene, accounts for the greater resistance observed in the desorption results. The measured desorption did not conform to the adsorption isotherm indicating hysteresis behaviour, which suggests perhaps partial irreversible binding of 2-methyl naphthalene to kaolinite.

The data used to construct Figures 4.9 and 4.10 is found in Tables B.4 to B.6, and B.16 to B.21 in Appendix B.

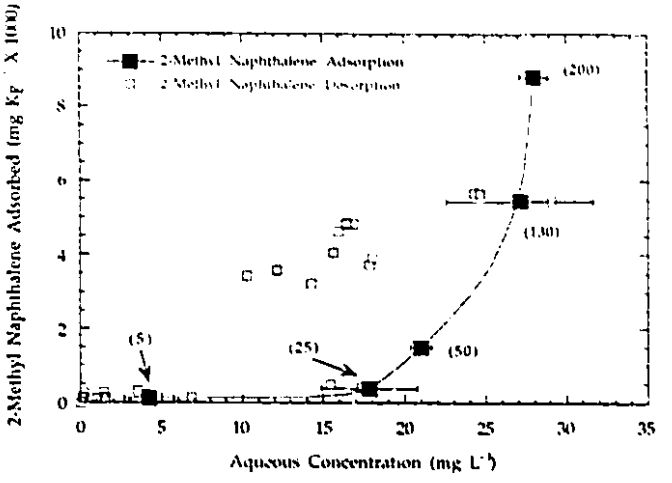


Figure 4.10. 2-Methyl Naphthalene adsorption-desorption curves showing the adsorption isotherm and experimental consecutive desorption points. Data are averages of two replicates. The initial 2-methyl naphthalene concentrations injected are indicated in parentheses as ppm.

2-Naphthol

The results of the adsorption experiment with 2-naphthol are plotted in Figure 4.11. The data are plotted in terms of aqueous 2-naphthol concentration in mg L^{-1}, versus amount of 2-naphthol adsorbed onto kaolinite as mg Kg^{-1}. The curves seen in Figure 4.11 show that 2-naphthol did not interact with the kaolinite surface whether in the presence or absence of cadmium.

The chemistry of 2-naphthol differs significantly from that of naphthalene and 2-methyl naphthalene. Table 1.1 lists some of the basic properties of all three PAHs, where it is clearly evident that 2-naphthol is considerably more soluble with a significantly lower partition coefficient than either naphthalene or 2-methyl naphthalene.

The hydroxyl substituent group on C_2 of naphthalene makes this compound a weak acid with a K_a value of 2.8 X 10^{-10} (pK_a of 9.55) [Solomons, 1984]. The hydroxyl group, like the methyl group, discussed above is an electron-releasing group, but it

enhances the electron density of the naphthalene structure via a resonance effect which stabilizes the conjugate base of the acid 2-naphthol. Under neutral conditions, the weakly acidic phenol group, experiences the following reaction:

The observed lack of adsorption of these ions at pH 7, is attributed to the formation of these anionic species. At a given pH, the ionic fraction (Φ_{ions}), of the deprotonated form of 2-naphthol, is given by equation

$$\Phi_{ions} = 1 / [1 + 10^{(pH-pKa)}]$$

Based on the above equation, 2-naphthol is almost 100% in its dissociated form.

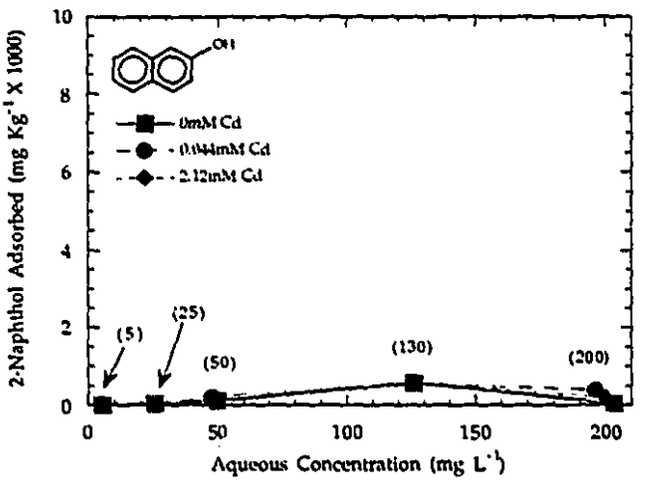


Figure 4.11. 2-Naphthol adsorption curves showing partition between the kaolinite and aqueous fractions after the addition o a known amount of 2-naphthol. The values in parentheses indicate the concentration of 2-naphthol injected. Adsorption observed in the presence of 0, 0.044, and 2.12 mmol L^{-1} Cd.

Highly soluble compounds tend to partition more in the liquid fraction and are less likely to experience hydrophobic sorption [Cerniglia, 1992; Hassett *et al*, 1981]. In the study by Xing *et al* (1994), it was observed that the partitioning of α-naphthol in soils cannot be predicted solely on the basis of its octanol/water partition coefficient (K_{ow}). The authors found that partitioning related particularly to the organic carbon content in the soil, where it was seen that partitioning became negligible in low organic matter content (OMC) or OMC consisting primarily of cellulosic materials.

Since 2-naphthol showed no interaction with the kaolinite surface, consecutive desorption analysis is not presented.

Tables B.7 to B.9 in Appendix B provide the data used to create Figure 4.11.

4.5 PAH Mineralization Experiments

The mineralization of [^{14}C]naphthalene, [^{14}C]2-methyl naphthalene, and [^{14}C]2-naphthol was measured as [^{14}C]CO_2 released in microcosms containing 2% kaolinite and MSM slurries. The mineralization experiments were expanded to include observing the impact cadmium may exert on PAH mineralization. Those samples looking at the cadmium effect, were amended with either 0.044 or 2.12 mmol L^{-1} final cadmium concentration. The controls for mineralization observed in the soil microcosms, were mineralization measured in the absence of kaolinite and in MSM only. All mineralization curves are plotted as the percent mineralized versus time given in days. Each point in these curves is the average of three replicates and the vertical bars represent the standard deviation. The concentrations of 25 and 130 ppm were the only concentrations examined for all three PAHs.

4.5.1 Mineralization Experiments in MSM (Controls)

This section includes the results of the mineralization of [^{14}C]naphthalene, [^{14}C]2-methyl naphthalene, and [^{14}C]2-naphthol by *P. putida* at the concentrations of 25 and 130 ppm in MSM. The mineralization results for all three PAHs at an initial concentration of 25ppm are shown in Figure 4.12. The curves shown in Figure 4.12 clearly show the ability of *P. putida* to effectively mineralize over 50% of naphthalene, 2-methyl naphthalene, and over 35% of 2-naphthol when provided at a concentration of 25ppm. None of the three PAHs mineralized experienced any lag phase. The mineralization of each PAH in the presence of a higher cadmium concentration, show a slightly higher percent mineralized. There are studies that have shown a slight stimulation of respiration or CO_2 release such as in the study by Capone *et al* (1983), where this effect was observed in the samples amended with the chloride salts $HgCl_2$, $PbCl_2$, $NiCl_2$, and $FeCl_2$. However, in the system in this study, it is reasonable to assume that the cadmium present was not interacting with the microorganisms based on the theoretical calculations on cadmium speciation (outlined in Appendix E). According to these equations, all cadmium should have been complexed with the phosphate anions present due to the high affinity cadmium has for phosphate. Metal salts added to solution have the effect of acidification and although the mineral salts medium used throughout these studies was a buffered medium, the pH dropped slightly (refer to Tables A.1, A.3, A.5 in Appendix A). The ideal growth medium for *P.putida* is nutrient broth having a final pH of 6.8. This pH level is approximately the pH range within the microcosms amended with a final cadmium concentration of 2.12 mmol L^{-1}. This pH variation between microcosms amended with the higher Cd concentration may be responsible for the increased percent mineralized observed, since this pH is more favourable for microbial activity.

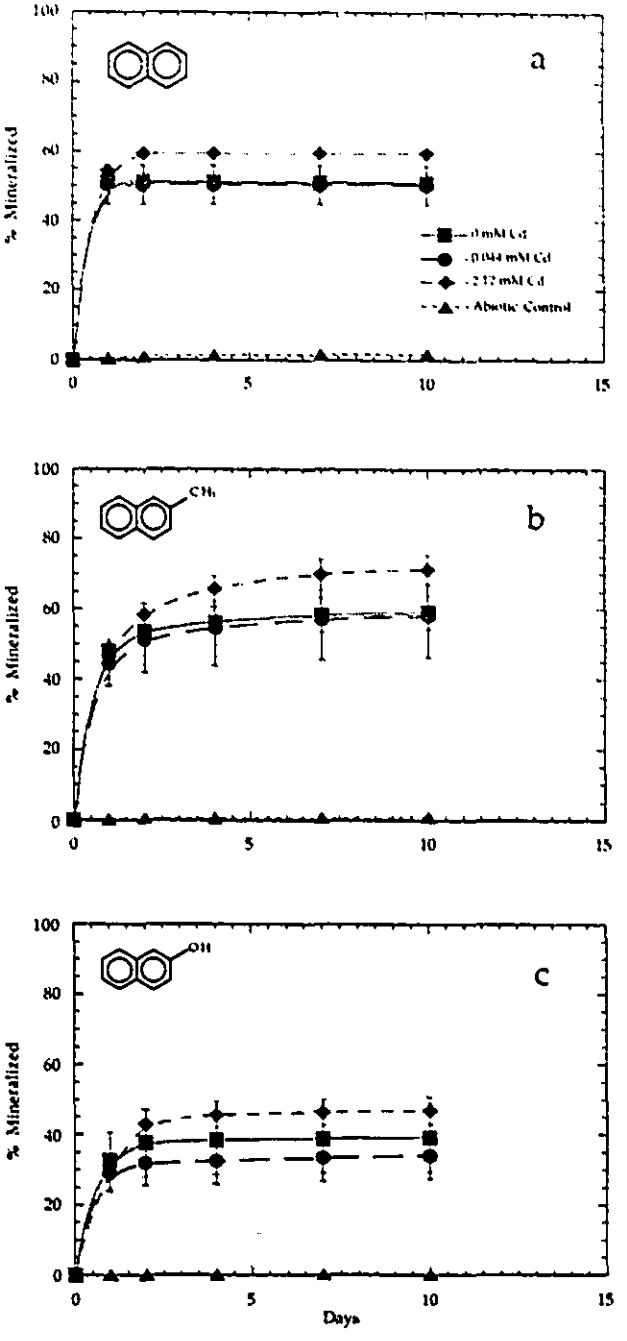


Figure 4.12. PAH mineralization in mineral salts medium from an initial PAH concentration of 25ppm. a) naphthalene, b) 2-methyl naphthalene, and c) 2-naphthol.

The mineralization results for all three PAHs at an initial concentration of 130 ppm are shown in Figure 4.13. The curves in Figure 4.13 show that *P. putida* was able to mineralize over 50% of naphthalene and 2-methyl naphthalene, when provided at the higher concentration of 130ppm. 2-Naphthol gave almost 0% mineralization, a result that corroborates well with the biomass curves for growth on 2-naphthol seen in Figure 4.4, where no biomass increase was observed. Clearly, at a concentration of 130ppm, 2-naphthol is toxic to *P. putida* and this may be related to its higher solubility.

The curves representing mineralization in the presence of a higher cadmium concentration, show a slightly elevated percent mineralized in comparison to the other curves. The cause for this slight increase is assumed to be identical to the reason outlined above for mineralization experiments with only 25ppm of each PAH. To discount any possible effect the excess nitrate may have caused in samples amended with the higher cadmium concentration (cadmium was supplemented as a nitrate salt), control samples were performed looking at the mineralzation of 130 ppm of each PAH amended with an equivalent concentration of nitrate (0.088 mmol L^{-1}). The results of these controls are given in Tables A.13 to A.15 in Appendix A. These results indicate that the total percent mineralized with excess nitrate added, did not differ from samples examining percent mineralization in the absence of cadmium.

In breakdown studies of 2-methyl naphthalene, a persistent yellow colour is observed in the medium. According to Cane and Williams (1982), the compound was identified as 2-hydroxy-5-methyl muconic semialdehyde (see Figure 2.2). A yellow colour in the mineralization of 2-methyl naphthalene in this study was also observed and did not disappear over time. It is a likely dead end product from the *meta*-cleavage of 4-methyl catechol.

The data used to construct Figures 4.12 and 4.13 is given in Tables A.1, A.2, A.3, A.4, A.5, and A.6 in Appendix A.

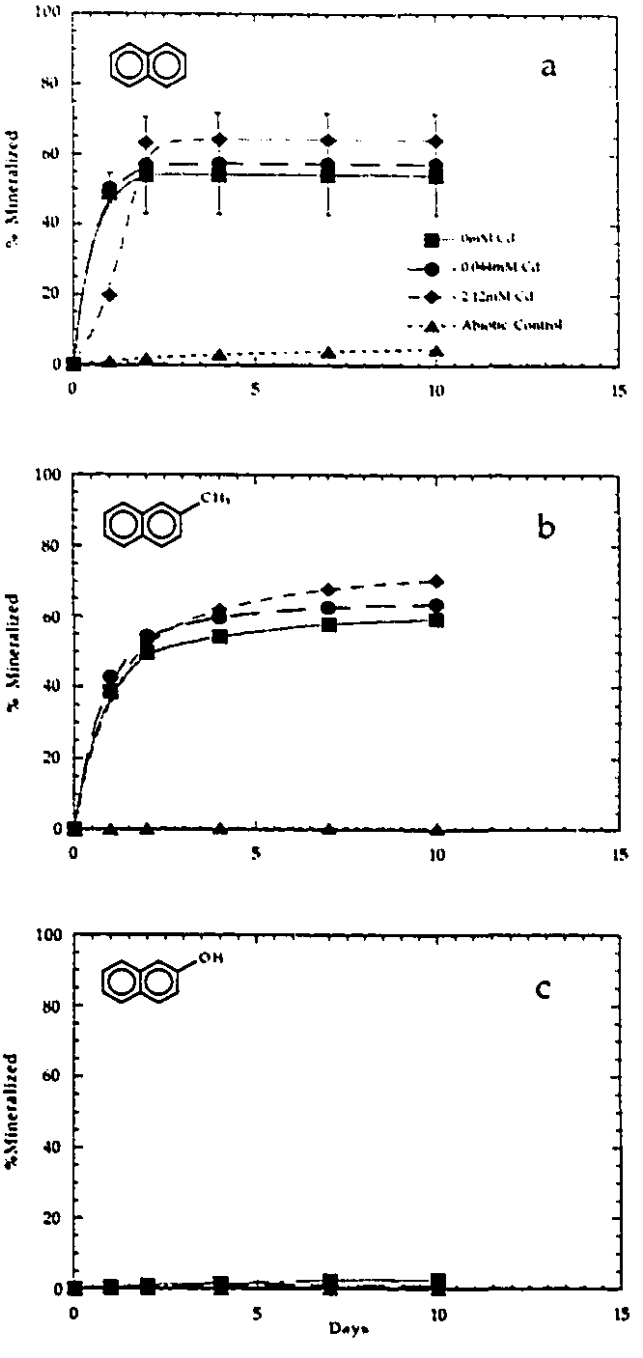


Figure 4.13. PAH mineralization in minimal salts solution from an initial PAH concentration of 130ppm. a)naphthalene, b) 2-methyl naphthalene, and c) 2-naphthol.

4.5.2 Mineralization Experiments in 2% Kaolinite and MSM

This section includes the results of the mineralization of [^{14}C]naphthalene, [^{14}C]2-methyl naphthalene, and [^{14}C]2-naphthol by *P. putida* at the concentrations of 25 and 130 ppm in the presence of kaolinite. The mineralization results for all three PAHs at an initial concentration of 25ppm are shown in Figure 4.14. The curves shown in Figure 4.14, relative to the curves in Figure 4.12, show a decrease in the rate of mineralization and the total percent mineralized. In the study by Stotzky and Rem (1966), kaolinite was found to decrease the respiration of systems even those that had been adjusted to a pH of 7. However it was found that this effect was not apparent for all microorganisms examined. The impact of clays often has to do with the ability or inability of the clay mineral to buffer the medium, to provide nutrients, or the presence of the clay can inhibit microbial activity depending on the presence of certain cations saturating the mineral surface [Filip, 1973]. It is reasonable to assume that the impact of kaolinite on reducing the mineralization rate of each PAH by *P. putida* in this study, was not due to a reduction in the buffering capacity since the pH was maintained around neutral in all samples. However, the pH did drop slightly in the samples amended with 2.12 mmol L^{-1} cadmium. This slight pH drop may account for the slightly enhanced mineralization in these samples, which as discussed in section 4.5.1, provided a more favourable pH for microbial growth. The mineralization rate for 2-methyl naphthalene, showed the greatest reduction and is likely due to its lower availability as seen by its greater affinity for the kaolinite surface (Figure 4.10).

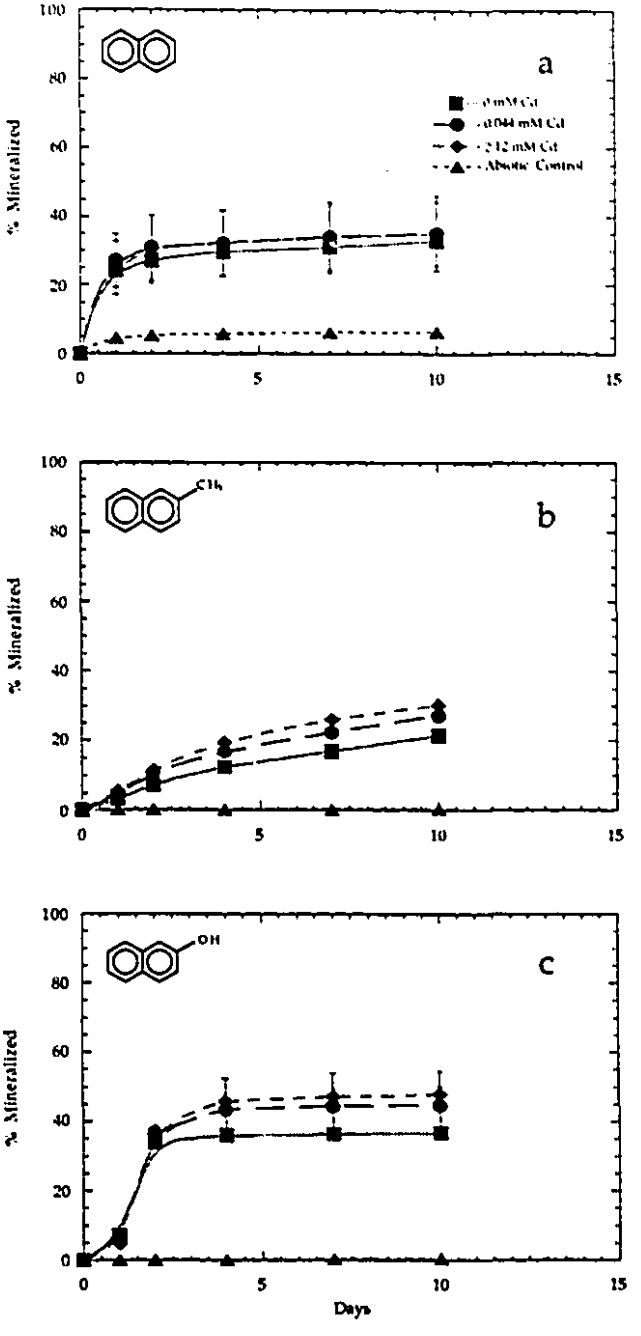


Figure 4.14. PAH mineralization in 2% kaolinite and MSM solution from an initial concentration of 25ppm.

The mineralization results for all three PAHs at an initial concentration of 130ppm, are shown in Figure 4.15. Similar to the behaviour discussed above for the mineralization of 25ppm of each PAH, the rate of mineralization in the presence of kaolinite was significantly reduced compared to the mineralization rates seen in the absence of kaolinite (Figure 4.13). The mineralization curves for naphthalene and 2-methyl naphthalene, show an increase in percent mineralization in the presence of a higher cadmium concentration. To discount any effect the escess nitrate added may have caused with samples amended with a total cadmium concentration of 2.12 mmol L^{-1} (Cd was added as a nitrate salt), equivalent nitrate controls were performed. No variation was observed in the total percent mineralized between samples amended with excess nitrate and those in the absence of cadmium. The results of these controls are presented in Tables A.16, A.17, and A.18 in Appendix A.

The pH for the naphthalene and 2-methyl naphthalene experiments with 130ppm, experienced a continual drop. The reduction in pH was due to the release of H^+ during compound breakdown and the production of acidic metabolites. The buffering capacity of the 2% kaolinite slurry was incapable of maintaining the pH at neutral unlike the microcosms in the absence of kaolinite (pH data shown in Appendix A). After 7 days the pH dropped to 5.4, which was still above the IEP of 4.2 determined for kaolinite hydrite PX [Yong and Ohtsubo, 1987]. The surface therefore, remained negative. The drop in pH was assumed not to have impacted microbial activity because no change in behaviour was observed in terms of the percent mineralization of naphthalene and 2-methyl naphthalene in comparison to the curves seen in Figure 4.14a and b.

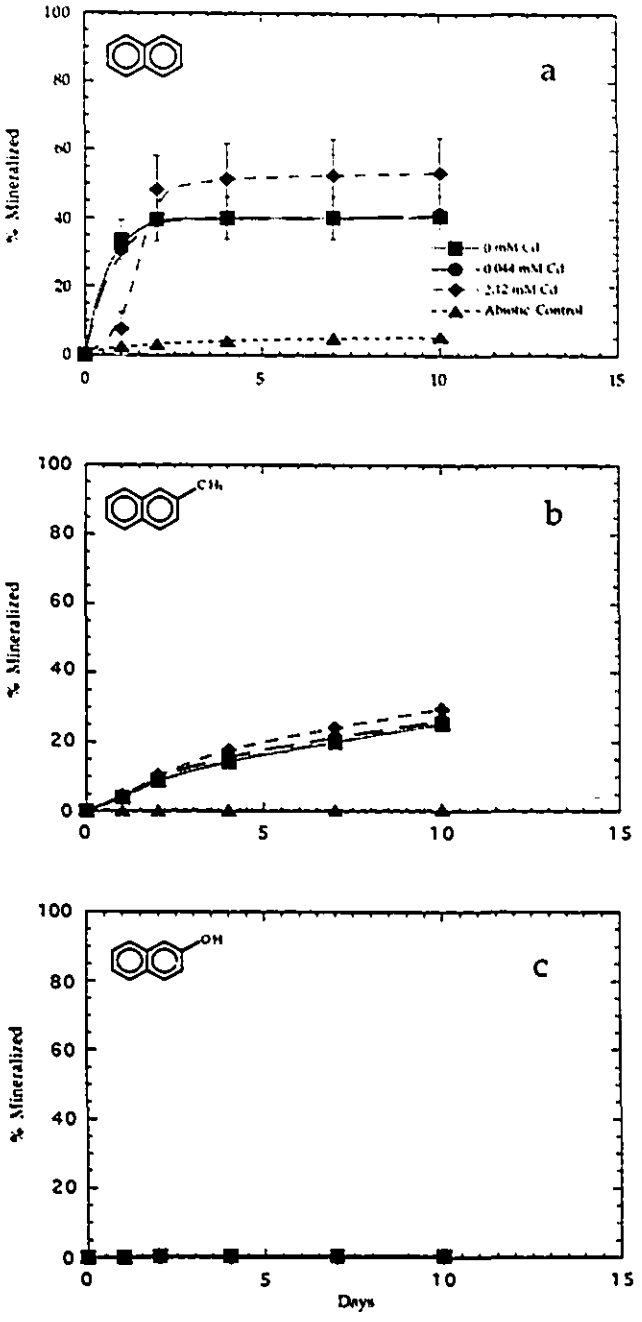


Figure 4.15. PAH mineralization in 2% kaolinite and MSM solution from an initial concentration of 130ppm.

The inability of the system to maintain the pH around neutral was likely due to the adsorption of phosphate onto the kaolinite surface as discussed earlier. The concentration of available phosphate was insufficient to buffer the H^+ ions released during biodegradation nor could the kaolinite surface adsorb H^+ ions being saturated wth Na^+ and heavy metals from the MSM.

The remaining possible assumptions relating to the observed reduction in the mineralization rates with kaolinite include:

- The reduction in the rate of mineralization may be a result of reduced availability of phosphate. In the study by Barrow *et al* (1980), results showed that the amount of phosphate adsorbed varied with the concentration and nature of the electrolytes and the pH of the solution. It was observed in the study by Bolland *et al* (1977), that the concentration of phosphate adsorbed onto geothite increased with the amount of Zn^{2+} adsorbed onto the surface. It is reasonable to assume however, that if phosphate availability were limited, that the effect would be enhanced in the mineralization of 130ppm naphthalene and 2-methyl naphthalene seen in Figure 4.15, where no significant variation or decrease was observed.
- The reduction in the rate of PAH mineralization may also have been due to a lack in availability of trace elements which were assumed to have adsorbed onto the kaolinite surface.

The greater partitioning of 2-methyl naphthalene compared to that of naphthalene onto the kaolinite surface seen in Figures 4.8 and 4.9, indicates 2-methyl naphthalenes greater affinity for kaolinite. This higher affinity for kaolinite was attributed to the increased reduction in 2-methyl naphthalene mineralization observed at both concentrations of 25 and 130ppm (Figures 4.14b and 4.15b).

Figure 4.15c like Figure 4.9c shows that 0% of 2-naphthol was mineralized indicating that this compound when present at elevated concentrations was toxic to *P.*

putida. As seen in Figure 4.11, 2-naphthol did not interact with the kaolinite surface, therefore the entire 130ppm of 2-naphthol was bioavailable.

It can be assumed that *P. putida*'s inability to mineralize the higher concentration of 2-naphthol, has to do with the location in which carbon breakdown occurs in the cell membrane. It is possible that the elevated concentration of 2-naphthol uncouples the oxidative phosphorylation process occurring in the cell membrane. Poisons such as 2,4-dinitrophenol are soluble in phospholipid membranes and are responsible for the dissipation of any transmembrane proton concentration or potential gradient [Darnel *et* al, 1986]. Essentially the membranes are made leaky to H^+ ions and prevent ATP synthesis. 2-Naphthol is a soluble contaminant which may easily dissolve into the phospholipid membrane and consequently affect the H^+ gradient of the phospholipid membrane.

Tables A.7, A.8, A.9, A.10, A.11, and A.12 in Appendix A, contain the data used to construct Figures 4.14 and 4.15.

Chapter 5
Conclusions and Future Research

5.1 Conclusions

The conclusions presented below are a summary of the results obtained in the mineralization and adsorption-desorption experiments performed.

- *Pseudomonas putida* was capable of mineralizing over 50% of naphthalene and 2-methyl naphthalene, and over 35% of 2-naphthol in solution (MSM). The mineralization of each PAH in the presence of kaolinite, experienced a significant reduction in the rate of mineralization relative to their rates in MSM only. This suggests the importance the presence of clay can exert not only on the attenuation of contaminants, but also the direct effects clay can exert on microbial activity. 2-Methyl naphthalene experienced the lowest mineralization rate in kaolinite because of its greater affinity for the kaolinite surface, which reduced its bioavailability. 2-Naphthol under the conditions in this system was not mineralized whether in the presence or absence of kaolinite indicating that it was toxic to *P. putida* at elevated concentrations.
- The adsorption-desorption of naphthalene, 2-methyl naphthalene, and 2-naphthol were observed to partition onto kaolinite in relation to their respective octanol/water partition coefficients. 2-Naphthol showed a negative interaction with the kaolinite surface due to its high solubility and the pH of the system. 2-Methyl naphthalene showed the highest affinity for the kaolinite surface and concurrently,

showed the slowest mineralization rates. The variation observed in the interactions of these compounds with clay, indicates that in a contaminated field site, experimental determination of the interaction behaviour between the contaminants and the soil, can contribute to predicting the bioavailability of the contaminants.

- Cadmium had no observable impact on the mineralization activity of *P. putida* which may have been accounted for by the complete complexation of cadmium with phosphate present in the MSM, as predicted by the thermodynamic equilibrium calculations. The slight increase in the total percent mineralization in the presence of a final cadmium concentration of 2.12 mmol L^{-1}, may have been an indirect effect. Those microcosms amended with a high cadmium concentration, experienced a slight drop in pH creating a more favourable pH range for growth. Estimations on the speciation of heavy metals present in a contaminanted site, can help predict the potential impact on the microorganisms present. The species of metal may exert either direct or indirect effects on the microorganisms and the physical-chemical status of their environment.

To summarize the preceding conclusions, it can be said that kaolinite had the potential to reduce the rates of mineralization for naphthalene and 2-methyl naphthalene, and had no impact on the rate of mineralization of 2-naphthol at 25ppm. The interaction behaviour of the PAHs with the kaolinite surface, related well to their physical/chemical properties. This also correlated well with their availability for microbial biodegradation as evidenced in the experimental mineralization results.

This study illustrates that biodegradation studies in conjunction with adsorption-desorption analyses, can provide information to adequately predict the behaviour of contaminants within a mixed contaminated system.

5.2 Future Research Suggestions

The following points outline possible future research suggestions that would significantly contribute to the development of the topic presented in this research.

- Extraction procedures along with GC MS analysis can be used to evaluate if the presence of heavy metals affects the rates of metabolite formation.
- Studies should focus on the interaction of contaminants within a biologically active system in order to more effectively evaluate their bioavailability.
- PAHs have not been observed to biodegrade under strictly anaerobic conditions, but 2-naphthol has been [Baker and Herson, 1994]. Soil systems consist of various REDOX environments, therefore biodegradation determination of substituted compounds under varying redox conditions can be performed.
- Studies simulating the manner in which contaminants enter a system, will significantly contribute to providing a better approximation of the dynamics of a contaminated site.

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Appendix A

Mineralization data for the controls in MSM and the 2% kaolinite and MSM samples.

Table A.1. Data for the Mineralization of 25ppm Naphthalene with 0; 0.044; 2.12 mmol L^{-1} Cd in Minimal Salts Solution

Days	0mmol L^{-1} Cadmium %Min*	Stan. Dev.	pH	0.044mmol L^{-1} Cadmium %Min*	Stan. Dev.	pH	2.12mmol L^{-1} Cadmium %Min*	Stan. Dev.	pH
0	0.0000	0.0000	6.99	0.0000	0.0000	7.00	0.0000	0.0000	6.85
1	51.193	2.5568	7.02	50.440	5.6312	7.02	54.484	2.0677	6.80
2	51.193	2.6847	7.00	50.440	5.6312	6.98	59.469	2.0012	6.80
4	51.193	2.6847	6.98	50.440	5.6312	6.99	59.469	2.0012	6.85
7	51.193	2.6847	6.99	50.440	5.6312	6.97	59.469	2.0012	6.85
10	51.193	2.6847	7.02	50.440	5.6312	7.00	59.592	2.0012	6.86

*value is based on the average of three samples

Table A.2. Data for the Mineralization of 130ppm Naphthalene with 0; 0.044; 2.12 mmol L^{-1} Cd in Minimal Salts Solution

Days	Cadmium Concentration: 0mmol L^{-1} %Min*	Stan. Dev.	pH	0.044mmol L^{-1} %Min*	Stan. Dev.	pH	2.12mmol L^{-1} %Min*	Stan. Dev.	pH
0	0.0000	0.0000	6.99	0.0000	0.0000	7.00	0.0000	0.0000	6.80
1	48.918	1.1588	6.89	50.204	4.3140	6.87	19.693	0.9965	6.88
2	54.169	0.94715	6.91	56.915	13.860	6.95	63.311	1.8976	6.85
4	54.169	0.94715	6.91	57.251	14.438	6.93	64.278	1.2347	6.91
7	54.169	0.9614	6.91	57.251	14.438	6.90	64.278	1.2347	6.88
10	54.169	0.9614	7.02	57.251	14.438	6.96	64.278	1.2347	6.80

*value is based on the average of three samples

Table A.3 Data for the Mineralization of 25ppm 2-Methyl Naphthalene with 0; 0.044; 2.12mmol L^{-1} Cd in Minimal Salts Solution

Days	0mmol L^{-1} Cadmium %Min*	Stan. Dev.	pH	0.044mmol L^{-1} Cadmium %Min*	Stan. Dev.	pH	2.12mmol L^{-1} Cadmium %Min*	Stan. Dev.	pH
0	0.0000	0.0000	7.00	0.0000	0.0000	7.00	0.0000	0.0000	6.90
1	47.857	1.9349	7.00	44.346	6.2569	7.20	48.697	2.6532	6.85
2	53.396	3.4973	7.05	50.808	8.9479	7.00	58.429	3.2511	6.85
4	56.278	4.3504	7.07	54.527	10.642	6.99	65.808	3.6552	6.84
7	58.280	4.7582	6.98	57.062	11.536	6.97	69.998	4.3399	6.84
10	59.185	4.8086	7.00	58.199	11.844	7.00	71.429	4.2043	6.85

*value is based on the average of three samples

Table A.4 Data for the Mineralization of 130ppm 2-Methyl Naphthalene with 0; 0.044; 2.12mmol L^{-1} Cd in Minimal Salts Solution

Days	0mmol L^{-1} Cadmium %Min*	Stan. Dev.	pH	0.044mmol L^{-1} Cadmium %Min*	Stan. Dev.	pH	2.12mmol L^{-1} Cadmium %Min*	Stan. Dev.	pH
0	0.0000	0.0000	7.00	0.0000	0.0000	7.00	0.0000	0.0000	6.89
1	38.707	1.6096	7.16	42.783	1.2482	7.01	37.865	2.1078	6.88
2	49.700	2.8248	7.02	54.440	4.2417	6.99	52.849	2.2940	6.85
4	54.295	1.7816	7.10	59.740	5.5478	7.01	61.901	2.3192	6.85
7	57.911	1.5880	7.00	62.540	6.0386	6.98	67.724	1.9003	6.86
10	59.356	1.6827	7.11	63.536	6.2895	7.07	70.327	1.9998	6.85

*value is based on the average of three samples

Table A.5 Data for the Mineralization of 25ppm 2-Naphthol with 0; 0.044; 2.12mmol L^{-1} Cd in Minimal Salts Solution

Days	0mmol L^{-1} Cadmium %Min*	Stan. Dev.	pH	0.044mmol L^{-1} Cadmium %Min*	Stan. Dev.	pH	2.12mmol L^{-1} Cadmium %Min*	Stan. Dev.	pH
0	0.0000	0.0000	7.00	0.0000	0.0000	7.00	0.0000	0.0000	6.80
1	32.428	8.2150	7.12	28.777	5.3423	7.12	29.548	2.5621	6.74
2	37.457	9.5045	7.20	31.673	6.3546	7.20	42.762	3.9525	6.80
4	38.338	9.7311	7.21	32.478	6.4978	7.10	45.610	3.7267	6.79
7	38.894	9.7828	7.19	33.366	6.6347	7.20	46.343	3.7013	6.81
10	39.218	9.7296	7.11	34.071	6.6858	7.11	46.905	3.8164	6.81

*value is based on the average of three samples

Table A.6 Data for the Mineralization of 130ppm 2-Naphthol with 0; 0.044; 2.12mmol L^{-1} Cd in Minimal Salts Solution

Days	0mmol L^{-1} Cadmium %Min*	Stan. Dev.	pH	0.044mmol L^{-1} Cadmium %Min*	Stan. Dev.	pH	2.12mmol L^{-1} Cadmium %Min*	Stan. Dev.	pH
0	0.0000	0.0000	7.00	0.0000	0.0000	7.00	0.0000	0.0000	6.74
1	0.58330	0.30572	7.19	0.51513	0.55819	7.09	0.11804	0.11865	6.76
2	1.0720	0.64132	7.20	0.83652	1.0271	7.10	0.14544	0.13858	6.80
4	1.5493	0.97872	7.11	0.99953	1.1582	7.20	0.15561	0.14765	6.83
7	2.4040	1.2841	7.00	1.0096	1.1490	7.18	0.16350	0.11553	6.80
10	2.5549	1.1494	7.11	1.0518	1.1245	7.07	0.17851	0.10416	6.80

*value is based on the average of three samples

Table A.7 Data for the Mineralization of 25ppm Naphthalene with 0; 0.044; 2.12 mmol L^{-1} Cd in 2% Kaolinite and Minimal Salts Solution

Days	0mmol L^{-1} Cadmium %Min*	Stan. Dev.	pH	0.044mmol L^{-1} Cadmium %Min*	Stan. Dev.	pH	2.12mmol L^{-1} Cadmium %Min*	Stan. Dev.	pH
0	0.0000	0.0000	7.12	0.0000	0.0000	7.18	0.0000	0.0000	6.79
1	24.002	0.71920	7.03	27.008	7.7519	7.18	24.913	7.7651	6.79
2	27.120	0.98884	6.84	30.810	9.4383	7.12	30.400	9.9733	6.78
4	29.589	1.2084	6.68	32.155	9.6320	6.94	32.356	9.5774	6.76
7	31.001	1.3690	7.01	34.001	10.373	6.97	33.832	9.3787	6.76
10	32.899	1.4376	XXX	35.219	10.978	XXX	35.092	9.2360	XXX

*value is based on the average of three samples

Table A.8 Data for the Mineralization of 130ppm Naphthalene with 0; 0.044; 2.12 mmol L^{-1} Cd in 2% Kaolinite and Minimal Salts Solution

Days	0mmol L^{-1} Cadmium %Min*	Stan. Dev.	pH	0.044mmol L^{-1} Cadmium %Min*	Stan. Dev.	pH	2.12mmol L^{-1} Cadmium %Min*	Stan. Dev.	pH
0	0.0000	0.0000	7.00	0.0000	0.0000	7.20	0.0000	0.0000	6.76
1	33.499	5.8133	6.86	30.972	0.98337	6.86	7.4144	4.9307	6.78
2	39.374	6.1637	6.74	39.116	1.6753	6.90	48.244	9.9732	6.77
4	39.964	6.1975	6.10	39.766	1.7904	6.59	51.438	10.471	6.56
7	40.184	6.3174	5.47	40.115	1.9101	6.75	52.610	10.415	6.63
10	40.499	6.3612	XXX	41.009	1.7020	XXX	53.466	10.343	XXX

*value is based on the average of three samples

Table A.9 Data for the Mineralization of 25ppm 2-Methyl Naphthalene with 0; 0.044; 2.12mmol L^{-1} Cd in 2% Kaolinite and Minimal Salts Solution

Days	0mmol L^{-1} Cadmium %Min*	Stan. Dev.	pH	0.044mmol L^{-1} Cadmium %Min*	Stan. Dev.	pH	2.12mmol L^{-1} Cadmium %Min*	Stan. Dev.	pH
0	0.0000	0.0000	7.20	0.0000	0.0000	7.20	0.0000	0.0000	6.78
1	3.2617	0.52020	7.07	4.6668	0.25977	7.10	5.4962	0.24582	6.80
2	7.2450	0.25319	7.10	10.490	0.19539	7.16	11.424	0.70433	6.80
4	12.464	0.44209	6.99	16.774	0.65628	7.16	19.399	1.0841	6.80
7	16.987	1.2013	6.95	22.380	1.4068	7.03	25.941	1.3252	6.80
10	21.474	1.9643	XXX	27.174	1.6793	XXX	30.140	1.5510	XXX

*value is based on the average of three samples

Table A.10 Data for the Mineralization of 130ppm 2-Methyl Naphthalene 0; 0.044; 2.12mmol L^{-1} Cd in 2% Kaolinite and Minimal Salts Solution

Days	0mmol L^{-1} Cadmium %Min*	Stan. Dev.	pH	0.044mmol L^{-1} Cadmium %Min*	Stan. Dev.	pH	2.12mmol L^{-1} Cadmium %Min*	Stan. Dev.	pH
0	0.0000	0.0000	7.11	0.0000	0.0000	7.20	0.0000	0.0000	6.80
1	3.8829	0.52471	6.95	4.1703	0.26056	7.06	4.4097	0.54085	6.76
2	8.8697	0.45269	6.81	9.7909	0.21725	6.99	10.350	0.72169	6.75
4	14.380	0.71282	6.29	15.654	0.22055	6.81	17.649	1.0799	6.60
7	20.066	1.5037	5.44	21.253	0.38392	6.42	24.059	1.4997	5.81
10	25.269	1.7628	XXX	26.192	0.56301	XXX	29.548	1.7833	XXX

*value is based on the average of three samples

Table A.11 Data for the Mineralization of 25ppm 2-Naphthol with 0; 0.044; 2.12mmol L^{-1} Cd in 2% Kaolinite and Minimal Salts Solution

Days	0mmol L^{-1} Cadmium %Min*	Stan. Dev.	pH	0.044mmol L^{-1} Cadmium %Min*	Stan. Dev.	pH	2.12mmol L^{-1} Cadmium %Min*	Stan. Dev.	pH
0	0.0000	0.0000	7.09	0.0000	0.0000	7.07	0.0000	0.0000	6.99
1	7.2311	2.7279	7.20	7.0983	1.5053	7.20	4.8280	0.56751	6.89
2	34.001	0.78264	7.03	36.666	1.7202	7.10	37.339	1.1549	6.92
4	36.062	1.0513	7.05	43.483	8.8814	7.20	45.863	2.1526	7.07
7	36.424	0.99099	6.82	44.428	9.5911	7.01	47.148	2.3517	6.95
10	36.766	0.83277	XXX	44.809	9.6264	XXX	47.825	2.3152	XXX

*value is based on the average of three samples

Table A12 Data for the Mineralization of 130ppm 2-Naphthol with 0; 0.044; 2.12mmol L^{-1} Cd in 2% Kaolinite and Minimal Salts Solution

Days	0mmol L^{-1} Cadmium %Min*	Stan. Dev.	pH	0.044mmol L^{-1} Cadmium %Min*	Stan. Dev.	pH	2.12mmol L^{-1} Cadmium %Min*	Stan. Dev.	pH
0	0.0000	0.0000	7.16	0.0000	0.0000	7.08	0.0000	0.0000	7.02
1	0.11827	0.082212	7.12	0.24616	0.23630	7.20	0.020499	0.045852	6.97
2	0.52805	0.15863	7.16	0.56104	0.26219	7.03	0.020499	0.10955	7.07
4	0.52956	0.16639	7.05	0.56104	0.26219	7.20	0.020499	0.11590	6.90
7	0.52956	0.16871	7.17	0.61227	0.27003	7.19	0.020499	0.10716	7.12
10	0.52956	0.16624	XXX	0.61227	0.27003	XXX	0.020499	0.11781	XXX

*value is based on the average of three samples

A.2. Mineralization Data for Microcosms Containing Sodium Nitrate to Account for Excess Nitrate Added as Cadmium Nitrate in 2.12mmol L^{-1} Cd Microcosms

Table A.13 Data for the Mineralization of 130ppm Naphthalene with Additional Nitrate added as Sodium Nitrate Salt ($[NO_3^-]$ is 4.2 X 10^{-3} M), in MSM Only

Days	*A*	*B*	*C*	*Average %*	*Standard Deviation*
1	54.97	52.68	57.91	55.18	2.62
2	58.61	58.39	65.22	60.74	3.88
4	58.61	58.56	65.66	60.94	4.082
7	58.61	58.56	65.66	60.94	4.082
10	58.61	58.56	65.66	60.94	4.082

Table A.14 Data for the Mineralization of 130ppm 2-Methyl Naphthalene with Additional Nitrate added as Sodium Nitrate Salt ($[NO_3^-]$ is 4.2 X 10^{-3} M), in MSM Only

Days	*A*	*B*	*C*	*Average %*	*Standard Deviation*
1	42.36	36.67	36.67	39.51	4.023
2	50.48	43.78	43.78	47.13	4.73
4	54.80	47.32	47.32	51.067	5.28
7	58.84	49.91	49.91	54.37	6.31
10	60.071	51.35	51.35	55.71	6.16

Table A.15 Data for the Mineralization of 130ppm 2-Naphthol with Additional Nitrate added as Sodium Nitrate Salt ($[NO_3^-]$ is 4.2 X 10^{-3} M), in MSM Only

Days	*A*	*B*	*C*	*Average %*	*Standard Deviation*
1	0.15	0.23	2.27	0.88	1.19
2	0.19	0.29	4.75	1.74	2.60
4	1.78	0.48	6.37	2.87	3.09
7	1.92	0.90	7.56	3.46	3.58
10	1.93	1.03	8.18	3.72	3.89

Table A.16 Data for the Mineralization of 130ppm Naphthalene with Additional Nitrate added as Sodium Nitrate Salt ($[NO_3^-]$ is 4.2 X 10^{-3} M), in 2%Kaolinite Slurry

Days	*A*	*B*	*C*	*Average %*	*Standard Deviation*
1	44.91	30.80	31.63	35.78	7.91
2	56.23	38.78	37.60	44.20	10.433
4	57.37	40.50	38.67	45.51	10.31
7	57.82	41.48	39.32	46.21	10.11
10	58.43	42.83	40.26	47.18	9.83

Table A.17 Data for the Mineralization of 130ppm 2-Methyl Naphthalene with Additional Nitrate added as Sodium Nitrate Salt ([NO_3^-] is 4.2×10^{-3} M), in 2%Kaolinite Slurry

Days	*A*	*B*	*C*	*Average %*	*Standard Deviation*
1	4.21	4.11	4.11	4.16	0.071
2	9.14	10.62	10.62	9.88	1.04
4	14.94	17.16	17.16	16.05	1.56
7	20.33	23.74	23.74	22.04	2.41
10	25.65	29.97	29.97	27.81	3.05

Table A.18 Data for the Mineralization of 130ppm 2-Naphthol with Additional Nitrate added as Sodium Nitrate Salt ([NO_3^-] is 4.2×10^{-3} M), in 2%Kaolinite Slurry

Days	*A*	*B*	*C*	*Average %*	*Standard Deviation*
1	0.033	0.12	0.15	0.10	0.061
2	0.68	0.90	1.18	0.92	0.24
4	0.72	0.94	1.26	0.97	0.27
7	0.76	0.94	1.27	0.99	0.26
10	0.76	0.94	1.28	0.99	0.26

Appendix B
Adsorption-Desorption Data

B.1. Adsorption Data for Naphthalene, 2-Methyl Naphthalene, and 2-Naphthol in the presence and absence of Cadmium

Table B.1 Adsorption data for naphthalene in the absence of cadmium

Initial Concentration	*mg/L A*	*mg/L B*	*Average ppm.*	*Standard Deviation.*	*Amount in Soil ppm*
5	3.21	3.73	3.47	0.37	90.14
25	22.25	22.68	22.46	0.30	196.31
50	27.78	28.10	27.94	0.23	1189.59
130	28.92	28.25	28.58	0.47	4945.64
200	29.69	28.90	29.29	0.56	8829.26

Table B.2 Adsorption data for naphthalene in the presence of an initial cadmium concentration of 0.944mmol L^{-1}

Initial Concentration	*mg/L A*	*mg/L B*	*Average ppm.*	*Standard Deviation.*	*Amount in Soil ppm*
5	4.65	5.26	4.96	0.43	15.92
25	21.97	20.52	21.25	1.02	257.16
50	27.61	28.22	27.91	0.43	1190.78
130	28.79	30.06	29.43	0.89	4903.49
200	34.62	31.81	33.21	1.98	8633.04

Table B.3 Adsorption data for naphthalene in the presence of an initial cadmium concentration of 2.12mmol L^{-1}

Initial Concentration	*mg/L A*	*mg/L B*	*Average ppm*	*Standard Deviation.*	*Amount in Soil ppm*
5	4.94	4.99	4.96	0.038	15.54
25	18.22	23.29	20.76	3.58	281.65
50	27.44	28.29	27.86	0.60	1193.43
130	28.25	28.63	28.44	0.26	4952.75
200	28.32	28.89	28.60	0.39	8863.51

Table B.4 Adsorption data for 2-methyl naphthalene in the absence of cadmium

Initial Concentration	*mg/L A*	*mg/L B*	*Average ppm*	*Standard Deviation.*	*Amount in Soil ppm*
5	3.10	5.25	4.17	1.51	119.94
25	19.89	15.74	17.82	2.93	383.82
50	20.54	21.44	20.99	0.63	1500.21
130	32.28	25.88	29.08	4.52	5430.69
200	28.60	27.34	27.97	0.89	8801.08

Table B.5 Adsorption data for 2-methyl naphthalene in the presence of 0.044mmol L^{-1} cadmium

Initial Concentration	*mg/L A*	*mg/L B*	*Average ppm*	*Standard Deviation.*	*Amount in Soil ppm*
5	5.26	5.21	5.24	0.035	66.85
25	16.46	14.43	15.44	1.43	502.56
50	19.34	20.65	19.99	0.92	1550.04
130	23.68	24.84	24.26	0.82	5671.73
200	26.05	24.91	25.48	0.80	8925.82

Table B.6 Adsorption data for 2-methyl naphthalene in the presence of 2.12mmol L^{-1} cadmium

Initial Concentration	*mg/L A*	*mg/L B*	*Average ppm*	*Standard Deviation*	*Amoun in Soil ppm*
5	4.42	4.91	4.66	0.34	95.50
25	17.64	17.03	17.34	0.43	407.91
50	20.29	18.47	19.38	1.28	1580.86
130	23.50	25.72	24.61	1.56	5654.21
200	25.90	24.76	25.33	0.81	8933.17

Table B.7 Adsorption data for 2-naphthol in the absence of cadmium

Initial Concentration	*mg/L A*	*mg/L B*	*Average ppm*	*Standard Deviation.*	*Amount in Soil ppm*
5	5.21	5.22	5.21	0.00802	14.021
25	24.83	25.63	25.23	0.56	44.97
50	49.43	49.38	49.43	0.073	107.60
130	126.27	126.07	126.17	0.14	576.30
200	202.24	204.12	203.18	1.32	43.89

Table B.8 Adsorption data for 2-naphthol in the presence of 0.044mmol L^{-1} cadmium

Initial Concentration	*mg/L A*	*mg/L B*	*Average ppm*	*Standard Deviation.*	*Amount in Soil ppm*
5	4.37	5.13	4.75	0.53	37.12
25	25.54	26.13	25.84	0.41	14.79
50	47.094	47.32	47.20	0.16	218.83
130	127.003	126.12	126.56	0.62	556.87
200	194.51	197.844	196.18	2.35	390.93

Table B.9 Adsorption data for 2-naphtol in the presence of 2.12mmol L^{-1} cadmium

Initial Concentration	*mg/L A*	*mg/L B*	*Average ppm*	*Standard Deviation.*	*Amount in Soil ppm*
5	5.16	4.44	4.80	0.50	34.55
25	24.92	25.71	25.31	0.55	40.89
50	50.54	48.99	49.76	1.09	90.79
130	126.69	125.85	126.27	0.59	571.40
200	200.58	199.72	200.15	0.60	192.25

B.2. Desorption Data for Naphthalene, 2-Methyl Naphthalene, and 2-Naphthol in the Presence and absence of Cadmium

Table B.10 Desorption data for naphthalene initial concentration of 25ppm in the absence of cadmium

Sample	*mg/L A*	*mg/L B*	*Average ppm*	*Standard Deviation.*	*Amount in Soil ppm*
1	22.25	22.68	22.46	0.30	196.31
2	1.90	2.12	2.01	0.15	95.71
3	0.098	0.15	0.12	0.043	89.25
4	0.079	0	0.039	0.056	87.25

Table B.11 Desorption data for naphthalene initial concentration of 130ppm in the absence of cadmium

Sample	*mg/L A*	*mg/L B*	*Average ppm*	*Standard Deviation.*	*Amount in Soil ppm*
1	28.92	28.25	28.58	0.47	4945.64
2	25.63	25.16	25.39	0.33	3675.82121
3	26.84	26.30	26.57	0.37	2346.93
4	24.27	20.73	22.50	2.50	1221.75

Table B.12 Desorption data for naphthalene initial concentration of 25ppm in the presence of 0.044mmol L^{-1} cadmium

Sample	*mg/L A*	*mg/L B*	*Average ppm*	*Standard Deviation.*	*Amount in Soil ppm*
1	21.97	20.52	21.25	1.02	257.16
2	2.70	2.23	2.47	0.32	133.62
3	0.13	0.057	0.098	0.057	128.69
4	0	0	0	0	128.69

Table B.13 Desorption data for naphthalene initial concentration of 130ppm in the presence of 0.044mmol L^{-1} cadmium

Sample	*mg/L A*	*mg/L B*	*Average ppm*	*Standard Deviation.*	*Amount in Soil ppm*
1	28.79	30.06	29.43	0.89	4903.49
2	25.89	24.81	25.35	0.76	3635.92
3	26.40	26.41	26.40	0.0071	2315.57
4	25.54	25.51	25.52	0.018	1039.18

Table B.14 Desorption data for naphthalene initial concentration of 25ppm in the presence of 2.12mmol L^{-1} cadmium

Sample	*mg/L A*	*mg/L B*	*Average ppm*	*Standard Deviation.*	*Amount in Soil ppm*
1	18.22	23.29	20.76	3.58	281.65
2	1.36	1.93	1.65	0.40	199.009
3	0.071	0.11	0.091	0.028	194.42
4	0.0084	0.0017	0.0051	0.0047	194.16

Table B.15 Desorption data for naphthalene initial concentration of 130ppm in the presence of 2.12mmol L^{-1} cadmium

Sample	*mg/L A*	*mg/L B*	*Average ppm*	*Standard Deviation.*	*Amount in Soil ppm*
1	28.25	28.63	28.44	0.26	4952.75
2	25.68	25.70	25.69	0.015	3667.85
3	25.67	25.96	25.81	0.20	2377.036
4	25.6	26.14	25.90	0.33	1081.94

Table B.16 Desorption data for 2-methyl naphthalene initial concentration of 25ppm in the absence of cadmium

Sample	*mg/L A*	*mg/L B*	*Average ppm*	*Standard Deviation.*	*Amount in Soil ppm*
1	19.89	15.74	17.82	2.93	383.82
2	9.10	4.610	6.85	3.17	128.5
3	1.28	0.75	1.01	0.37	109.67
4	0	0	0	0	0

Table B.17 Desorption data for 2-methyl naphthalene initial concentration of 130ppm in the absence of cadmium

Sample	*mg/L A*	*mg/L B*	*Average ppm*	*Standard Deviation.*	*Amount in Soil ppm*
1	32.28	25.88	29.08	4.52	5430.69
2	19.04	12.83	15.94	4.38	4633.69
3	17.35	18.29	17.82	0.66	3742.43
4	0	12.65	6.32	8.95	3426

Table B.18 Desorption data for 2-methyl naphthalene initial concentration of 25ppm in the presence of 0.044mmol L^{-1} cadmium

Sample	*mg/L A*	*mg/L B*	*Average ppm*	*Standard Deviation.*	*Amount in Soil ppm*
1	16.46	14.43	15.44	1.433	502.56
2	4.26	2.75	3.51	1.06	326.95
3	0.82	2.03	1.43	0.85	255.43
4	0.43	0	0.21	0.30	244.60

Table B.19 Desorption data for 2-methyl naphthalene initial concentration of 130ppm in the presence of 0.044mmol L^{-1} cadmium

Sample	*mg/L A*	*mg/L B*	*Average ppm*	*Standard Deviation.*	*Amount in Soil ppm*
1	23.68	24.84	24.26	0.82024387	5671.73
2	16.69	17.003	16.85	0.21643616	4829.21
3	17.94	18.07	18.01	0.095	3928.69
4	15.52	13.03	14.27	1.75	3214.82

Table b.20 Desorption data for 2-methyl naphthalene initial concentration of 25ppm in the presence of 2.12mmol L^{-1} cadmium

Sample	*mg/L A*	*mg/L B*	*Average ppm*	*Standard Deviation.*	*Amount in Soil ppm*
1	17.64	17.03	17.34	0.43	407.91
2	4.68	3.32	4.00	0.96	207.73
3	0.60	2.41	1.50	1.27	132.43
4	0	0.28	0.14	0.19	125.39

Table B.21 Desorption data for 2-methyl naphthalene initial concentration of 130ppm in the presence of 2.12mmol L^{-1} cadmium

Sample	*mg/L A*	*mg/L B*	*Average ppm*	*Standard Deviation.*	*Amount in Soil ppm*
1	23.50	25.72	24.61	1.56	5654.21
2	16.31	16.51	16.41	0.13	4833.56
3	14.34	16.90	15.62	1.80	4052.13
4	11.89	12.42	12.16	0.37	3443.95

Table B.22 Desorption data for 2-naphthol initial concentration of 25ppm in the absence of cadmium

Sample	*mg/L A*	*mg/L B*	*Average ppm*	*Standard Deviation.*	*Amount in Soil ppm*
1	24.83	25.63	25.23	0.566	44.97
2	0.83	0.72	0.77	0.082	11.57
3	0.03	0.07	0.05	0.027	10.79
4	0.11	0.34	0.22	0.15	7.83

Table B.23 Desorption data for 2-naphthol initial concentration of 130ppm in the absence of cadmium

Sample	*mg/L A*	*mg/L B*	*Average ppm*	*Standard Deviation.*	*Amount in Soil ppm*
1	126.27	126.07	126.17	0.14	576.30
2	5.65	5.62	5.63	0.024	294.42
3	0.38	0.30	0.34	0.063	277.18
4	0.19	0.11	0.15	0.057	269.40

Table B.24 Desorption data for 2-naphthol initial concentration of 25ppm in the presence of 0.044mmol L^{-1} cadmium

Sample	*mg/L A*	*mg/L B*	*Average ppm*	*Standard Deviation.*	*Amount in Soil ppm*
1	25.54	26.13	25.84	0.41	14.79
2	1.18	1.079	1.13	0.07	0
3	0.06	0.05	0.060	0.008	0
4	0	0	0	0	0

Table B.25 Desorption data for 2-naphthol initial concentration of 130ppm in the presence of 0.044mmol L^{-1} cadmium

Sample	*mg/L A*	*mg/L B*	*Average ppm*	*Standard Deviation.*	*Amount in Soil ppm*
1	127.003	126.12	126.56	0.62	556.87
2	5.16	6.13	5.65	0.68	274.3
3	0.24	0.29	0.27	0.036	260.79
4	0	0.0758	0.037	0.053	258.90

Table B.26 Desorption data for 2-naphthol initial concentration of 25ppm in the presence of 2.12mmol L^{-1} cadmium

Sample	*mg/L A*	*mg/L B*	*Average ppm*	*Standard Deviation.*	*Amount in Soil ppm*
1	24.92	25.71	25.31	0.55	40.89
2	0.97	1.09	1.037	0.082	5.80
3	0.062	0.058	0.060	0.002	4.24
4	0	0	0	0	4.24

Table B.27 Desorption data for 2-naphthol initial concentration of 130ppm in the presence of 2.12mmol L^{-1} cadmium

Sample	*mg/L A*	*mg/L B*	*Average ppm*	*Standard Deviation.*	*Amount in Soil ppm*
1	126.69	125.85	126.27	0.59	571.40
2	5.51	5.67	5.59	0.109	291.59
3	0.22	0.23	0.23	0.005	280.08
4	0.001	0.064	0.033	0.043	278.42

B.3. Adsorption Data for Cadmium in the absence of the Carbon Contaminants

Table B. 28 Adsorption data for cadmium in the absence of the carbon contaminants.

Initial Cd Conc mmol L^{-1}	*A*	*B*	*C*	*Average mmol L^{-1}*	*Standard Deviation*	*Cd in soil mmol kg^{-1}*
0.044	0.021	0.020	0.020	0.020	0.00	1.11
0.936	0.020	0.028	0.016	0.021	0.005	45.71
2.12	0.014	0.018	0.017	0.017	0.002	110.18
3.51	0.024	0.020	0.027	0.024	0.003	174.08

B.4. Desorption Data for Cadmium in the presence and absence of the Carbon Contaminants

Table B. 29 Desorption data for cadmium in the absence of the carbon contaminants (initial concentration 0.044 mmol L^{-1}).

Sample	*A*	*B*	*Total Average left mmol L^{-1}*	*Standard Deviation*	*Cd in soil mmol kg^{-1}*
1	0.022	0.022	0.022	0	1.14
2	0.012	0.012	0.012	0.00041	0.62
3	0.008	0.007	0.0076	0.00062	0.38
4	0.0057	0.004	0.0052	0.00073	0.26

Table B. 30 Desorption data for cadmium in the absence of the carbon contaminants (initial concentration 2.12 mmol L^{-1}).

Sample	*A*	*B*	*Total Average left mmol L^{-1}*	*Standard Deviation*	*Cd in soil mmol kg^{-1}*
1	0.016	0.016	0.016	0	110.2
2	0.017	0.012	0.015	0.0037	109.45
3	0.013	0.0093	0.011	0.0062	108.89
4	0.0088	0.0070	0.0079	0.0075	108.50

Table B.31 Desorption data for cadmium from an initial concentration of 0.044mmol L^{-1} in the presence of naphthalene (25ppm)

Sample	*A*	*B*	*Total Average left mmol L^{-1}*	*Standard Deviation*	*Cd in soil mmol kg^{-1}*
1	0.023	0.024	0.023	0.0003	1.19
2	0.015	0.015	0.015	0.0003	0.76
3	0.011	0.011	0.011	0.0002	0.56
4		0.0094	0.009	0.0003	0.46

Table B.32 Desorption data for cadmium from an initial concentration of 2.12mmol L^{-1} in the presence of naphthalene (25ppm)

Sample	*A*	*B*	*Total Average left mmol L^{-1}*	*Standard Deviation*	*Cd in soil mmol kg^{-1}*
1	0.017	0.017	0.017	0	110.16
2	0.009	0.009	0.0095	0.00035	109.68
3	0.0064	0.0069	0.0066	0.0007	109.35
4	0.0047	0.0058	0.0053	0.0014	109.09

Table B.33 Desorption data for cadmium from an initial concentration of 0.044mmol L^{-1} in the presence of naphthalene (130ppm)

Sample	*A*	*B*	*Total Average left mmol L^{-1}*	*Standard Deviation*	*Cd in soil mmol kg^{-1}*
1	0.023	0.023	0.023	0.0003	1.17
2	0.015	0.014	0.014	0.0006	0.740
3	0.011	0.010	0.010	0.0006	0.52
4	0.0087	0.007	0.008	0.0007	0.41

Table B.34 Desorption data for cadmium from an initial concentration of 2.12mmol L^{-1} in the presence of naphthalene (130ppm)

Sample	*A*	*B*	*Total Average left mmol L^{-1}*	*Standard Deviation*	*Cd in soil mmol kg^{-1}*
1	0.015	0.015	0.015	0.0002	110.27
2	0.008	0.008	0.0087	0.00010	109.83
3	0.006	0.006	0.0064	0.0002	109.51
4	0.0054	0.0054	0.0054	0.0002	109.23

Table B.35 Desorption data for cadmium from an initial concentration of 0.044mmol L^{-1} in the presence of 2-methyl naphthalene (25ppm)

Sample	*A*	*B*	*Total Average left mmol L^{-1}*	*Standard Deviation*	*Cd in soil mmol kg^{-1}*
1	0.024	0.024	0.024	0.0003	1.22
2	0.016	0.015	0.016	0.0004	0.81
3	0.012	0.012	0.012	0.0004	0.61
4	0.010	0.009	0.010	0.0005	0.51

Table B.36 Desorption data for cadmium from an initial concentration of 2.12mmol L^{-1} in the presence of 2-methyl naphthalene (25ppm)

Sample	*A*	*B*	*Total Average left mmol L^{-1}*	*Standard Deviation*	*Cd in soil mmol kg^{-1}*
1	0.026	0.014	0.020	0.0087	110.02
2	0.01	0.0094	0.009	0.00943	109.52
3	0.008	0.0069	0.0075	0.0102	109.14
4	0.0019	0.0020	0.0020	0.0101	109.04

Sample	*A*	*B*	*Total Average left mmol L^{-1}*	*Standard Deviation*	*Cd in soil mmol kg^{-1}*
1	0.023	0.020	0.0221	0.0022	1.106
2	0.016	0.010	0.013	0.0036	0.67
3	0.0116	0.0057	0.0087	0.00418	0.43
4	0.0093	0.0030	0.0062	0.0044	0.31

Table B.38 Desorption data for cadmium from an initial concentration of 2.12mmol L^{-1} in the presence of 2-methyl naphthalene (130ppm)

Sample	*A*	*B*	*Total Average left mmol L^{-1}*	*Standard Deviation*	*Cd in soil mmol kg^{-1}*
1	0.013	0.016	0.015	0.0018	110.28
2	0.011	0.0099	0.0104	0.0010	109.76
3	0.0073	0.0066	0.0069	0.0005	109.41
4	0.005	0.0053	0.0055	0.00025	109.13

Table B.39 Desorption data for cadmium from an initial concentration of 0.044mmol L^{-1} in the presence of 2-naphthol (25ppm)

Sample	*A*	*B*	*Total Average left mmol L^{-1}*	*Standard Deviation*	*Cd in soil mmol kg^{-1}*
1	0.024	0.024	0.024	0	1.22
2	0.016	0.016	0.016	0.0001	0.80
3	0.012	0.012	0.012	0.0001	0.60
4	0.010	0.010	0.010	0.0001	0.50

Table B.40 Desorption data for cadmium from an initial concentration of 2.12mmol L^{-1} in the presence of 2-naphthol (25ppm)

Sample	*A*	*B*	*Total Average left mmol L^{-1}*	*Standard Deviation*	*Cd in soil mmol kg^{-1}*
1	0.018	0.017	0.017	0.0007	110.15
2	0.0094	0.0087	0.009	0.0012	109.70
3	0.007	0.0063	0.0067	0.0017	109.36
4	0.0055	0.0045	0.0050	0.0024	109.11

Table B.41 Desorption data for cadmium from an initial concentration of 0.044mmol L^{-1} in the presence of 2-naphthol (130ppm)

Sample	*A*	*B*	*Total Average left mmol L^{-1}*	*Standard Deviation*	*Cd in soil mmol kg^{-1}*
1	0.025	0.021	0.023	0.0031	1.16
2	0.017	0.011	0.01455944	0.0042	0.72
3	0.013	0.0068	0.010	0.0047	0.51
4	0.011	0.0043	0.0079	0.0051	0.39

Table B.42 Desorption data for cadmium from an initial concentration of 2.12mmol L^{-1} in the presence of 2-naphthol (130ppm)

Sample	*A*	*B*	*Total Average left mmol L^{-1}*	*Standard Deviation*	*Cd in soil mmol kg^{-1}*
1	0.014	0.019	0.016	0.0032	110.19
2	0.0082	0.0089	0.0085	0.0037	109.77
3	0.0062	0.0064	0.0063	0.0039	109.45
4	0.0042	0.0050	0.0046	0.0045	109.22

Appendix C

Data used to construct the equilibrium determination curves for each PAH and cadmium in kaolinite.

Table C.1 Equilibrium data for cadmium in 2% kaolinite and MSM samples

Time (days)	*mmol L^{-1}*	*mmol L^{-1}*	*mmol kg^{-1}*	*mmol kg^{-1}*	*Average in Aqueous Fraction*	*Variation*	*Average in Soil Fraction*
24	0.015	0.018	1.39	1.20	0.017	0.0025	1.29
48	0.019	0.019	1.19	1.16	0.019	0.00045	1.18
72	0.019	0.019	1.18	1.17	0.019	0.0001	1.17

Table C.2 Equilibrium data for PAHs in 2% kaolinite and MSM samples

PAH	*Naphthalene*				*2-Methyl Naphthalene*				*2-Naphthol*			
Initial Concen-tration	*25ppm*		*130ppm*		*25ppm*		*130ppm*		*25ppm*		*130ppm*	
Time (days)	*mg L^{-1}*	*mg Kg^{-1}*	*mg L^{-1}*	*mg Kg^{-1}*	*mg L^{-1}*	*mg Kg^{-1}*	*mg L^{-1}*	*mg Kg^{-1}*	*mg L^{-1}*	*mg Kg^{-1}*	*mg L^{-1}*	*mg Kg^{-1}*
0	14.304	566.038	19.990	5411.08	8.619	819.01	15.848	6045.06	21.249	243.76	132.42	316.01
24	13.930	584.709	21.810	5320.06	11.104	694.76	19.945	5840.23	23.179	147.25	132.40	317.27
48	13.312	615.630	20.376	5391.80	11.745	662.73	20.715	5801.73	22.721	170.15	134.43	215.66
72	12.866	637.932	22.705	5275.32	11.821	658.90	20.013	5836.84	22.981	157.17	133.86	244.47

Appendix D

Optical density readings used to construct the biomass curves for *Pseudomonas putida*.

Table D.1 Optical density readings at 600_{nm} for growth of *P. putida* in a 1% glucose and mineral salts solution with cadmium.

[Cadmium] (mmol L^{-1})	*0*		*Ave.*	*0.044*		*Ave.*	*2.12*		*Ave.*	*4.23*		*Ave.*
Time (hours)	A	B		A	B		A	B		A	B	
0	0.053	0.056	0.054	0.048	0.045	0.047	0.085	0.086	0.086	0	0	0
6	0.137	0.148	0.143	0.093	0.09	0.092	0.093	0.118	0.105	0.004	0.017	0.01
16	0.327	0.349	0.338	0.262	0.25	0.256	0.203	0.218	0.211	0.066	0.118	0.92
24	0.456	0.465	0.461	0.38	0.389	0.385	0.264	0.285	0.275	0.092	0.159	0.126
40	0.642	0.578	0.61	0.53	0.534	0.532	0.37	0.404	0.387	0.212	0.248	0.23
48	0.687	0.588	0.638	0.595	0.596	0.596	0.386	0.412	0.399	0.236	0.264	0.25

Table D.2 Optical density readings at 600_{nm} for growth of *P. putida* at 25ppm of each PAH in mineral salts medium.

PAH	*Naphthalene*			*2-Methyl Naphthalene*			*2-Naphthol*		
Time (hours)	*A*	*B*	*Average*	*A*	*B*	*Average*	*A*	*B*	*Average*
0	0.027	0.028	0.027	0.052	0.029	0.035	0.023	0.032	0.028
6	0.06	0.05	0.055	0.077	0.05	0.065	0.031	0.042	0.037
16	0.074	0.078	0.077	0.116	0.075	0.097	0.056	0.077	0.067
24	0.079	0.084	0.082	0.119	0.117	0.118	0.063	0.086	0.077
40	0.081	0.087	0.086	0.121	0.120	0.121	0.077	0.091	0.087
48	0.08	0.100	0.094	0.125	0.126	0.126	0.068	0.102	0.096

Table D.3 Optical density readings at 600_{nm} for growth of *P. putida* at 130ppm of each PAH in mineral salts medium.

PAH	*Naphthalene*			*2-Methyl Naphthalene*			*2-Naphthol*		
Time (hours)	*A*	*B*	*Average*	*A*	*B*	*Average*	*A*	*B*	*Average*
0	0.056	0.059	0.058	0.04	0.07	0.045	0.024	0.03	0.027
6	0.078	0.082	0.08	0.07	0.092	0.071	0.024	0.021	0.023
16	0.15	0.16	0.155	0.077	0.117	0.086	0.027	0.027	0.028
24	0.191	0.196	0.194	0.092	0.135	0.102	0.027	0.028	0.033
40	0.181	0.191	0.186	0.159	0.195	0.167	0.033	0.032	0.033
48	0.184	0.188	0.186	0.177	0.21	0.184	0.035	0.031	0.033

Appendix E
Sample Calculation

The following presents the sample calculations to illustrate the potential speciation of cadmium in the mineral salts medium. It should be noted that these calculations are present results that are only qualitative in nature.

The following calculations use concentrations instead of activities which will introduce a margin of error.

Sample Calculations:

The following equations represent the complexation of cadmium with the most likely ligands present in mineral salts medium. The following equations were presented in the **Introduction** section.

$$Cd^{2+} + H_2O \rightleftharpoons CdOH^+ + H^+$$

$$Cd^{2+} + NO_3^- \rightleftharpoons CdNO_3^+$$

$$Cd^{2+} + 2H_2O \rightleftharpoons Cd(OH)_2 + 2H^+$$

$$Cd^{2+} + H_2PO_4^- \rightleftharpoons CdHPO_4 + H^+$$

The preceding equations are rearranged below to include the formation constants taken from Lindsay (1979).

$$\frac{[CdOH^+][H^+]}{[H_2O][Cd^{2+}]} = K\,(7.95 \times 10^{-11})$$

$$\frac{[CdNO_3^+]}{[Cd^{2+}][NO_3^-]} = K\,(2.04)$$

$$\frac{[Cd(OH)_2][H^+]}{[H_2O]^2[Cd^{2+}]} = K\,(5.01 \times 10^{-21})$$

$$\frac{[CdHPO_4]\,[H^+]}{[Cd^{2+}]\,[H2PO_4^-]} = K\,(1 \times 10^{-4})$$

The following parameters were used for calculating cadmium speciation:

Total cadmium concentration (Cd_T) = 0.044 mmol L^{-1}
Total phosphate concentration (HPO_4^{2-}) = 19.4 mmol L^{-1}
Nitrate concentration (NO_3^-) = 0.088 mmol L^{-1}
pH = 7 therefore, $H^+ = 1 \times 10^{-7}$
$[OH] = 1 \times 10^{-7}$
Keq = 1.8×10^{-16} mol L^{-1} at 25°C

After replacing the appropriate values into the above equations, the concentration of Cd^{2+} was calculated to equal 2.27×10^{-6} M using Cd_T = 0.00004M). By substituting Cd^{2+} back into the above equations, the following concentrations for the cadmium complexes is calculated:

$[CdOH^+] = 5.8 \times 10^{-3}$ $[Cd^{2+}] = 1.3 \times 10^{-8}$

$[CdNO_3^+] = 2.24 \times 10^{-4}$ $[Cd^{2+}] = 5.085 \times 10^{-10}$ M

$[Cd(OH)_2] = 2.67 \times 10\ 10^{-5}$ $[Cd^{2+}] = 2.67 \times 10^{-5}$ M

$[CdHPO_4] = 19.4$ $[Cd^{2+}] = 4.4 \times 10^{-5}$ M

Theoretically, the amount of cadmium removed from solution includes,

$[Cd^{2+}] + [CdOH^+] + [CdNO_3^+] + [Cd(OH)_2] + [CdHPO_4]$

which gives a total concentration of 0.000042M (0.042 mmol L^{-1}). The amount calculated is almost virtually the same concentration of the total amount of cadmium added which was 0.044 mmol L^{-1}.