3. LITERATURE REVIEW

A. Chemical Characteristics of Monitored PAHs

Creosote contains a substantial amount of PAHs, about 85% by weight (Table 2). Understanding the chemical characteristics of PAHs and the associated implications for bioremediation is very important (Table 3). In general, as PAH molecular weight increases, aqueous solubility decreases. Typically, mass transfer of contaminant from solid to liquid phase is required for removal of soil contamination. Many PAHs are considered to be biodegradable, but the rate of PAH biodegradation appears to be limited by dissolution rates, which are directly correlated to aqueous solubility (Brubaker *et al.*, 1992).

Log K_{OW} and log K_{OC} are both equilibrium constants that provide an indication of constituent sorption onto soil or organic matter, respectively. K_{OW} is defined as the concentration of a constituent in octanol (soil) divided by the aqueous concentration of the constituent. The K_{OW} of a constituent is a measure of hydrophobicity. K_{OC} has been defined as K_D divided by f_{OC}. The Freundlich isotherm constant, K_D, is the mass of contaminant adsorbed per unit mass of media divided by the contaminant concentration in solution. F_{OC} is the fraction of organic carbon in a soil sample. In general, as PAH molecular weight increases, log K_{OW} and K_{OC} also increase. Larger values of both constants indicate preference of the constituent to be sorbed onto soil or organic matter than be present in the aqueous phase. The higher the affinity of a constituent to sorb to soil, the less likely it will be available for bioremediation.

Table 2: Predominant Polycyclic Aromatic Hydrocarbon (PAH) Constituents in Creosote

PAH Constituent	Percent by weight of Total PAHs
Naphthalene	13
Anthracene	13
2-Methylnaphthalene	13
Phenanthrene	13
Biphenyl	8
Fluorene	8
1-Methylnaphthalene	8
2,3-Dimethylnaphthalene	4
2,6-Dimethylnaphthalene	4
Acenaphthene	4
Fluoranthene	4
Chrysene	2
Pyrene	2
Anthraquinone	1
2-Methylanthracene	1
2,3-Benzo(b) fluorene	1
Benzo(a)pyrene	1
Total % of PAHs in creosote	**100**

Source: Mueller *et al.* (1989)

Table 3: Chemical Characteristics of the Ten Monitored PAHs

PAH	Chem. Formula	Molecular Weight	Water Solubility, mg/L	Log K_{ow}	Log K_{oc}
Naphthalene	$C_{10}H_8$	128	31.69	3.37	2.97
Acenaphthylene	$C_{12}H_8$	152	3.93	4.07	1.40
Acenaphthene	$C_{12}H_{10}$	154.21	3.93	3.98	3.66
Fluorene	$C_{13}H_{10}$	166.2	1.68-1.98	4.18	3.86
Phenanthrene	$C_{14}H_{10}$	178.2	1-1.6	4.45	4.15
Anthracene	$C_{14}H_{10}$	178.2	0.0446	4.45	4.15
Fluoranthene	$C_{16}H_{10}$	202.26	0.206	4.90	4.58
Pyrene	$C_{16}H_{10}$	202.3	0.129-0.165	4.88	4.58
Chrysene	$C_{18}H_{12}$	228.3	0.0015-0.0022	5.61	5.30
Benzo(b) fluoranthene	$C_{20}H_{12}$	252.3	0.0012	6.04	5.74

Sources: Futoma *et al.* (1981); Tiehm *et al.* (1997);Verschueren (1983); and ATSDRb (1993).

Two PAHs, such as naphthalene and benzo(b)fluoranthene, can possess very different chemical properties and behave quite differently in air/water/soil systems. Naphthalene is the most soluble of the monitored PAHs. Naphthalene also has the highest vapor pressure of the 10 PAHs and a characteristic mothball smell. Naphthalene does not adhere strongly to soils or sediments and can pass through sandy soils with relative ease and contaminate ground water supplies (ATSDRb, 1993). Conversely, benzo(b)fluoranthene has the lowest solubility of the monitored PAHs. Benzo(b)fluoranthene is a non-volatile PAH that adheres very strongly to soil and organic matter. Contrasting the chemically-related parameters of a LMW and HMW PAH demonstrates the difficulty associated with the remediation of complex mixtures of PAHs, such as creosote.

B. Remediation of Creosote/PAHs in Soil Systems

The loss of PAHs in soil can occur by many mechanisms. Biodegradation, sorption, and desorption/solubilization are the most common mechanisms. Soil bioremediation is dependent upon contaminant desorption from the solid to the liquid phase. Aqueous phase contaminants are considered to be bioavailable and subject to remediation. *Ex situ* aerobic slurry bioreactors provide the best overall PAH removal (Wilson *et al.*, 1993; Ellis *et al.*, 1991). Bioremediation can be severely limited due to a lack of bioavailable compounds, insufficient oxygen or nutrients, and nonideal moisture levels. Under denitrifying conditions, anaerobic degradation of some PAHs occurred in

the presence of excess nitrate (Milhelcic *et al*., 1988b). In studies performed by various researchers, PAHs persisted under most other anaerobic conditions with varying microorganisms and electron acceptors. The bioavailability of creosote-associated PAHs must be considered and is dependent on many factors. The most important factor is the contact time experienced between a PAH and soil. "Bioavailability is a function of numerous factors intrinsic to the biological system as well as environmental conditions. It is, at first, determined by the way in which microorganisms transport substrates across the cell membranes. In general, only dissolved molecules can be transported across the cell membranes" (Zhang *et al*., 1998). The organic matter content and composition of a soil also contribute to the bioavailability of many PAHs. Cometabolism of HMW PAHs in the presence of LMW PAHs holds promise for enhanced biodegradation of HMW PAHs. If the concentration of bioavailable LMW PAHs becomes limiting, the biodegradation of HMW PAHs could be inhibited.

i. Aerobic Bioremediation

Aerobic biodegradation is the most efficient form of PAH bioremediation. Complete contaminant degradation results in the formation of CO_2 and water. Loehr (1992) listed the following factors that may prevent microbial degradation and bioremediation: 1) chemical concentrations that may be toxic to microorganisms; 2) conditions that are too acidic or alkaline; 3) lack of essential nutrients such as nitrogen, phosphorous, potassium, sulfur, and/or trace elements; 4) unfavorable moisture conditions (too wet or too dry); and 5) lack of oxygen or other electron acceptors. Typically, a lack of nutrients and/or oxygen

would be a rate limiting parameter at the beginning of an *in situ* PAH bioremediation project. As time progresses, the total mass of readily degradable constituents is decreased and thus the oxygen requirements for active bioremediation are also decreased (Brubaker *et al*., 1992). Over time, dissolved oxygen supplied by natural ground water advection will be an adequate source of oxygen for continued *in situ* bioremediation (Brubaker *et al*., 1992). However, the rate of PAH constituent desorption from the soil matrix/organic matter can quickly become the factor limiting biodegradation. Brubaker *et al*. (1992) concluded that PAH desorption rates and the extent of PAH bioavailability are site specific and not readily predictable. In many instances, small populations of microorganisms capable of degrading PAHs are present, but simply cannot access the PAH to oxidize it for an energy source. Greater amounts of bioavailable carbon may be required to achieve a microbial population capable of PAH degradation.

Ellis *et al*. (1991) examined the bioremediation of PAHs associated with creosote production at an inactive production facility in Sweden. The performance of an off-site treatment bed was evaluated and discussed. In addition, several laboratory experiments were performed. Thirty-two trial pits were dug around the site. Eighty samples were collected and analyzed. Total creosote concentration was comprised of naphthalene, 1-methylnaphthalene, 2-methylnaphthalene, acenaphthylene, dibenzofuran, fluorene, phenanthrene, fluoranthene, pyrene, anthracene, and chrysene. Total creosote concentration ranged from less than 10 to greater than 32000 mg/kg.

Soil total organic carbon (TOC) ranged between 0.81 to 6.25% for five samples. Contamination was present in two main areas of the site. At a depth of 0.9 to 4.5 meters in these areas, creosote contamination was evident and appeared to be associated with wood chips and/or sawdust in the fill.

In a lab experiment, soil from the contaminated site was used as an inoculum source in a series of microbial culture experiments. Mineral salts and carbon sources, Standard and soil extracted creosote, were added to each culture vessel. Batch culture vessels were incubated at a range of temperatures (10 to 25 C) with shaking (180 rpm) in the dark. *Pseudomonas cepacia* reduced most of the major components of creosote. In comparison to the control, total creosote reductions in Standard and soil-extracted creosote were 21 and 42% over a 35-day period. Chrysene reduction in Standard creosote was low, approximately 20%.

In a single substrate aerobic culture experiment with an initial concentration of 200 mg/L and three types of inocula, *Pseudomonas cepacea*, *Pseudomonas fluorescens*, and *Pseudomonas putida*, the degradation of 10 PAHs was assessed. The most successful bacteria was *Pseudomonas cepacia* which reduced concentrations of all substrates, except chrysene and pyrene. Since pyrene and chrysene were degraded in a mixed substrate environment containing LMW PAHs, the authors suggest that cometabolic activity is an important degradation mechanism for pyrene and chrysene.

At an initial total creosote concentration of 2300 mg/kg, a soil leaching test was performed in glass columns. The columns were subject to leaching with 6 L of water under constant hydrostatic head. Only approximately 9% of

the total soil creosote could be leached with water. In soil pot studies, a 1 Liter glass container was filled with 500 grams of contaminated site soil that had been homogenized by sieving and partially air-dried. A range of soil amendments was evaluated. Soil moisture was maintained at 20 to 30% (w/w) and the soil was mixed every 2 days. Treatments were replicated 5 times. The sealed pots were incubated at several temperatures between 4 and 25 C in the dark. After 9 weeks at 10 C, a 30.3% creosote reduction was achieved in the control, which was moistened and mixed. At 25 C and over a 28-day period, a 15% reduction occurred in the control system, in comparison to a 59% reduction when inorganic nutrients were supplied. Degradation rates were not improved by the addition of creosote-degrading microorganisms. After 10 weeks of 15 C incubation, the control system experienced a 23% reduction in total creosote and the alternative and most successful treatment, organic nutrients + inorganic nutrients + microbial supplements, yielded a 71% reduction. However, chrysene concentrations were not reduced in any of the trials. In fact, reported concentrations were higher than initial concentrations. Pyrene concentrations followed the same trend in the control, but approximately a 50% reduction was achieved with the alternative treatment. Fluoranthene concentrations were only slightly reduced in both treatments. Fluorene and phenanthrene were modestly degraded in the control treatment and greatly (>50%) reduced in the alternative treatment.

Excavated site soil was screened and placed into an off-site treatment bed to a depth of 0.75 meters. Bed area was 80 by 60 meters. The soil was underlain by a 10 cm gravel layer. Bed leachate was collected in a sump and

periodically sprayed back onto the top of the soil. Treatment involved moistening the soil to 20 % (w/w) and rotovation using a tractor-pulled spading machine twice weekly. Inorganic nutrients and microorganisms in solution were added to the bed from a tractor-mounted spraying boom. After 52 and 88 days of treatment, a 52% and 68% reduction of total creosote was achieved. Chrysene concentrations at time = 0, 52, 88 days were: 130.3, 89.5, and 65.7 mg/kg. Pyrene concentrations at those time intervals was: 177, 109.1, and 82.9 mg/kg. Respective fluoranthene concentrations were: 202.2, 121.6, and 85.9 mg/kg. Phenanthrene and fluorene concentrations at the time intervals were: 196.7, 78.9, 50.0 and 72.1, 17.9, and 6.9 mg/kg, respectively.

Elmendorf *et al*. (1994) used a consortia of Prince William Sound microorganisms to degrade crude oil. Flasks containing indigenous microorganisms, nutrients, and crude oil at 1% loading were incubated and continuously shaken for 168 days. Flasks were prepared in triplicate and sampled every 14 days. Over a 6-month period, they determined that unsubstituted forms of PAHs were degraded more readily than substituted forms. Unsubstituted PAHs disappeared in the following order: naphthlenes, fluorenes, phenanthrenes, and lastly chrysenes. Unsubstituted napthalene, fluorene, and phenanthrene were completely degraded at the end of the experiment. However, chrysene biodegradation was limited to approximately 18% depletion. Bioremediation of unsubstituted and substituted chrysene members was marginal. “Chrysene is one of those hydrocarbons that is generally considered to be nonbiodegradable.” Biodegradation of chrysene did occur, but not at rates comparable to that of the LMW PAHs.

Mueller *et al*. (1991) studied the feasibility of landfarming creosote-contaminated materials in a lab study using specially designed “landfarming chambers.” Sediment and surface soil from the American Creosote Works Superfund Site was used in the experiment. Creosote wood treating facilities were operational from 1902 until 1950. A mixture of creosote, pentachlorophenol (PCP), and copper-chromium-arsenic (CCA) were used to treat wood from the 1950’s until closure of the plant in 1981. Saturated air was passed through the chambers containing 3 kg of surface soil (SS) or sediment (SD). Two treatments were tested, unamended and amended with inorganic nutrients. Soil moisture was adjusted accordingly and ranged between 8 and 15 %. Solids were tilled on a weekly basis. Subsamples from the SS and SD chambers were collected initially and subsequent to tilling following 1, 2, 4, 8, and 12 weeks of chamber incubation at 23 C. In the SS chambers, losses due to leaching or sorption were not observed. At the end of 12 weeks, unamended and amended SS chambers exhibited substantial decreases in the LMW PAHs and modest decreases in most HMW PAHs. In general, the extent of biodegradation was greater in the amended chambers. After 12 weeks in the SD chambers, the extent of LMW PAH biodegradation was basically equivalent in the amended and unamended treatments. However, nutrient supplementation appeared to augment HMW PAH removal in the SD chamber. The authors suggested that nutrient amendment of creosote-contaminated surface soils would augment bioremediation of certain creosote constituents. However, the results also indicated that removal of HMW PAHs and other recalcitrant constituents was not sufficient.

ii. Anaerobic Bioremediation

Several researchers have studied the effects of PAH reduction under anaerobic conditions. Milhelcic *et al*. (1988b) found that PAHs persisted under sulfate-reducing conditions. Aqueous phase concentrations of naphthalene and acenaphthene were stable over a 50 and 70 day test duration, respectively. The authors reminded readers that denitrification is a more favorable process than oxidation of organic solutes via sulfate reduction or methane fermentation at a neutral pH.

Coates *et al*. (1996) demonstrated that sulfate reduction was necessary for PAH mineralization in heavily-contaminated (33 mg/kg) sediment. Sediment samples were obtained from a naval station site. Samples were placed in an air-tight bottle and spiked with radiolabeled naphthalene and phenanthrene. The bottles were agitated on a vortex mixer and incubated at 25 C in the dark. Based on $^{14}CO_2$ production, naphthalene and phenanthrene were oxidized without a lag in the sediment solution. The authors felt that many previous studies failed to detect PAH degradation under sulfate-reducing conditions, because the environmental media had not been subject to long-term PAH exposure. They suggest that long-term PAH exposure may be necessary for establishment of a significant PAH-degrading community under sulfate-reducing conditions.

Mihelcic *et al*. (1988a) investigated microbial degradation of acenaphthene and naphthalene under denitrification conditions in soil-water systems. Soils containing microorganisms acclimated and unacclimated to naphthalene were evaluated. When nitrate was supplied in excess, acenaphthene and naphthalene were degraded microbially under denitrifying conditions to nondetectable

levels. As suspected, an acclimation phase was observed in the unacclimated soil, whereas immediate degradation occurred in the acclimated soil. It was suggested that the acclimation phase allowed an initially small population of PAH-degrading microorganisms to increase to levels sufficient for utilization of acenaphthene and naphthalene.

The effects of organic carbon on PAH biodegradation were also examined in the study. Denitrifying microorganisms will utilize the simplest form of available carbon for energy. Soil organic carbon available for mineralization and PAHs in the aqueous phase compete for utilization by the microbial population. When electron acceptors (i.e. nitrate) are in excess, the microorganisms can utilize both forms of carbon without inhibiting PAH biodegradation. However, when the electron acceptor was limiting, preferred mineralization of bioavailable soil organic matter was witnessed. Thus, the PAH compounds were not degraded. The authors suggested that the availability of nitrate and other mineralizable carbon sources are two significant factors that need to be evaluated.

Methanogenic bacteria have been found in the anaerobic zones of creosote-contaminated aquifers (Godsy *et al.*, 1992, Ehrlich *et al.*, 1983). Known methanogens can only use a restricted number of substrates, so biodegradation of creosote to simpler forms of carbon is occurring prior to methanogen uptake (Ehrlich *et al.*, 1983). Ehrlich *et al.* (1983) concluded that application of in situ methanogenic fermentation for removal of creosote appears limited. The carbon structure of PAHs is too complex for direct biodegradation of creosote compounds by methanogens.

iii. Cometabolism of PAHs

It has been hypothesized that presence of LMW PAHs augments biological removal of HMW PAHs. Pollard *et al*. (1994) state, " For the high molecular weight PAH (> 4 rings), co-oxidation may be a major degradation mechanism." In a laboratory study, Juhasz *et al*. (1996) witnessed improved aerobic degradation of five- and seven-ring PAHs by *Pseudomonas Cepacia* when LMW PAHs were present. They suggested that the presence of LMW PAHs resulted in an increase in microbial activity, thus enhancing biodegradation of HMW PAHs.

Keck *et al*. (1989) witnessed a more rapid disappearance of 4- and 5-ring PAHs in the presence of complex wastes containing 3-ring PAHs. The authors suggested that a consortia of microbes are likely to exist in contaminated soil systems that allow degradation of otherwise recalcitrant compounds, like HMW PAHs. A decrease in half-lives of the recalcitrant PAHs were attributed to an overall increase in microbial activity due to addition of carbon-containing biodegradable forms of oil and grease.

Schwab *et al*. (1995) conducted a laboratory experiment with pyrene and phenanthrene in the presence of rhizosphere soils and associated organic acids. In the presence of common rhizosphere organic acids (succinic and formic acids), phenanthrene addition to soil apparently induced cometabolism of pyrene. The addition of organic acids allowed further growth of microbial populations which increased mineralization of pyrene.

The presence of LMW PAHs is likely to aid in the biological removal of HMW PAHs. The results of Schwab *et al*. (1995) suggest that relatively simple forms of carbon,

i.e. LMW linear alkanes, could also have a positive effect on the bioremediation of HMW PAHs. All forms of bioavailable carbon simpler than the PAH of interest could provide a beneficial effect to site bioremediation.

iv. PAH Bioavailability

There are three major steps involved in the biodegradation process. Contaminant desorption from the soil matrix to the aqueous phase, mass transfer to biologically accessible regions, and biological uptake and subsequent transformation (Zhang *et al*., 1998). Contaminant sorption and desorption from soil has been reported to follow a bi-phasic pattern, a rapid phase followed by a slower phase (Loehr *et al*., 1996). The mass transfer limitations associated with the release of contaminants limit the biodegradation of PAHs (Luthy *et al*., 1994). It has been suggested by many researchers that contaminants experience an “aging” phenomena over time, which involves irreversible binding of contaminant to the soil matrix resulting in contaminant unavailability to microorganisms (Pollard *et al*., 1994; Hatzinger *et al*., 1995; Weissenfels *et al*., 1992).

Many researchers have consistently suggested that the longer PAHs or other hydrophobic chemicals reside in soil, the less bioavailable they become to microorganisms. In this instance, a larger fraction of the contaminant is unable to desorb from the soil or organic carbon within the soil matrix. Ramaswami *et al*. (1994) determined that “aged” coal tar had a lower mass transfer rate of naphthalene to the aqueous phase than “fresh” coal tar. The presence of organic carbon in the soil matrix alone can severely reduce desorption rates and bioavailability.

Loehr *et al*. (1996) suggested that “the effect of contact time on sorption and desorption may be more pronounced in soils with high organic carbon contents.” Surfactant addition to aged site soils has been suggested to induce further solubilization of heavily sorbed PAHs. In reality, surfactant addition is unlikely to aid in enhancing biodegradation, because the contaminants have actually sorbed into solid materials (Zhang *et al*., 1998).

A laboratory column experiment using highly contaminated creosote soil (Σ 16 PAHs=6,300 mg/kg) from a former creosote work site was performed to determine the effectiveness of in situ remediation techniques (Breedvald *et al*., 1994). Three glass columns were packed with soil. Moisture contents, oxygen flow rates, and inorganic nutrient additions were varied in each column. The following treatments were administered in column A, B, C, and D, respectively: water circulation with air and nutrient addition to the water phase; forced air aeration; forced air aeration and addition of a nutrient solution, and control. After 170 days, soil extractions of the column soil revealed mean reductions of the total PAHs (16 PAHs or TPAH) of 14, 67, 38, and 34% in the four columns in alphabetical order. Microbial enumeration revealed an increase in the creosote-degrading population in column A. TPAH reduction occurred primarily in the 2-, 3-, and 4-ring PAHs, while the 5- and 6-ring PAHs exhibited minimal reductions. The soil contained a large population of microorganisms capable of degrading creosote, yet environmental conditions limited PAH degradation in the soil. Chemical analyses revealed that the creosote-related pollutants had been in the soil for many decades without

being degraded. Relatively high concentrations of 2- and 3-ring PAHs were found in the soil. There was obviously a high residual PAH content that was biologically unavailable. The authors suggest that a high organic carbon content, 4.17 %, in the soil is contributing to the limited availability of PAH compounds to indigenous microorganisms.

Cornelissen *et al*. (1998) studied the desorption kinetics of 15 PAHs from sediments before and after bioremediation in a bioreactor and landfarm. PAH contaminated sediments were collected from 2 spots in Petrol Harbor, Amsterdam, The Netherlands, designated PH-A and PH-B. A bioreactor with 4 and 30 cubic meters internal volume contained soil from PH-A and PH-B, respectively. Both bioreactors were continually stirred, aerated, and kept at 20 C. Bacteria and substrates were not added to either reactor. Cessation of detectable degradation and, thus, a phase of resistant biodegradation was reached in 4 months. Bioreactor remediation was terminated.

Sediment from Wemeldinge, The Netherlands, was treated for 2 years in a landfarm remediation system. After dredging, sediment was spread over the land in a 25-cm layer and plowed monthly. In the last 9 months of landfarming, the initial, rapid phase of biodegradation had ceased. Initial concentrations of organic carbon in PH-A, PH-B, and the land-applied soil were 5.4, 8.2, and 2.3 percent, respectively. All PAHs in the PH-A reactor were significantly degraded, including chrysene. In the PH-B reactor, acenaphthene, fluorene, phenanthrene, and fluoranthene were all significantly degraded, while pyrene and chrysene were not. In the landfarming experiment and in order of increasing hydrophobicity, all PAHs up to

benzo(b)fluoranthene were degraded. Chrysene and pyrene concentrations were reduced by approximately 33 and 43 percent.

PAH desorption kinetics were determined at 20 C using a solid-phase Tenax TA extraction method. For the three sediments, average rate constants for rapid and slow desorption were calculated for degraded and nondegraded PAHs. In general, the rapid desorption rate constants were 100 to 3000 times larger that the slow desorption rate constants. Rapidly desorbing PAHs (mainly two- to four-ring compounds) appear to be degraded preferentially, because the slowly desorbing fraction is unavailable for microbial degradation. However, rapid desorption of five- and six-ring PAHs occurred before and after bioremediation. Therefore, persistence of these HMW PAHs may not be due to a lack of bioavailability. The authors suggest that PAH degradation can be roughly predicted by the fraction that rapidly desorbs. This suggestion assumes that creosote-degrading microorganisms are present and able to degrade these constituents. It is likely that microbial factors limited biodegradation during the initial, rapid phase desorption of PAHs. Conversely, it is probable that the reverse occurred during the slow desorption phase.

Weissenfels *et al.* (1992) investigated PAH biodegradation of contaminated soils from two different industrial sites. Soil A was obtained from a former wood impregnation plant with contamination levels of 1.8 g PAHs per kg soil. Soil A was a predominantly sandy soil. Soil B was a heterogeneous soil from a former tar oil refinery with contamination of about 1 g PAHs per kg soil. Soil A contained more LMW PAHs than soil B. In comparison to soil A, soil B contained a greater proportion of HMW PAHs with

respect to LMW PAHs. Two hundred grams of soil was placed in a percolator and intermixed with rashig rings. The column was trickled with mineral salt medium at 4 circulations per minute at room temperature.

After 8 weeks, overall PAH concentrations had decreased 62% in soil A with the indigenous microbial population. Soil B did not experience significant degradation of PAHs with molecular weights larger than fluorene. After innoculation of a bacterial culture previously demonstrated to degrade PAHs, significant soil PAH degradation was still not witnessed. Microbial enumeration of both soils indicated that a larger population of aromatic-degrading bacteria was present in soil A. The bacteria population and carbon dioxide production in soil B were low. Carbon dioxide production rates for soil A and B were 21.7 and 2.6 mg CO_2 per kg soil $\times$ hour, respectively.

After a soxhlet extraction of both soils, both soils were contaminated with the organic compounds of soil B. Subsequent to incubation, average PAH microbial degradation in soil A was 80 %. Biodegradation of soil B was not complete and resulted in an undegradable, residual PAH fraction of 28 %. The authors suggested that inhibition of PAH biodegradation was due to some sort of binding within the matrix of soil B.

Soil organic carbon levels (w/w) in soil A and B were 1 and 13.6 %, respectively. Accordingly, the effect of time on PAH soil sorption was investigated. As exposure time of anthracene oil on soil B increased, the degree of PAH extractability decreased continuously. There was an initial fast sorption phase followed by a much slower

sorption phase of the oil to the soil matrix. A biotoxicity test indicated that as soil organic carbon levels increased, the toxicity of soil elutes decreased. The authors suggested that the constituents responsible for toxic effects were bound to the organic carbon. This study suggests that PAH binding was a function of contaminant age and soil organic matter content.

Erickson *et al*. (1993) studied the bioremediation of PAHs at a former MGP site under varying microcosm conditions. The PAHs were present in the soil for at least 30 to 40 years. The initial concentrations of 3- and 4-ring PAHs ranged from 1 to 47 mg/kg. The microcosms were incubated at 20 C and monitored for PAH constituents as a function of incubation time. Microcosm studies investigated the extent of PAH biodegradation by varying temperature, nutrient amendments, organic amendments, and microbial inoculum. Combinations of these parameters were also evaluated. The authors concluded that PAHs in the MGP soil were not degraded even under the most ideal conditions. PAHs in the water soluble fraction of the soil were non-detectable at a detection limit of 5 µg/L. Microtox© analyses indicated that this water soluble fraction was non-toxic. Contaminant aging has deemed the PAH constituents unavailable for biological removal and unlikely to be lost through leaching.

Yeom *et al*. (1996) studied the dissolution of PAHs from a weathered coal tar contaminated soil. Tenax®-TA polymeric beads were used to assess the mass transfer of PAHs. The release of PAHs, such as naphthalene, fluorene, phenanthrene and pyrene, from the weathered soil was a very slow, nonequilibrium process. Diffusional mass transfer

within the soil-contaminant matrix and, more specifically, diffusivities of PAHs within the soil/tar matrix, were determined to be the factor limiting PAH desorption. In a lab study, large doses (5.5 g/L) of a surfactant, Brij 30, substantially increased the rate of PAH dissolution. The authors suggested that swelling of the weathered tar matrix by infiltrating surfactant molecules improved diffusivities of the PAH components and did not necessarily increase the aqueous solubility of these components.

C. Phytoremediation

Phytoremediation, or green plant-based remediation, is an innovative treatment strategy currently being implemented at sites to further understanding of contaminant removal mechanisms and determine specific compounds amenable to removal. A search for inexpensive and effective soil bioremediation methods has fueled research interests in phytoremediation. Plants have a proven ability to accumulate metals, in particular nickel, zinc, and cadmium, from the aqueous phase and store them in plant material for eventual removal via harvesting (Matso, 1995). Phytoextraction, accumulation of contaminants within a plant structure, should be distinguished from phytoremediation, which entails conversion of pollutants to nontoxic materials (Cunningham *et al*., 1995). Plants have been proven to aid in removal of herbicides, TNT and other explosives (Davis *et al*., 1998; Schnoor *et al*., 1995). "Phytoremediation is most effective at sites with shallow contaminated soils" (Schnoor *et al*., 1995).

Just as with other forms of bioremediation, Contaminant bioavailability is a critical factor. Bioavailability depends on contaminant related factors, such as lipophilicity and age, and soil characteristics, such as pH, clay and organic matter content (Cunningham *et al*., 1995). Cunningham *et al*. (1995) state that, "Contaminants that are tightly adsorbed to soil particles and resist uptake by microbes or plants make poor targets for phytodegradation." In addition, Schnoor *et al*. (1995) state that chemical desorption of contaminants from the soil, when compared to the relatively fast degradation rate of organics in conjunction with plant enzymes, may be rate-limiting.

Following plant death, plant enzymes are released into the soil and may positively influence the phytoremediation process (Cunningham *et al.*, 1995). Soeder *et al.* (1996) studied the influence of two plant-produced surfactants, quillaya saponin (QS) and soya lechithin (SL), on biodegradation. As expected, both surfactants increased the solubility of phenanthrene and fluoranthene. The authors concluded that despite enhanced solublization of the 2 PAHs via QS, bioavailability was not improved for either compound. With the SL surfactant, enhanced growth of three bacteria strains did not increase the rate PAH elimination. In situ flushing of soil with surfactant solutions to enhance solubilization of PAHs has been implemented in many bench and pilot studies (Luthy *et al.*, 1994).

i. Contaminant Removal Mechanisms

Plants can augment contaminant removal in soils by two different routes, indirect and direct. Indirect phytoremediation involves the rhizosphere, a biologically-active soil region adjacent to plant roots. The rhizosphere is an area of increased microbial activity due to the presence of root exudates and decayed plant and root-associated material (Banks *et al.*, 1997). The surface area of the root structure is a key factor that allows augmentation of a microbial community. Schwab *et al.* (1995) suggested that highly branched, fine root systems with higher total rhizoplane area appear to enhance biodegradation more than taproot systems. Plant-microbe interaction in the rhizosphere can increase concentrations of biomass by an order of magnitude or more (Anderson *et al.*, 1993). In a greenhouse experiment with alfalfa,

Lee *et al*. (1993) experienced substantially higher microbial counts in soils with plants than soils without plants. "Plants sustain large microbial populations in the rhizosphere by secreting substances such as carbohydrates and amino acids through root cells and by sloughing root epidermal cells" (Anderson *et al*., 1993). Indirect plant contributions to microbial communities via recycling of plant-derived materials, including humic and fulvic acids, and decomposition of dead roots, should be considered an important part of the phytoremediation process (Davis *et al*., 1998). Plants can deposit carbon and nitrogen to the rhizosphere that can be used by microbes. Production of subsequent byproducts can then be utilized by varying types of microbes (Davis *et al*., 1998). "Rhizosphere soil supports a higher population of biomass due to increased availability of nutrients in the form of root exudates" (Davis *et al*., 1998).

Direct phytoremediation utilizes internal plant metabolic pathways to degrade contaminants (Schnoor *et al*., 1995). Organic contaminants are taken up by the plant roots, metabolized and then translocated to the shoots (Cunningham *et al*., 1996). Plants obtain soil contaminants most readily from the liquid phase (Cunningham *et al*., 1996). In the case of PAH uptake, vapor and soil phase uptake by plants is a virtually negligible pathway. Schnoor *et al*. (1995) suggest that contaminants with a log K_{OW} ranging from 0.5 to 3 are the most amenable for direct uptake and subsequent contaminant mineralization. Lipophilicity is an extremely important chemical property that determines ease of movement across plant membranes, thus allowing contaminant access into and within a plant (Cunningham *et al*., 1996). Large K_{OW} values, i.e. most

PAHs, are associated with compounds that have low water solubility and high lipophilicity. Once contaminants have been taken up by the plant, relatively lipophilic contaminants are attacked by enzymes to produce more water-soluble compounds (Cunningham *et al.*, 1996). As a final step, transport of metabolized products is incorporated into various plant cell components. "Most PAHs cannot move appreciably into plants from the soil, and remediation depends almost totally on rhizosphere microbes" (Davis *et al.*, 1998).

ii. Grass Phytoremediation Case Studies

Banks *et al.* (1997) evaluated the ability of three types of grasses to remediate a petroleum contaminated soil. Initial total petroleum hydrocarbon (TPH) soil concentrations ranged from 1700 to 3600 mg/kg. Soil composition was 60% sand, 21% silt, and 19% clay. The organic carbon content was 22 mg/kg. The field study area contained 24 plots. Bermuda, fescue, and white clover were each planted in 6 of the plots. The six remaining plots served as unamended, unvegetated controls. After nine months of plant growth, a 2.5 cm diameter soil core was taken to a depth of 15 cm from each plot. It was concluded that the rate of TPH degradation was significantly greater in the vegetated plots versus the control plots. After nine months of growth, the clover, fescue, bermuda, and control plots experienced the following respective percent reductions of TPH: 24, 27, 23 and 12 percent. After one year of growth (Banks *et al.*, unpublished), the following percent reductions of TPH were witnessed in clover, fescue, bermuda, and control plots: 37, 35, 31 and 25 percent. After 68 hours of incubating indigenous microorganisms

extracted from plot soil, it was determined that the microbial communities in the clover plots utilized the highest percentage of aromatics, carboxylic and amino acids. The bermuda, fescue, clover, and controls utilized the following percentages of aromatics: 75, 50, 76, 0%. The authors suggested that the microbial structure of the rhizosphere may differ for each type of plant species evaluated in the study.

Ferro *et al*. (1997) evaluated the feasibility of phytoremediation in a greenhouse experiment with rye grass and wood preservative contaminated soil. Soil was obtained from a Superfund site contaminated with creosote and pentachlorophenol (PCP). Three treatments were evaluated in the greenhouse study: 1)rye-amended; 2)unplanted-amended; and 3)unplanted-unamended. Nutrients (nitrogen, phosphorous, potassium, and sulfur) and dolomite (3 mg/kg) were initially added to amended soils. After addition of initial amendments, planted soil columns were watered with a nutrient solution to prevent nutrient depletion from the soil. Soil moisture was constantly measured and adjusted accordingly.

At time zero, 72 glass columns (8×50 cm) were started. At 2 month intervals, replicate sets of columns were harvested for each treatment. The article focused on discussion of one specific PAH, pyrene. The initial concentration of pyrene was 85.1 mg/kg. At t=64, 130, 189, and 258 days, the average pyrene concentrations in the planted-amended plots were approximately 9, 9.5, 6, and 5 mg/kg. At the same time intervals, the approximate average pyrene concentrations in the unplanted-amended and the unplanted-unamended plots were: 17, 18, 11, 5 mg/kg and 34,

21, 19, 11 mg/kg. The most substantial decrease in pyrene concentration occurred for the planted-amended plots during the first 64 days of the experiment. However, at t=258 days, the planted-amended and the unplanted-amended plots had approximately the same pyrene concentrations. A form of intrinsic bioremediation had occurred in the unplanted-unamended plots. The researchers suggest that aeration and optimal soil moisture in the greenhouse study may have accelerated biodegradation that would have unlikely occurred under field conditions. Similar results were obtained for acenaphthene, fluoranthene, and chrysene. Table 1A in the Appendix contains PAH percent reductions over the study period.

iii. Poplar Tree Phytoremediation

Hybrid forms of the poplar tree have been utilized at sites with soil organic chemical contamination. Most hybrid varieties are fast-growers, perennial, long-lived (25-50 years) and tolerant of organic contamination (Schnoor *et al.*, 1995). Poplar roots can extend towards the water table and establish root mass that can potentially consume rather large quantities of water (Schnoor *et al.*, 1995). According to Edward Gatliff, founder of Applied Natural Sciences, poplar trees have the ability to reach deep aquifers and pump 50 to 350 gallons per day (gpd) per tree (Matso, 1995). In amenable soils and temperate conditions, hybrid poplars can grow 2 meters in the first growing season and reach a height of 5 to 8 meters after 3 years (Schnoor *et al.*, 1995).

In a study at the University of Iowa (Schnoor *et al.*, 1995), exudates from hybrid poplar roots contained 10 to 120 mg/L of dissolved organic carbon and 1 to 10 mg/L of

acetic acid. An increased amount of bioavailable substrates in the root zone is likely to support growth of larger populations, if other factors are not limiting. Therefore, microbial activity could also be increased if poplars are implementated into a treatment strategy.

Jordahl *et al*. (1997) reported the first evaluation of hybrid poplar tree effects on microbial populations in the rhizosphere. The rhizosphere soils of seven-year–old Imperial Carolina poplars were used to enumerate five specific phenotypes. Total heterotrophs, denitrifiers, pseudomonads, BTX degraders, and atrazine degraders were enumerated for 3 rhizosphere samples previously exposed to nitrate and atrazine. The phenotypes were also enumerated in soil samples devoid of roots from an adjacent cornfield. Rhizosphere soil was collected from 4 feet below the land surface and soil within 2 to 10 mm of the root surface was used in the analysis. All investigated types of microbial populations were higher in the poplar rhizosphere soil than unplanted soils. All increases were significant at the 90% level and the corresponding rank in concentration was: total heterotrophs > denitrifiers > pseudomonads > BTX degraders > atrazine degraders. Organic carbon was distributed to the rhizosphere due to root exudation. The poplars in this study exuded 17 ± 8 mg/L of dissolved organic carbon (DOC) into the rhizosphere soil. The DOC is likely to aid in biodegradation of more complex organic contaminants by serving as primary substrates for cometabolism. However, increased amounts of DOC increases the number of sites contaminants can reversible or irreversibly sorb. The study concluded that the poplar rhizosphere enhanced the viability of beneficial

microorganisms and the proliferation of desirable phenotypes in the rhizosphere is conducive to contaminant removal. Even though this study holds promise for poplar rhizosphere and subsequent contaminant bioremediation, much has yet to be learned about the microbiology of the poplar rhizosphere and its relation to contaminant removal. Anderson *et al*. (1993) state, “Further understanding of the critical factors influencing the plant-microbe-toxicant interaction in soils will permit more rapid realization of this new approach to *in situ* bioremediation.”