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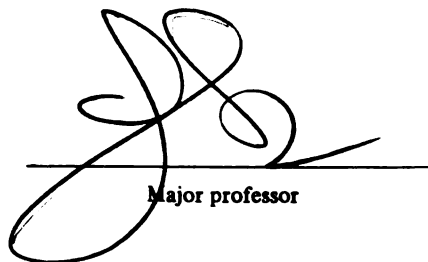
PHYTOREMEDIATION OF POLYCYCLIC AROMATIC HYDROCARBON-
CONTAMINATED SOIL USING NATIVE MICHIGAN PLANT SPECIES

presented by

Cindy Shiu Mai Wan

has been accepted towards fulfillment
of the requirements for

M.S. degree in Crop & Soil Sciences



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**PHYTOREMEDIATION OF POLYCYCLIC AROMATIC HYDROCARBON -
CONTAMINATED SOIL USING NATIVE MICHIGAN PLANT SPECIES**

By

Cindy Shiu Mai Wan

A THESIS

**Submitted to
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ABSTRACT

PHYTOREMEDIATION OF POLYCYCLIC AROMATIC HYDROCARBON - CONTAMINATED SOIL USING NATIVE MICHIGAN PLANT SPECIES

By

Cindy Shiu Mai Wan

Phytoremediation is the use of plants to degrade, detoxify, or remove environmental contaminants. The Rouge Manufacturing Complex (Dearborn, MI) Coke Oven area is contaminated with polycyclic aromatic hydrocarbons (PAHs), which were formed from 60 years of industrial coal processing. PAHs are carcinogenic, mutagenic, and teratogenic organic contaminants with low water solubility. PAHs sorb strongly to organic matter in soils and sediments and consequently, are not readily available for biodegradation. In this study, 18 native Michigan plant species and an unplanted control were evaluated for their abilities to reduce PAHs in amended Coke Oven area soil over one growing season in a field demonstration plot. Four plant species treatments significantly decreased soil total PAH concentration ([tPAH]) over time; two plant species treatments had lower soil [tPAH] compared to the unplanted control in July, and the soil [tPAH] for one plant species treatment was lower than that for the unplanted control in September. By contrast, the unplanted control soil [tPAH] did not decrease over time. This study identifies plant species with superior PAH-phytoremediation abilities for further laboratory studies and field applications.

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INTRODUCTION

Phytoremediation, the use of plants to degrade, detoxify, remove or contain environmental contaminants, is an emerging field in environmental rehabilitation.

Phytoremediation is a sub-discipline of bioremediation, which more commonly describes the use of microbes for treatment of contaminants. The use of plants to remediate a site has advantages over traditional engineered cleanup or bioremediative techniques. Plants can stabilize soil by intercepting the impact of raindrops, absorbing and taking up water from the soil, thereby minimizing soil erosion. Vegetation increases organic matter in the soil and prevents the loss of organic matter by wind erosion by decreasing the soil surface area exposed to convection. Unlike microbes, plants are able to reduce leaching of water-soluble contaminants because they utilize water from soils. In addition, plants harvest and utilize the sun's energy, whereas most engineering remediation technologies require the expensive input of energy for operating machinery to decontaminate soils.

Studies on phytoremediation of inorganic and organic contaminants have focused on plant selection, emphasizing screening for superior species, selected plants, and symbiotic interactions between plants and microorganisms. Recently, research has investigated the influence of factors such as soil amendments. Most phytoremediation research has been conducted under laboratory or greenhouse conditions. This thesis describes laboratory-scale screening of a variety of Michigan native plant species followed by field-scale application of selected species.

The Ford Rouge Manufacturing Complex in Dearborn, MI has been manufacturing steel and automobiles for eighty years. By-products from these activities have led to contamination of parts of the site. Areas of the site are contaminated with

polycyclic (polynuclear) aromatic hydrocarbons (PAHs or PNAs), which were formed from the production of coke for the smelting of iron ores. PAHs are carcinogenic, mutagenic, and teratogenic. They are environmentally persistent, due in part to their low water solubility, which cause them to sorb strongly organic matter in soils and sediments. Consequently, they are not readily bioavailable and are resistant to biological degradation. As a part of the Rouge Heritage Initiative Renovation designed by Bill McDonough & Partners (Charlottesville, VA), the Ford Motor Company Environmental Quality Office, Ford Land Corporation, and Michigan State University's Phytoremediation Lab are collaborating to develop a phytoremediation strategy for the Ford Rouge Facility.

The objectives of this thesis were to evaluate the efficacy of plant species for the phytoremediation of PAHs and to broaden our understanding of the phytoremediation of organic contaminants with low water solubility in soils. This study was designed to characterize potential plant species to be used for PAH phytoremediation. Plant species that exhibit the greatest rate of PAH reduction could be applied in future laboratory studies and large-scale environmental rehabilitation efforts such as at the Rouge Manufacturing Complex. Furthermore, determining which species have the highest PAH-soil decontaminating capacities is a primary step in identifying plant species for further research, which could lead to greater understanding of the biochemical reactions and mechanisms involved in PAH phytoremediation.

CHAPTER I

REVIEW OF LITERATURE

1. INTRODUCTION

Environmental contamination of land and water natural resources with hazardous materials is a worldwide concern. The source of contamination on hazardous sites is frequently anthropogenic, typically resulting from industrial and military activities. As of August 2001 there were 1235 Superfund National Priorities List sites in the United States being cleaned up under the Superfund program (EPA, 2002b). A brownfield is an urban site that is either abandoned or under-used because it has real or perceived environmental contamination, though potential for redevelopment or reuse. The number of brownfield sites has been estimated to be more than 2900 in Canada (National Round Table on the Environment and the Economy, 1998). In United States there are over 5000 brownfields (EPA, 2002a). In many cases, contaminated sites are abandoned, have low soil fertility and are poorly vegetated. Such areas can further deteriorate via wind and water erosion resulting in the loss of soil nutrients and organic matter. Environmental rehabilitation is necessary to prevent further land degradation, and to protect humans and wildlife from exposure to hazardous pollutants.

Persistent organic pollutants (POPs) are toxic chemicals that do not readily undergo biogeochemical reactions, remain in soils for a long time, and are prone to

biomagnify through the food chain. Biomagnification refers to the increase in contaminant concentration at sequentially higher levels of the food web. PAHs are harmful to humans and wildlife because they are carcinogenic, mutagenic and teratogenic. Examples of POPs include the pesticide 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT), polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs). Unlike synthetic pesticides and PCBs, PAHs are formed naturally in the environment as a result of forest fires, volcanic eruptions, thermal geologic reactions, and plant and bacterial reactions (Blumer, 1976). Since the 1800s, the beginning of the industrial revolution, anthropogenic activities have led to the production of vast amounts of PAHs, which have exceeded the levels that are naturally degraded, and created an imbalance between PAH formation and degradation (Hites, 1977; Suess, 1976). Anthropogenic sources of PAHs include the burning of fossil fuels, railroad industries, manufactured gas plants, and coke production.

Current strategies for remediation of PAH-contaminated soil include physical, chemical and or biological treatment, though each has its drawbacks. Isolation and containment use physical, chemical or hydraulic barriers to inhibit the distribution of the contaminant, but do not reduce the level of the contaminant. For example, capping involves mixing the soil with clay to reduce hydraulic conductivity (Cunningham and Berti, 1993). *In situ* thermal desorption treatment of soil for petroleum hydrocarbons involves the application of heat and vacuum via thermal wells to vaporize, decontaminate, or transport contaminants to the surface for further treatment (Conley, 2000). Thermal desorption may be effective, but this treatment requires the high input of energy and the installation of wells. Soil washing consists of mixing the soil, and

separation of the pollutant portion (silt and clay) of the soil from the portion with less pollutant (sand and gravel). Soil washing is a commonly used strategy that requires intensive labor and money (EPA, 2001). Excavation of contaminated material is often favored because it rapidly removes contamination from a site, but this method of remediation only transfers the contamination from one location to another where the pollutant will persist (Cunningham and Berti, 1993).

Biological treatments of contaminated soil include natural attenuation, microbial bioremediation, and phytoremediation. Natural attenuation is the use of indigenous soil processes without intervention. Natural attenuation has reduced organic contaminants via microbial degradation (Hiebert, 2000). Natural biodegradation of hydrocarbons and chlorinated hydrocarbons occurred at an oilfield service facility. The intermediates and products of microbial degradation of hydrocarbons and chlorinated hydrocarbons were observed to increase over time. Elevated levels of methane were detected indicating microbial degradation of chlorinated hydrocarbons and ethene (by product of biodegradation of tetrachlorethene (PCE) and trichloroethene (TCE)). Carbon dioxide, the final product of biodegradation of hydrocarbons and some chlorinated hydrocarbons, increased (Hiebert, 2000). Natural attenuation is low-cost, but may not be effective if initial contaminant concentration is high or toxic to plants and microorganisms. Microbial bioremediation uses microorganisms to metabolize complex organic molecules. Microbial degradation of PAHs has been extensively demonstrated in research literature (Cerniglia, 1992; Cerniglia, 1979; Bumpus, 1985; Field *et al.*, 1992). Microbial remediation typically requires nutrient inputs and adjustment of soil properties, such as pH or temperature, so that the degrading bacteria and or fungi can persist, a process

known as biostimulation. The contaminated soil and microorganisms are sometimes mixed *in situ*, which is a disruptive, though sometimes beneficial, procedure. In some cases, reactors are used for bioremediation (Civilini and Sebastianutto, 1996; Civilini *et al.*, 1996; Lilja *et al.*, 1996). The use of a bioreactor involves transport of the soil, possibly destroying the site and often incurring high operating costs. Landfarming of contaminated materials has also been shown to be a feasible method of remediation and involves routine soil tillage (8 -12" depth) and the addition of fertilizer to enhance microbial degradation of organic contaminants in the absence of plants (Sayles *et al.*, 1999; Reilley *et al.*, 1996). This technique is often used for petroleum hydrocarbons, but dissipation slows over time (Sims and Overcash, 1983).

Remedial programs can also combine biological and chemical methods. For instance, soil contaminated with PAHs and pentachlorophenol was treated in a laboratory experiment using chemical oxidation by adding Fenton's reagent (ferrous iron and hydrogen peroxide) to generate free hydroxyl radicals followed by indigenous microbial biodegradation of the chemically oxidized compounds (Allen and Reardon, 2000). For large cleanup operations, the cost of this method may be prohibitively high.

Phytoremediation, or vegetated treatments, is a method of environmental rehabilitation that could potentially reduce the concentration of contaminants and improve soil quality simultaneously. Phytoremediation is the use of plants to degrade, detoxify or remove inorganic and organic contaminants (Cunningham and Berti, 1993). Phytoremediation has been demonstrated to accelerate contaminant biodegradation during natural attenuation, microbial bioremediation, and landfarming. Previous studies showed vegetated soil leads to greater rates of PAH reduction compared with unplanted

soil (Aprill and Sims, 1990; Nedunuri *et al.*, 2000; Pradhan *et al.*, 1998; Yateem *et al.*, 2000). Vegetated landfarming was 30-44% more effective for PAH reduction than soil landfarming with no plants (Reilley *et al.*, 1996). Few studies, however, have reported the individual effects of a variety of plant species on phytoremediation of PAHs. Plants may secrete different compounds that support soil microflora, and some plant species may favor the PAH-degrading microorganisms via exudation of specific compounds.

The Ford Rouge Manufacturing Complex in Dearborn, MI, once the largest integrated industrial facility in the world, has areas contaminated with byproducts from eighty years of steel and automobile manufacturing. PAHs have accumulated in areas of the facility used during coal processing for coke production for iron ore smelting. PAHs are highly hydrophobic organic contaminants that tend to sorb strongly to the soil and sediment organic matter fraction. As a consequence, PAHs are difficult to biodegrade and remain in the soil for extended periods. PAHs pose health hazards to humans and wildlife because they are carcinogenic, mutagenic, and teratogenic. The purpose of this thesis is to evaluate the effectiveness of various native Michigan plant species for phytoremediation of PAHs in soil from the Rouge Manufacturing Complex. It is hoped that information gained from this study will advance our understanding of processes involved in phytoremediation of PAH pollutants.

2. LITERATURE REVIEW

2.1 Phytoremediation

Phytoremediation is the use of plants to degrade, detoxify or remove environmental contaminants (Cunningham and Berti, 1993) and has been reviewed in numerous papers (Alkorta and Garbisu, 2001; Cunningham and Berti, 1993; Macek *et al.*,

2000; Salt *et al.*, 1995). Bioremediation refers to the using biological means to remove contamination. In the literature, “Bioremediation” typically refers to the use of bacteria and fungi to remove pollutants from the environment. Phytoremediation is a subdiscipline of bioremediation and consists of a variety of strategies based on different mechanisms of contaminant removal. Phytoextraction is the use of plants to remove inorganic contaminants, typically metals, from soil by concentrating them in harvestable plant parts. Phytoextraction of useful or valuable metal pollutants (e.g. Zn, Cu) with subsequent harvesting and recovery is referred to as biomining (Cunningham and Berti, 1993) or phytomining (Pletsch *et al.*, 1999). For plants to decontaminate a site within a reasonable number of harvests, it has been proposed that plants must accumulate 1 to 3% of a metal per dry weight aboveground biomass (Cunningham and Ow, 1996). Plants that can accumulate a contaminant in high concentrations are known as hyperaccumulators. Phytostimulation, or plant-assisted bioremediation, is the enhancement of microbial biodegradation in the rhizosphere. Rhizofiltration is the use of plant roots to absorb mineral or heavy metal contaminants from water and aqueous waste streams and subsequent disposal of laden biomass. Phytostabilization is the use of plants to reduce motility of pollutants in the environment by sequestration, lignification, or humification in plant or soil matrices. Phytostabilization is usually used on metal–contaminated sites to prevent erosive particles from increasing the area of contamination. Phytovolatilization is the use of plants to uptake a contaminant and then convert it to a volatile form that is released into the atmosphere. Phytodegradation (also known as phytotransformation) has been defined as the absorption and conversion by catabolism or anabolism in the plant root or shoot. Phytodegradation has also been defined as the use of plants and associated

microorganisms to degrade organic pollutants (Cunningham *et al.*, 1995), however in this thesis the previous definition of phytodegradation will be used because the terms phytodegradation and phytostimulation distinguish between plant degradation of the contaminant and plant-assisted microbial degradation, respectively.

Plants have been demonstrated to be an effective approach for remediation of inorganic pollutants. Lead can be removed from soil by phytoextraction by hyperaccumulators such as *Thlaspi rotundifolium* (Cunningham and Ow, 1996; Reeves and Brooks, 1983). Lead uptake by *Brassica juncea* (Indian mustard) was enhanced when the synthetic chelator ethylene diamine tetraacetic acid (EDTA) was added to hydroponic solution (Vassil *et al.*, 1998) or soil (Blaylock *et al.*, 1997). Selenium (Se) can be phytoextracted by *Brassica napus* (canola) (Banuelos and Mayland, 2000). The Se-enriched shoots of *B. napus* may then be harvested and used as forage for Se-deficient livestock (Banuelos and Mayland, 2000). *B. juncea* has also been shown to phytovolatilize selenium (de Souza *et al.*, 1998). Soils contaminated with arsenic can be remediated by phytoextraction using *Pteris vittata* (brake fern) (Ma *et al.*, 2001) or *B. juncea* (Pickering *et al.*, 2000). Arsenic uptake is enhanced by addition of dimercaptosuccinate, a chelator of dithiol arsenic (Pickering *et al.*, 2000). Phytoextraction of nickel can be accomplished by *Thlaspi goesigense* (Kramer *et al.*, 1997; Persans *et al.*, 1999) and several Brassicaceae species (Baker, 1989). *Thlaspi caerulescens* (Brassicaceae), a hyperaccumulator, can phytoextract zinc (Tolra *et al.*, 1996) and cadmium (Whiting *et al.*, 2000). Phytoremediation may take 2–20 years depending on clean-up goals, the volume of contaminated soil, the distribution and concentrations of contaminant, soil characteristics, depth of contamination, plant growth rate, and climate

(Naval Facilities Engineering Center, 2002). Phytoremediation of metals in soil costs \$25-\$100 per ton of soil. Conventionally-used remediation techniques can cost considerably more: soil washing (\$50-150/ton), *in situ* soil flushing (\$75-\$210/ton), *ex situ* solidification/stabilization (\$75-\$150/ton), *in situ* solidification/stabilization (\$111-205/ton), thermal desorption (\$150-\$500/ton), thermal treatment (\$200-\$450/ton), and landfilling (\$100-\$500/ton)(Schnoor, 2002). The excavation of one acre of sandy loam soil to a depth of 50 cm would cost \$400 000 for excavation and storage using conventional soil removal methods. By contrast, phytoextraction of the same soil would cost \$60 000 – \$100 000 (Salt *et al.*, 1995).

Phytoextraction can be also used to remediate soils contaminated with radionuclides. Redroot pigweed (*Amaranthus retroflexus*) has been shown to hyperaccumulate radioactive cesium ^{137}Cs , a byproduct of nuclear fission (Lasat *et al.*, 1998). A recent phytoextraction study showed that *A. retroflexus*, *B. juncea* and *Phaseolus acutifolus* A. Gray (teparty bean) removed ^{90}Sr and ^{137}Cs from soil in a field study (Fuhrmann *et al.*, 2002). Radionuclide concentration ratios (plant contaminant concentration divided by that in soil) for that *A. retroflexus*, *B. juncea* and *Phaseolus acutifolus* were 2.58, 0.46, 0.17 for ^{137}Cs , respectively and 6.5, 8.2, 15.2 for ^{90}Sr , respectively (Fuhrmann *et al.*, 2002). A plant to soil concentration ratio greater than one indicates that the plant is accumulating the contaminant. High levels of ^{134}Cs were taken up by *Agrostis capillaris* (bent grass) (Sanchez *et al.*, 1999). *Brassica narinosa* (Chinese mustard), *Brassica chinensis* (Chinese cabbage) and *B. juncea* have demonstrated

hyperaccumulation potential of uranium in the presence of citric acid (Huang *et al.*, 1998).

Phytoremediation has been used to treat soils containing organic contaminants such as TCE (trichloroethene), BTEX compounds (benzene, toluene, ethylbenzene, xylene), TNT (2,4,6-trinitrotoluene), RDX (Royal Demolition Explosives; 1,3,5-trinitro-1,3,5-triazine), pesticides and PAHs principally by phytodegradation or phytostimulation. Numerous field studies used hybrid poplars (*Populus trichocarpa* x *Populus deltoides* and *P. trichocarpa* x *P. maximowiczii*) to metabolize TCE to metabolites such as chloral hydrate, trichloroethanol, di- and trichloroacetic acid, and carbon dioxide (Newman *et al.*, 1997). Hybrid poplars were shown to degrade TCE due to plant dehalogenase enzyme activity (Schnoor *et al.*, 1995). Phytoremediation has also been demonstrated for nitroaromatic compounds, such as nitrobenzene (McFarlane *et al.*, 1990) and hybrid poplar (*P. deltoides* x *P. nigra*) metabolism of TNT (Thompson *et al.*, 1998). Plants have potential for PAH phytodegradation since they possess oxygenase, peroxidase, and laccase enzymes, but this ability has not been clearly demonstrated (Criquet *et al.*, 2000). These studies indicate there is potential for phytodegradation to effectively remediate organic contaminants.

In addition to phytodegradation, plants can also remediate organic contaminants by phytostimulation. Poplars can phytostimulate microbial degradation of TCE (Walton and Anderson, 1990). Microorganisms in the rhizosphere can degrade TCE to form metabolites such as cis-1,2-dichloroethylene and vinyl chloride, and degrade TCE completely to carbon dioxide (Walton and Anderson, 1990). Greater mineralization of ¹⁴C-TCE and microbial respiration were observed in rhizosphere soil than in unvegetated

soil (Walton and Anderson, 1990). Plant roots have been shown to increase the microbial count and enhance mineralization in soils contaminated with the pesticides parathion and diazinon (Hsu and Bartha, 1979). Plants such as *Morus rubra* (Mulberry), *Rhus aromatica* (sumac), *Malclura pomifera* (osage orange), *Helianthus maximillani* (perennial sunflower) can provide PCB-degrading bacteria with cometabolites such as the phenolic compounds flavonoid and coumarin (Donnelly *et al.*, 1994; Fletcher *et al.*, 1995; Fletcher and Hegde, 1995). Plants can provide cometabolites, e.g. phenolics or terpenes, for PAH-degrading microbes (Hegde and Fletcher, 1996). Phytoremediation of PAHs will be discussed in more detail later in this chapter.

The cost of phytoremediation of organic contaminants is lower compared with other remediation strategies. Phytoremediation using fine-rooted grasses costs \$10-\$35 per ton of soil (Schnoor, 2002). By contrast, the costs of other approaches are: *in situ* bioremediation \$50-\$150/ton, soil venting \$20-\$220/ton soil washing \$80-\$200/ton, thermal treatment \$120-\$300/ton, solidification/stabilization \$240-340/ton, and incineration \$200-1500/ton (Schnoor, 2002).

Biotechnological methods have been employed for improvement of plants for environmental clean-up. Plant may be genetically altered to change plant morphology to favor remediation processes. Plants can be genetically transformed by using *Agrobacterium rhizogenes* to produce increased root biomass (Stomp *et al.*, 1993; Stomp *et al.*, 1994; de Araujo *et al.*, 2002; Shanks and Morgan, 1999). This transformation would enhance the root surface area, possibly increase root exudation, which in turn could increase microbial activity and contaminant biodegradation. Increased root biomass may also lead to increased contaminant uptake (Nedelkoska and Doran, 2000b;

Nedelkoska and Doran, 2000a). Plants can also be genetically altered to produce enzymes that can degrade or transform contaminants. For instance, *Arabidopsis thaliana* plants and *Nicotiana tabacum* (tobacco) were transformed with the bacterial *merA* gene, which encodes mercuric reductase, and *merB* gene, that encodes organomercurial lyase (Rugh *et al.*, 1996; Rugh *et al.*, 1998; Bizily *et al.*, 2000). *MerB* enzyme catalyzes the degradation of organic mercury to Hg(II) and the *MerA* enzyme catalyzes the reduction of Hg(II) to Hg(0), a much less toxic form of mercury that volatilizes to the atmosphere (Summers, 1986). The *mer* gene transformed plants evolved substantial amounts of elemental mercury compared with the control and were able to tolerate 25-100 μM HgCl_2 , levels toxic to untransformed plants (Rugh *et al.*, 1996). Transgenic plants have been developed for the phytoremediation of organochlorides such as TCE. Plants engineered to express to a mammalian cytochrome P450 gene were capable of 400 times greater degradation of TCE than wildtype plants (Doty *et al.*, 2000). Transgenic poplar plants have also been developed that can overexpress γ -glutamylcysteine synthetase, the rate-limiting step in glutathione synthesis (Rennenberg, 1997; Gullner *et al.*, 2001), Glutathione binds organochlorides, which makes them less toxic and tags them for vacuolar import (Edwards *et al.*, 2000). Transgenic tobacco plants expressing the bacterial nitroreductase gene from *Enterobacter cloacae* showed increased tolerance and detoxification of TNT (2,4,6-trinitrotoluene) compared to wildtype (Hannink *et al.*, 2001). Field studies and research experiments to evaluate the safety and cross-fertilization of transgenic plants with wild populations need to be conducted before these biotechnological advances can be practically used in phytoremediation.

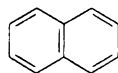
Phytoremediation is a remediation strategy that has many advantages over other clean-up technologies. Phytoremediation may be implemented with minimal disturbance to a site, simultaneously rehabilitating the soil, and with reduced risk of contaminant distribution. Phytoremediation can enhance bioremediation by providing carbon sources, cometabolites, and improved soil properties, such as decreased pH, increased porosity, decreased bulk density. Transgenic plants may be easier to control compared with transgenic microorganisms. Phytoremediation requires relatively low maintenance, is aesthetically pleasing, and is compatible with restoration ecology. In the United States, the use of conventional technologies for cleanup of existing contaminated sites is estimated to cost \$10 billion and treatment of hazardous wastes to be at least \$400 billion (Salt *et al.*, 1995). The costs of phytoremediation are expected to be lower than standard engineering-based approaches.

Despite the potential for ecological and economic advantages, phytoremediation has its limitations. Vegetated treatments cannot access deep contaminants, may take longer than most other methods, and are restricted to the growing season. Plant-based remediation may not be effective at high levels or for all contaminants. In spite of these shortcomings, phytoremediation is a relatively new field with potential to enhance and complement other remediation strategies.

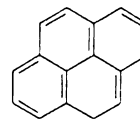
2.2 Polycyclic Aromatic Hydrocarbons (PAHs)

Polycyclic (polynuclear) aromatic hydrocarbons are widely distributed environmental pollutants. PAHs consist of two or more fused benzene or furan rings arranged linearly, angularly or in clusters (Blumer, 1976) (Fig. 1.1). Heterocyclic aromatic compounds are formed when the carbon in the benzene is substituted with

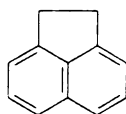
Naphthalene



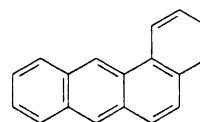
Pyrene



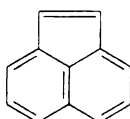
Acenaphthylene



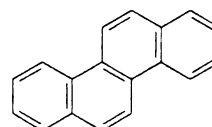
Benz(a)anthracene



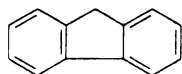
Acenaphthene



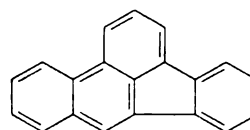
Chrysene



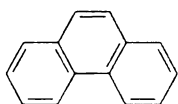
Fluorene



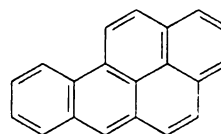
Benzo(b)fluoranthene



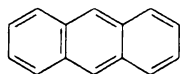
Phenanthrene



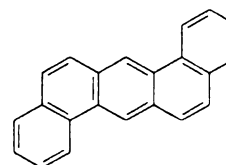
Benzo(a)pyrene



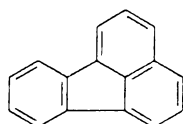
Anthracene



Dibenzo(ah)anthracene



Fluoranthene



Benzo(ghi)perylene

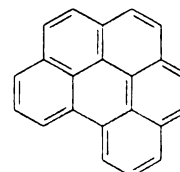


Figure 1.1. Structures of some PAHs.

Table 1.1. PAH chemical and toxicological properties.

PAHs	MW	¹ Aqueous solubility (mg/L) @ 25 °C	¹ log K _{ow}	² MI soil cleanup Criteria (mg/kg)	² MI Groundwater cleanup Criteria (µg/L)	³ Carcinogenicity
Naphthalene	128.16	30	3.37	8.00×10^4 ⁴	520	-
Acenaphthene	154.21	3.47	4.33	2.00×10^5 ⁵	1300	-
Acenaphthylene	152.2	3.93	4.07	8.00×10^3 ³	52	-
Fluorene	166.21	1.98	4.18	1.30×10^5 ⁵	880	-
Phenanthrene	178.23	1.29	4.45	8.00×10^3 ³	52	-
Anthracene	178.22	0.07	4.46	1.00×10^6 ⁶	43	-
Fluoranthene	202	0.26	5.33	1.80×10^5 ⁵	210	-
Benz(a)anthracene	228	0.014	5.61	100	2.1	+
Chrysene	228.2	0.002	5.61	1.00×10^4	5	+
Pyrene	202.26	0.14	5.32	1.10×10^5 ⁵	140	-
Benzo(b)fluoranthene	252	0.0012	6.57	100	2	+
Benz(a)pyrene	252.3	0.0038	6.04	10	5	+
Dibenz(a,h)anthracene	278.35	0.0005	5.97	10	5	+
Benz(ghi)perylene	276	0.00026	7.23	9.10×10^3 ³	5	-

¹ Sims and Overcash (1993).

² MDEQ, <http://www.deq.state.mi.us/documents/deq-erd-sec6bl.pdf>.

³ Note: + carcinogen according to IARC (1987); - nonmutagenic according to (Malachova 1999).

nitrogen, sulfur, oxygen, or other elements (Blumer, 1976). PAHs are formed as a result of incomplete combustion of organic material such as when there is an insufficient supply of oxygen (Heil, 1998). Temperature and combustion conditions, rather than fuel type, influence the specific PAHs formed (Giger, 1974; Jenkins *et al.*, 1996; Blumer, 1975). There are some general chemical and toxicological trends for PAH compounds. PAHs of successively higher molecular weights have lower aqueous solubility, greater hydrophobicity as indicated by higher log K_{ow} values, and greater carcinogenicity (Table 1.1). The log K_{ow} is a term that describes the hydrophobicity of a compound and is the logarithm of the concentration of a compound dissolved in the octanol phase divided by the concentration in the water phase in a partitioning assay.

PAHs are formed by both natural and anthropogenic processes. Prior to the twentieth century, there was a natural balance between the production and degradation of PAHs (Hites, 1977; Suess, 1976). PAHs occur naturally as a result of thermal geologic reactions associated with fossil fuel and mineral production, during the burning of vegetation in forest and brush fires, and also by some plant and bacterial reactions (Blumer, 1976). Human activities such as combustion of wood and fossil fuels, petroleum refining, coal and oil shale conversion, and chemical manufacturing lead to the formation of PAH compounds creating areas of high PAH deposition and accumulation (Hites, 1977; Suess, 1976).

Background levels of PAHs prior to the industrial age were substantially lower than levels currently seen around the world. The main source of terrestrial PAHs is from atmospheric deposition of PAHs from the combustion of fossil fuels. At the Rothamsted Experimental Station in southeast England, PAH concentrations in soil core samples from

the mid-1800s to the present were analyzed (Jones, 1989). The total PAH concentration found in soil samples from the mid 1800s was 350 ng/g dry weight and has since increased at an accelerating rate to 1770 ng/g dry weight reflecting the rise in anthropogenic activities, such as fossil fuel combustion and growing worldwide industrialization (Jones, 1989).

PAHs are hazardous compounds and major environmental problems. Several PAHs are carcinogenic, teratogenic, and mutagenic (Shabad, 1975; Sims and Overcash, 1983; Dipple, 1990). Fish from industrially contaminated water with benzantracene and other contaminants had four times more tumors than fish from unpolluted waters (Brown, 1973). Since PAHs can biomagnify, accumulation in organisms such as oysters (Lee, 1978) and fruit flies (Southworth, 1978), has consequences for animals higher in the food web. PAHs are environmentally persistent due to their tendency to partition to organic matter. This results in strong sorption to soils and sediments, hindering their degradation and resulting in their persistence as stable soil complexes (Means, 1980). The biogeochemical fate of PAHs in soil is controlled in part by surface adsorption (Reilley *et al.*, 1996) and sorption to other soil components. Research has shown that even when soil microbes were abundant, PAHs were not degraded due to their lack of bioavailability (Carmichael and Pfaender, 1997).

Regulatory agencies have set environmental limits for PAHs that attempt to balance economic and health interests. The U.S. Environmental Protection Agency states that particulate concentrations of PAHs in air above $150 \mu\text{g}/\text{m}^3$ are unhealthy and above $420 \mu\text{g}/\text{m}^3$ are hazardous (Heil, 1998). As indicated by the Michigan Department of Environmental Quality (MDEQ) clean-up regulations, PAHs are toxic at different levels.

According to the MDEQ, the 4-ring PAHs - benz(a)anthracene, chrysene, pyrene - and the 5 and 6 ring PAHs are permitted in soils and waters at particularly low levels (Table 1.1). The MDEQ has designated acceptable levels for PAH industrial and commercial long-term ingestion groundwater cleanup criteria, and dermal exposure to contaminated soil (Table 1.1). These criteria provide targets for environmental rehabilitation efforts.

2.3 PAHs in soil

Microbial degradation

Biodegradation of PAHs is positively correlated with water solubility (Aronstein *et al.*, 1991). PAHs with more rings and higher molecular weight have lower water solubility and tend to sorb strongly to organic matter and soil (Bossert and Bartha, 1986; Reilley *et al.*, 1996; Carmichael and Pfaender, 1997). Subsequently, two- and three-ring PAHs biodegrade more readily than those of four-, five- and six-ring PAHs (Bossert and Bartha, 1986).

Microorganisms (bacteria, fungi, algae) can degrade PAHs under aerobic conditions using oxygenases to incorporate oxygen into the carbon ring (Dagely, 1975). Microbial degradation of PAHs has been well summarized in a variety of comprehensive reviews (Sims and Overcash, 1983; Wilson and Jones, 1993; Cerniglia, 1993; Walton, 1994).

There are two classes of oxygenase enzymes: monooxygenase and dioxygenase. Eukaryotes, including mammals and fungi, possess monooxygenases, which incorporate one oxygen atom into the aromatic substrate to form arene oxides (epoxides) followed by enzymatic addition of water to yield trans-dihydrodiols and phenols (Cerniglia, 1993; Wilson and Jones, 1993). The trans-dihydrodiol is oxidized to a catechol, which is then

subjected to ring cleavage enzymes (Sims and Overcash, 1983). Further catabolic activities lead to the production of tricarboxylic acid cycle (TCA) intermediates such as succinic, fumaric, pyruvic, acetic acids and acetaldehyde (Heitkamp, 1988a; Heitkamp, 1988b). Since the monooxygenase PAH biodegradation pathway forms epoxides, this may be a cause of PAH ecotoxicity. Epoxides have been shown to bind DNA and RNA initiating the formation of tumors in carcinogenesis (Sims and Overcash, 1983).

The dioxygenase pathway does not lead to the formation of mutagenic and carcinogenic epoxides. Dioxygenases are found in prokaryotes, including bacteria and some blue-green algae, and incorporate both atoms of molecular oxygen (O_2) into an aromatic substrate (Sims and Overcash, 1983). Dioxygenase attack on an aromatic ring results in the production of cis-dihydrodiol (Cerniglia, 1993; Wilson and Jones, 1993). The cis-dihydrodiol is oxidized to catechol followed by catabolism to TCA cycle intermediates.

Most fungal metabolism mechanisms of PAHs are cometabolic, which means that PAHs are not the primary substrate and that intermediate compounds are formed rather than carbon dioxide and water (Wilson and Jones, 1993). The two main groups of fungi involved in PAH degradation are those that use monooxygenases (e.g. cytochrome P-450) and those that use lignin peroxidases to initiate attack on PAHs (Cerniglia, 1993). Lignin peroxidases oxidize PAHs and initiate a free radical attack by a single electron transfer forming quinones (Reddy, 1995). A third group of fungal enzymes involved in the degradation of PAHs, called laccases, are considered to contribute less to the degradation of PAHs because of their relaxed substrate specificity (Cerniglia, 1993; Harayama, 1997).

Bacterial biodegradation of PAHs is considered to be the dominant process for PAH reduction in soils (Reilley *et al.*, 1996). Bacteria can degrade PAHs either as the sole carbon source or by cometabolic processes (Wilson and Jones, 1993).

Bacterial degradation of PAHs is predominantly due to dioxygenase activity, which does not lead to the formation of epoxides or mutagenic, carcinogenic, or teratogenic intermediates (Sims and Overcash, 1983). This factor may favor soil bacteria over fungi for bioremediation of PAHs.

Photodecomposition, oxidation, hydrolysis, leaching and volatilization of PAHs

Absorption of ultraviolet radiation leads to photolysis of PAHs (Sims and Overcash, 1983). PAHs also react with ozone (Zeng *et al.*, 2000), other oxidants, nitrogen oxides, and sulfur oxides to cause decomposition (Sims and Overcash, 1983). The formation of singlet oxygen and radicals generated by photolytic cleavage of trace carbonyl compounds or from enzymatic reactions in aqueous systems, alkylperoxy ($\text{RO}_2\bullet$) and hydroperoxy ($\text{HO}_2\bullet$), can result in the oxidation of PAHs (Sims and Overcash, 1983). PAHs generally do not undergo hydrolysis. Photodecomposition, oxidation and hydrolysis of PAHs are not considered significant pathways for PAH reduction in soils (Sims and Overcash, 1983).

Leaching is not considered an important pathway leading to the reduction of PAHs in soil due to their low aqueous solubility (Reilley *et al.*, 1996). Leachate from pots in a greenhouse experiment with contaminated soils was analyzed and did not contain detectable PAHs ($<10 \text{ g/L}$) (Schwab, 1994). Volatilization also is not considered an important PAH reduction process from soils. PAHs with three or more rings have very low vapor pressures (Reilley *et al.*, 1996) and the high $\log K_{\text{ow}}$ of most PAHs suggest

soil sorption of PAHs would be far greater than volatilization (Sims and Overcash, 1983) (Table 1.1; Fig. 1.1). Park et al. (1990) observed that volatilization accounted for 30% decrease of naphthalene 48h after PAH addition to soil, though did not significantly reduce PAHs of higher molecular weight.

Plant uptake and accumulation of PAHs

PAHs detected in plants may be the result of atmospheric deposition, plant biosynthesis, adsorption, or uptake. Atmospheric deposition of PAHs on plants could account for background plant PAH levels (Lodovici *et al.*, 1994). PAHs have been reported to form by plant biosynthetic processes (Borneff, 1968), though this phenomenon has not been demonstrated by others (Sims and Overcash, 1983). Using predictive mathematical models based on previous literature values for various plant physiological and biochemical parameters, compounds with $\log K_{ow}$ 0-1 may be taken up by roots and translocated because they are water-soluble; compounds with $\log K_{ow}$ between 1 to 4 can be taken up by roots and transported in the xylem, and compounds with $\log K_{ow}$ greater than 4 would adsorb to roots (Trapp, 2002; Cunningham and Berti, 1993). The graphical relationship between translocation and $\log K_{ow}$ is bell-shaped indicating that there is an optimum lipophilic range of $\log K_{ow}$ 1.5 to 2.5 for plant translocation of organic compounds (Briggs *et al.*, 1982).

Plants can accumulate PAHs via sorption onto plant roots, e.g. naphthalene (Schwab *et al.*, 1998; Schwab, 1994), or volatilization through the plant (Watkins, 1994). The more lipophilic a compound, the greater the likelihood it is to concentrate in roots.

Mentha pulegium plants accumulate polymeric dyes, which are PAH analogs, into

lignifying tissues (Strycharz and Shetty, 2002). It was not determined if the aromatic substrate was cross-linked to the cell wall or if it merely accumulated in plant tissue. Some studies suggest that higher plants may not translocate PAHs. In one experiment various plants grown hydroponically with 8.0 µg/kg benzo(a)pyrene (B(a)P) showed no (<3 µg/kg) B(a)P ($\log K_{ow} = 6.04$) was translocated in any of the plants or plant tissues including leaf and stem tissues for green beans (Blum, 1977). In a three-year field study, a mixture of 14 PAHs with $\log K_{ow}$ ranging 3.37 to 7.66 did not accumulate to detection limit levels in grass tissues (Qiu *et al.*, 1997). Similarly, insignificant amounts of anthracene and pyrene were taken up by alfalfa after 24 weeks in spiked field soil with initial starting concentration of 100 mg/kg in a greenhouse study (Schwab, 1994).

Plant biostimulation of microbial PAH-degradation

Plants may stimulate microbial degradation of PAHs and thereby enhance biological remediation. Vegetation improves physical and chemical properties of soils, promotes soil microbial activity, and increases contact between root-associated microbes and soil contaminants. Roots benefit soil structure by enhancing soil porosity and subsequent water and gas movement. Increased oxygen in soil may be important for bioremediation of PAHs since the initial step in the main PAH-degradation pathway requires oxygen. Roots can grow into dense soil aggregates, and thereby increase the volume of soil exploited by plants and microorganisms (Aprill and Sims, 1990). The rhizosphere promotes microbial activity by enhancing transport of water, air and providing carbon substrates via decaying organic matter and root exudation (Brady and Weil, 1999). In addition to enhancement of water infiltration, plants can also remove excess water, an important role for biodegradation of PAHs since aerobic conditions are

required. Plants may provide carbon sources in the form of carbohydrates or organic acids to increase microbial activity and or numbers. Plants may enhance the rate of microbial PAH degradation by providing cometabolites, e.g. phenolics or terpenes, for PAH-degrading microbes (Hegde and Fletcher, 1996). Plants can also secrete surfactants (lipids and sterols) that lubricate the root, solubilize contaminants, and thereby increase contaminant bioavailability (Siciliano and Germida, 1998a). Root exudates have been experimentally demonstrated to enhance bioremediation of PAHs. The addition of root exudates of *Avena barbata* Pott ex Link (slender oat) resulted in lower phenanthrene concentrations compared to unamended controls after 20 days in a growth chamber experiment (Miya and Firestone, 2001). This decrease may have been because the plant exudates provided nutrients, enhanced phenanthrene solubility (e.g. exudates were biosurfactants), or served as primary substrates for cometabolic metabolism of phenanthrene or served as cometabolites themselves. Root exudates increased heterotrophic and microbial phenanthrene-degraders in the soil compared to soil amended with only root debris, likely because root exudates provided higher carbon and nitrogen than root debris (Miya and Firestone, 2001). Plants possess oxygenase, peroxidase, and laccase enzymes which may allow phytodegradation of PAHs, though this has not been clearly demonstrated (Criquet *et al.*, 2000). As a result, phytostimulation, rather than phytodegradation alone, is likely the main phytoremediation mechanism for PAH biodegradation.

2.4 Phytoremediation of PAHs in soil

Numerous studies have been performed to evaluate the effectiveness of plants for PAH biodegradation. Planted treatments using *Festuca arundinacea* Schreb. (fescue),

Sorghum vulgare L. (sudan grass), and *Panicum virgatum* L. (switchgrass) resulted in significantly lower soil concentrations of pyrene and anthracene and higher soil microbial counts in a greenhouse study after 24 weeks compared to the unplanted treatment (Schwab, 1994). In a follow-up to the preceding study, the same research group added another species, *Medicago sativa* L. (alfalfa) to the previous three plant species mix which was shown to reduce the soil PAH concentration more effectively than the unvegetated treatments under greenhouse conditions (Reilley *et al.*, 1996). This research team conducted phytoremediation field trials for treatment of industrially contaminated PAH soils and demonstrated that planted treatments significantly enhanced dissipation of the target pollutants (Fiorenza *et al.*, 2000). The authors of this report suggested that the successful planted trials may have been due to persistence of drought-tolerant species during the drought conditions of the study period, rather than comparison of relative abilities between the three species tested. Another research team also demonstrated that PAHs (benz(a)anthracene, benzo(a)pyrene, chrysene and dibenz(ah)anthracene) disappearance was greatly enhanced in vegetated soils (Aprill and Sims, 1990). A phytoremediation study conducted on a crude oil spill site showed that *Lolium annual* (rye grass) and *Stenotaphrum secundatum* L. (St. Augustine grass) were superior to *Sorghum biocolor* L. (sorghum) and an unvegetated control in reducing contaminant concentration (Nedunuri *et al.*, 2000). These studies support the use of phytoremediation of PAH-contaminated soil as an effective environmental rehabilitation technology.

2.5 Potential Sources of Variation in PAH Phytoremediation

Plant species and soil amendments may affect the results of PAH phytoremediation. When designing a phytoremediation strategy, a broad range of plant

species and soil amendments should be thoroughly researched for effectiveness prior to full-scale implementation. Different plant species have varied toxicity tolerances to PAHs. Not all plants can germinate and grow well in PAH-contaminated soils. Plant species were shown to differ in germination rates in PAH-contaminated soil derived from petroleum in a screen of 22 plant species (grasses, herbs, legumes) (Adam and Duncan, 1999). Certain plants may possess physiological characteristics beneficial to phytoremediation of PAHs. It has been proposed that flood-tolerant or wetland species may enhance aerobic PAH microbial metabolism by more efficiently transporting air to the rhizosphere and thereby improve the soil conditions for the initial oxidative step in bacterial PAH metabolism (Shimp *et al.*, 1993). Plants can provide varying amounts and types of carbon sources that support microorganisms in general (Yoshitomi, 2001). Different plant species produce different types and quantities of exudates, such as cometabolites, that may potentially favor PAH-degrading microbes (Leigh *et al.*, 2002; Miya and Firestone, 2001; Siciliano and Germida, 1998b). In contrast, some root secreted compounds may inhibit PAH-degrading bacteria by providing allelochemicals (Brady and Weil, 1999; Leigh *et al.*, 2002). When evaluating phytoremediation as a potential environmental rehabilitation strategy, it is essential to examine numerous plant species due to the wide range of potential rhizosphere contributions and plant adaptations to varied climate and edaphic conditions.

Different plant species have been demonstrated to possess varying abilities to decrease PAH concentration in soils. Nine plant species were tested individually for pyrene degradation on soil spiked with 86.6 mg/kg pyrene. Plant species treatments had pyrene concentration reductions of 55-74% in the soil compared to reductions of no more

than 40% from the unplanted soil over a period of 8 weeks (Liste and Alexander, 2000). Degradation rates of total petroleum hydrocarbon-contaminated soil by three different plant species treatments were compared and the leguminous plants resulted in the most degradation (Yateem *et al.*, 2000).

Since each contaminated site condition is unique, it is essential to pre-test plant and soil amendment combinations to achieve optimal phytoremediation. Soil additives such as compost can have several beneficial effects on phytoremediation of contaminated soil. Amendments can increase nutrient levels, organic matter content, cation exchange capacity, and nutrient availability, and decrease bulk density and pH (Brady and Weil, 1999). Compost can increase the microbial activity and specifically the number of pollutant degraders in soil. Addition of steer manure compost to contaminated soil was shown to enhance degradation of 1,3-dichloropropene after 8 weeks of treatment (Ibekwe, 2001). It has been demonstrated that the bacterial and fungal populations were increased in numbers and diversity in pesticide-contaminated soil amended with yard compost compared to unamended soil (Cole, 1994). Poultry litter and peat moss soil amendments have been demonstrated to increase plant biomass and percentage accumulation of radioactive contaminants, ^{137}Cs and ^{90}Sr , compared to controls with no amendments (Entry *et al.*, 2001).

Addition of soil amendments, however, does not always lead to improved remediation. Amending the soil with chemical cometabolites, inorganic/organic nutrients and surfactants in an attempt to enhance bioremediation led to decreased mineralization of ^{14}C -phenanthrene and ^{14}C -pyrene in 5 spiked soils (Carmichael, 1997). These supplements increased the population of heterotrophic microorganisms, but not that of

PAH-degrading microorganisms (Carmichael, 1997). It was hypothesized that degrader-microbes used the amendments as carbon sources rather than degrading the target contaminant (Carmichael, 1997). Furthermore, organic contaminants can become less available for microbial degradation if organic amendments are used because organic contaminants sorb to organic matter. The use of amendments in environmental rehabilitation should be evaluated for effectiveness in greenhouse experiments and pilot-field studies prior to large-scale application because amendments may not always improve remediation.

SUMMARY & CONCLUSION

Persistent organic pollutants (POPs), such as polycyclic aromatic hydrocarbons (PAHs) are hydrophobic chemicals that biomagnify through the food chain. As a result of their low water solubility, PAHs sorb strongly to soil and sediment and become unavailable for biodegradation.

Phytoremediation is a form of environmental remediation that uses plants to treat organic and inorganic contaminants. Phytoremediation has several advantages over other remediation strategies; it is minimally destructive, cost-effective, aesthetically pleasing, and potentially self-sustaining. Soil PAHs are reduced predominantly by microbial degradation, which has been observed to be enhanced in rhizosphere conditions. Abiotic processes are considered relatively insignificant for natural reduction of soil PAHs. Phytoremediation has been successfully demonstrated by numerous laboratory and field studies as a promising technology for treatment of PAH-contaminated soils.

Selection of plant taxa and application of soil amendments should be researched carefully because not all treatments have been shown to be beneficial for

phytoremediation. In this thesis study, a variety of native Michigan plant species were evaluated for PAH phytoremediation in industrially-impacted soils collected from the Rouge Manufacturing Complex. It is hoped that this study will enhance our knowledge of PAH-phytoremediation processes and produce results that will be useful for large-scale environmental rehabilitation efforts.

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CHAPTER II

FIELD STUDY OF MICHIGAN NATIVE PLANTS FOR PHYTOREMEDIATION OF PAH-CONTAMINATED SOIL

ABSTRACT

Field Study of Michigan Native Plants for Phytoremediation of PAH-Contaminated Soil

Phytoremediation of polycyclic aromatic hydrocarbons (PAHs) was evaluated as a strategy to rehabilitate soil contaminated from coking operations of the Ford Rouge Manufacturing Complex (Dearborn, MI). A Phytoremediation Demonstration Site consisting of 3 plots (wetland, upland, and control) was constructed in Sept 2000 at the Allen Park Claymine (Allen Park, MI). The effects of 18 Michigan native plant species on the concentrations of 14 PAHs in amended Rouge soil were evaluated in the upland plot over the 2001 growing season. The soil total PAH (sum of 14 PAHs) concentration (soil [tPAH]) decreased at a rate of 10 mg/kg per 2 months in the entire plot from May to September 2001. The soil [tPAH] of the plot in May, July and September were approximately 109 mg/kg, 100 mg/kg, and 91 mg/kg, respectively. Four of the 18 plant species significantly reduced soil [tPAH] in the upland plot from July to September, unlike unplanted control cells which showed no significant reduction. Only one treatment (*Eupatorium purpureum*) had significantly lower soil [tPAH] than the unplanted control at the end of the 2001 season. Two planted treatments (*Andropogon scoparius* and *Eupatorium perfoliatum*) had significantly lower soil [tPAH] than the unplanted treatment in July, but this early-season effect did not persist for the September samples. The soil [tPAH] results agree with those from preliminary greenhouse studies conducted in collaboration with Applied Phytogenetics research labs. The study will run for 3 years, during which time vegetated soils could continue or even accelerate PAH biodegradation. Early observations from this field experiment indicate phytoremediation may be an effective strategy for rehabilitation of PAH-contaminated soils.

INTRODUCTION

Former industrial sites are often impacted with hazardous materials, are poorly vegetated, and possess infertile soils and sediments. If such land remains abandoned, wind and water erosion can accelerate soil degradation to the point at which natural soil rehabilitation is not possible. The Ford Rouge Manufacturing Complex (Dearborn, MI, U.S.A.) was once the world's largest integrated industrial site and is a historical icon of the American industrial age. Eighty years of automobile and steel manufacturing, however, have led to contamination of the facility with byproducts of these activities. Phytoremediation, the use of plants to degrade, detoxify, or remove environmental contaminants, is one method of environmental rehabilitation that is being considered as a potential remedial treatment for PAH-contaminated soils in unused areas of the Complex.

The primary contaminants found at the Coke Oven area of the Rouge Manufacturing Complex are polycyclic (polynuclear) aromatic hydrocarbons (PAHs or PNAs), which were formed by the processing of coal during coke production for the smelting of iron ores. PAHs consist of two or more fused benzene and furan rings arranged linearly, angularly or in clusters (Fig. 1.1). PAHs are formed as a result of incomplete, oxygen-deficient combustion of organic materials (Heil, 1998). Temperature and combustion processes rather than fuel type determine which PAHs are formed (Giger, 1974; Jenkins *et al.*, 1996; Blumer, 1975). PAHs are derived from both natural and anthropogenic sources (Hites, 1977). PAHs are formed naturally as a result of thermal geologic reactions, forest and brush fires, and plant and bacterial reactions (Blumer, 1976). Anthropogenic activities over the last century, such as the combustion of fossil fuels, wood burning, industrial coke production, petroleum refining, coal and oil

shale conversion and chemical manufacturing, have led to high levels of PAH discharges disrupting the natural balance between PAH production and degradation (Hites, 1977; Suess, 1976). PAHs are highly hydrophobic and have low solubility in water (Table 1.1) causing them to be highly recalcitrant to decay in soils and sediments (Harayama, 1997). PAHs pose environmental hazards because they are carcinogenic, teratogenic, and mutagenic (Shabad, 1975; Sims and Overcash, 1983; Dipple, 1990). It is therefore necessary to remediate land contaminated with PAHs to prevent harmful exposure to humans and wildlife.

Phytoremediation, vegetation-based environmental detoxification, could be an effective method of reducing soil PAH concentration. Microbial bioremediation, the use of fungi and bacteria to degrade contaminants, has been used to treat PAH-contaminated soils. Plants have been shown to be beneficial for PAH-bioremediation of contaminated soils. Soils planted with *Festuca arundinacea* Schreb. (fescue), *Sorghum vulgare* L. (sudan grass), and *Panicum virgatum* L. (switchgrass) resulted in significantly lower soil concentrations of pyrene and anthracene compared to unplanted soils after 24 weeks in a greenhouse study (Schwab, 1994). Plants were also shown to accelerate the disappearance of several high molecular weight PAHs compared to unvegetated soils (Aprill and Sims, 1990). Vegetated soil had significantly lower soil PAH concentrations compared with the unvegetated control (Reilley *et al.*, 1996). This study showed the presence of plant roots and added organic substrates, such as organic acids, were essential for sustained PAH degradation. This research suggests that effective PAH biodegradation may depend on consistent supplies of root exudates and other readily metabolizable

carbon resources (Reilley *et al.*, 1996). Collectively, these studies indicate that vegetated soils may be superior to unplanted soils for treatment of PAH-contaminated soils.

Plants may assist soil bioremediation by several mechanisms, including induction of microbial degradation gene activity and enhancement of microbial biomass and community diversity. Several studies have demonstrated that plant-produced compounds may stimulate microbial PAH degradation. Plants secrete carbon compounds such as organic acids and carbohydrates for utilization by microorganisms (Yoshitomi, 2001). Root exudates may promote microbial cometabolic degradation of organic pollutants (Leigh *et al.*, 2002). High molecular weight PAHs or PCBs may be degraded by microorganisms, but cannot function as the sole carbon source. Some root secreted compounds may serve as both metabolizable carbon sources and inducers for expression of PAH-degradation pathway genes. Plants have been shown to release exudates similar in structure to organic contaminants and lead to increased microbial activity and contaminant degradation (Siciliano and Germida, 1998). Roots of *Morus rubra* (red mulberry) to produce phenolic compounds which were demonstrated promote the growth and activity of PCB degrading microbes (Hegde and Fletcher, 1996). PCB-degrading bacteria, *Alcaligenes eutrophus* H850, *Corynebacterium sp.* MB1, and *Pseudomonas putida* LB400, were observed to be increased in numbers and degrading activity by plant root compounds (Donnelly *et al.*, 1994). Plant phenolic compounds may also cometabolically induce PAH-degrading bacteria as well as PCB-degrading bacteria (Donnelly *et al.*, 1994; Hegde and Fletcher, 1996). In addition, plants possess the enzymes oxidase, laccase, and peroxidase, which may contribute to PAH oxidation and metabolism (Criquet *et al.*, 2000). Plant root 2,7-diaminofluorene-peroxidases were

demonstrated to increase in abundance by the presence of anthracene and mycorrhizal fungi (Criquet *et al.*, 2000). These studies have shown that plants themselves may have enzymes capable of PAH degradation and also produce root exudates that may act as cometabolic carbon sources for induction of microbial PAH-degrading genes.

Rhizosphere soils have been shown to possess higher microbial counts compared to unplanted soils resulting in more effective PAH bioremediation. Vegetated soils were observed to possess 1400×10^5 bacterial colony forming units (CFU) per gram of soil, while soil in the absence of plants had 6×10^5 CFUs (Schwab, 1994). Similarly, researchers showed that vegetated contaminated soil had 4-8 log CFU/g compared with non-vegetated contaminated soils, which had a log CFU/g soil range from 3-6, a ten- to hundred-fold increase (Yateem *et al.*, 2000). For both studies, the observed increase in bacterial numbers for vegetated soils was proposed to be responsible for enhanced PAH degradation.

PAH phytoremediation is not currently a well-developed environmental rehabilitation technology. A limited number of plants species have been demonstrated and reported to be effective for PAH degradation in field conditions. Different plant taxa are known to possess widely different capabilities for PAH phytoremediation with little understanding of the range of potential mechanisms. Previous PAH phytoremediation studies have made either very general, empirical observations or focused on limited numbers of plant species. Most research publications report PAH phytoremediation experiments using only a single species (Qiu *et al.*, 1997), few individual species (Nedunuri *et al.*, 2000; Pradhan *et al.*, 1998; Reilley *et al.*, 1996), or a mixture of small numbers of species (Aprill and Sims, 1990; Fiorenza *et al.*, 2000).

Phytoremediation remains an attractive method of soil rehabilitation, though few studies have tested a comprehensive range of different plant species under field conditions. The project reported in this thesis attempts to use a broad spectrum of Michigan native plants to evaluate each species' relative effectiveness for soil PAH phytoremediation. Eighteen selected plant species were evaluated for phytoremediation of 14 PAH compounds in a pilot-scale field study. It is expected that this experiment will provide additional resources to enhance our understanding of PAH phytoremediation and its utility for rehabilitation of PAH-contaminated soils.

MATERIALS AND METHODS

Plant species selection

A preliminary 14-week greenhouse study was conducted to identify plant taxa with potential to reduce PAHs. The experiment tested ~40 native Michigan native plant species for soil PAH reduction in collaboration with the Applied PhytoGenetics laboratory (APGEN; Athens, GA). Soil was obtained from the Rouge Coke Oven area, amended with 30 % perlite (v/v), placed in 400 cc plastic pots and planted with plant plugs. Planted and unplanted control pots were watered and fertilized weekly with 6% of a stock solution of Peter's N-P-K (20-20-20). Untreated control pots were not amended and were maintained in the greenhouse without fertilization or watering. Native Michigan plants were obtained from Wildtype Native Plant Nursery (Mason, MI). Soil and plant tissue sampling was performed after treatment periods of 4, 6, 10 and 14 weeks. Single, whole-pot samples were analyzed at each interval for each species.

Twenty species were selected from preliminary data after 6 weeks of the APGEN study (Table 2.1). For ease of presentation and discussion, 6 letter abbreviations were

assigned for plant species based on the first 3 letters of the both the first and second names of the plant scientific name (Table 2.1).

Table 2.1. Species planted in Phytoremediation Demonstration Facility. For Plot: W = Wetland, U = Upland, C = Control.

Scientific Name	Common Name	Abbreviations	Plot
<i>Amorpha canescens</i>	Leadplant	AMOCAN	U, C
<i>Andropogon gerardii</i>	Big Bluestem	ANDGER	U, C
<i>Andropogon scoparius</i>	Little Bluestem	ANDSCO	U, C
<i>Aster novae-angliae</i>	New England Aster	ASTNOV	W, U, C
<i>Carex sprengelii</i>	Sprengel Sedge	CARSPR	W, U, C
<i>Ceanothus americanus</i>	New Jersey Tea	CEAAME	U, C
<i>Cirsium discolor</i>	Pasture Thistle	CIRDIS	U, C
<i>Eupatorium perfoliatum</i>	Boneset	EUPPER	W, U, C
<i>Eupatorium purpureum</i>	Joe-Pye Weed	EUPPUR	U, C
<i>Geum triflorum</i>	Prairie Smoke	GEUTRI	U, C
<i>Hystrix patula</i>	Bottlebrush Grass	HYSPAT	U, C
<i>Lobelia cardinalis</i>	Cardinal Flower	LOBCAR	U, C
<i>Mimulus ringens</i>	Monkey-Flower	MIMRIN	W, C
<i>Physocarpus opulifolius</i>	Common Ninebark	PHYOPU	U, C
<i>Scirpus atrovirens</i>	Bulrush	SCIATR	W, U, C
<i>Silphium teribinthinaceum</i>	Prairie-dock	SILTER	U, C
<i>Solidago patula</i>	Swamp goldenrod	SOLPAT	W, C
<i>Spartina pectinata</i>	Prairie Cordgrass	SPAPEC	W, U, C
<i>Spirea alba</i>	Meadowsweet	SPIALB	U, C
<i>Viburnum dentatum</i>	Arrowwood Viburnum	VIBDEN	U, C

Field Trial of Selected Native Michigan Plant Species

The Rouge Manufacturing Complex is still in operation for steel production and is not suitable for field experimentation. Therefore, a Phytoremediation Demonstration (Phyto Demo) site was constructed at the Allen Park Claymine (Allen Park, MI) in conjunction with URS Corporation under the direction of Michael Coia, Project Engineer (Willow Grove, PA) in the summer of 2000 (Fig. 2.1). Pits were excavated for the construction and installation of the treatment plots measuring 20' x 50' each for the

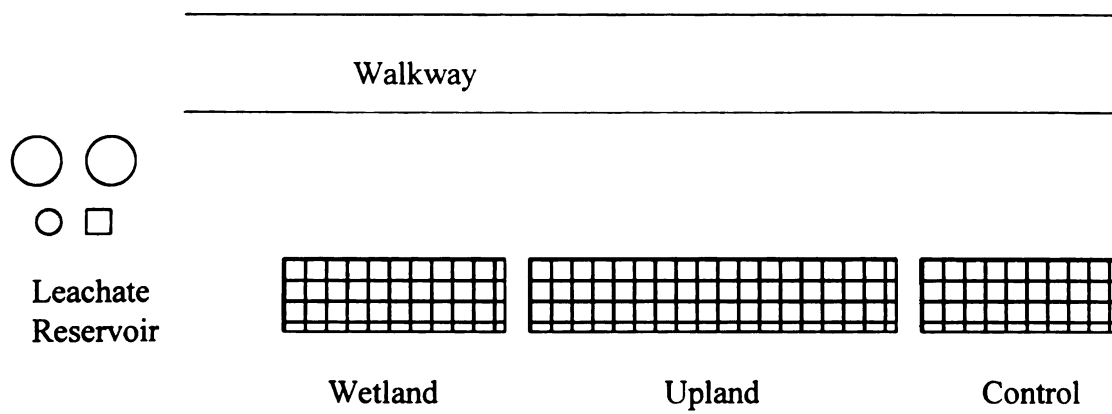


Figure 2.1. Overhead view of Phytoremediation Demonstration Site. Distances not drawn to scale.

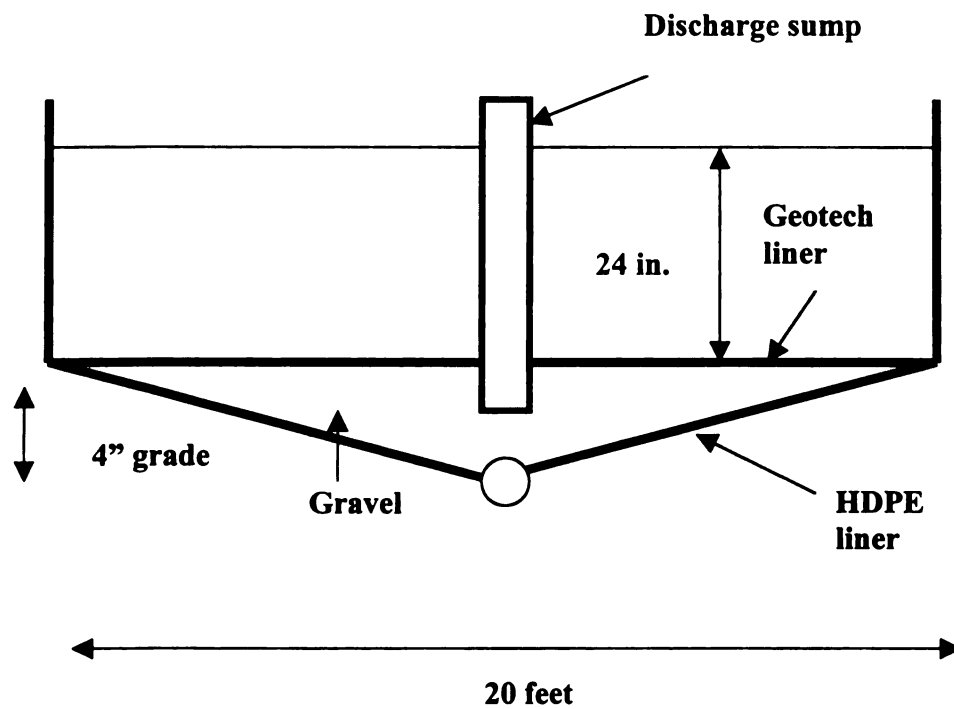


Figure 2.2. Phyto Demo Treatment Plot cross-section schematic. HDPE refers to high density polyethylene.

wetland and control plots and 20' x 100' for the upland plot. The upland plot was twice as large as the wetland because most of the terrain at the Rouge Manufacturing Complex is upland and a potential remediation installation at this site would be for this habitat. Each plot was bordered by ~1' berm of uncontaminated soil and overlaid with 60 mil high-density polyethylene (HDPE) liner to prevent escape of contaminated soil or leachates from the treatment plots. Approximately 4" of pea gravel was laid on the HDPE-lined plot in the graded slope at the bottom of each plot (3% incline from long axis side to center) to facilitate drainage and overlaid with geotech fabric to contain the soils (Fig. 2.2). Sump tanks and float-ball actuated pumps were installed to remove excess leachate to nearby holding tanks for testing and eventual discharge into a sanitary sewer.

PAH-contaminated soil was obtained from the area adjacent to the Coke Oven system of the Ford Rouge Manufacturing Complex (Dearborn, MI). Uncontaminated control soil was topsoil from the claymine landfill. PAH-contaminated and uncontaminated control soils were amended with (5% v/v) poultry manure (Herbruck's Poultry Ranch, Saranac, MI) and (10% v/v) yard compost (Charter Township of Ypsilanti, MI). All soil and compost amendment components for the Phyto Demo site were sieved then mixed using a mechanized soil shaker-screen (2" debris exclusion) and a front-end loader. Compost-amended, Coke Oven soil was placed in the wetland and upland plots and amended, uncontaminated soil was placed in the control plot. Compost-amended soil from the Phyto Demo site and native Coke Oven soil were tested for agronomic and physical properties (Tables 2.2 & 2.3) by MDS Harris Laboratories (Lincoln, Nebraska). Both the native Coke Oven soil and the Phyto Demo soil were

Table 2.2. Properties [mean, standard deviation (SD)] of Rouge Manufacturing Complex Coke Oven soil (unamended–no yard compost or poultry manure) and Phytoremediation Demonstration site soil [amended with 10% yard compost and 5% poultry manure (v/v)], $N = 2$. Amended soils were sieved (4.75 mm). NA means standard deviation was not available because $N = 1$. Soil pH and soluble salt determined using 1:1 water to soil ratio.

	% Sand	% Silt	% Clay	Bulk density	pH	Soluble salts (mmhos/cm)	Sodium (mg/kg)	% OM	CEC (meq / 100g)
Unamended Rouge soil									
Mean	72	24	4	1.1	8.3	0.3	140	7.85	17.05
SD	2.8	2.8	0	0.07	0	0.02	5.67	0.07	0.92
Amended Rouge soil									
Mean	70	24	6	1.2	8.1	1.2	124	2.8	18.7
SD	NA	NA	NA	0	0	0.14	2.83	0.28	1.56

Table 2.3. Concentrations of nutrients [mean, standard deviation (SD)] of soil Rouge Manufacturing Complex Coke Oven soil (unamended–no yard compost or poultry manure) and Phytoremediation Demonstration site soil [amended with 10% yard compost and 5% poultry manure (v/v)], $N = 2$. Amended soils were sieved (4.75 mm). Note, N is nitrate determined by cadmium reduction method, P is determined by Bray I or Olsen method, cations (Ca, Mg, K, Na) extracted by modified ammonium acetate method, trace elements (Zn, Mn, Cu, Fe) extracted by modified diethylenetriaminepentaacetic acid (DTPA) method; organic matter determined by loss-on-ignition, sulfur (S) and boron (B) were determined by inductively coupled plasma (ICP) emission spectroscopy.

	Nutrient (mg/kg)										
	N	P	K	Mg	Ca	S	Zn	Mn	Cu	Fe	B
Unamended Rouge soil											
mean	2.5	3.5	92	240.5	2877	29.5	16.6	22.2	5	27.7	1.1
SD	0.7	0.7	9.9	33.2	79.2	3.5	2.0	0.9	0.9	5.0	0.1
Amended Rouge soil											
mean	72	82	570	348.5	2756	89.5	17.35	18.5	3.9	50.4	2.0
SD	5.7	0	59.4	38.9	213.6	7.8	2.1	2.3	0.4	5.9	0.2

classified as sandy loam (MDS Harris Laboratories, Lincoln, NE). Amended soils were placed into each of the plots at a loose-filled depth of 24" (Fig. 2.2). The plots were overlaid with wood planks on the soil surface to demarcate individual cells (~3.5' x 4' = ~20 sq. ft). The wetland and control plots consisted of 30 cells and the upland plot consisted of 60 cells (Fig. 2.3). Individual cells were assigned plant treatments designated alphanumerically as shown (Fig. 2.3).

Plants were planted at the Rouge Phyto Demo site in September 2000. Plug- or quart-sized plants were taken out of pots, roots cleaned of potting soil, and transplanted into the individual cells on ~9" centers (12 plants per cell). Plants were planted in 3 cells per species in either or both upland and wetland plots according to their ecological habitat range (Table 2.1). The control plot had one cell per plant species and was used to assess herbivory or pathogen problems and background soil PAH concentrations. The Phyto Demo field plots were fertilized with an N-P-K solution (20-20-20) at a concentration of ~475 ppm weekly. A substantial number of plants (about 50%) were replaced in May 2001 due to overwinter mortality.

Sample Handling

For the summer 2001 growing season, soil samples were collected three separate times at 9-week intervals in mid May, July and September. At each sampling time, three 2" diameter-cores of 8" depth were removed from each cell. Each core sample excluded the first 2 inches of the surface to avoid soil PAH content altered by photolysis or evaporation. Rocks were separated from core samples in plastic tubs and the soil placed in 150 mL amber jars with teflon caps. The amber jars containing the soil samples were transported from the field in coolers containing ice-packs and stored in the 4°C walk-in

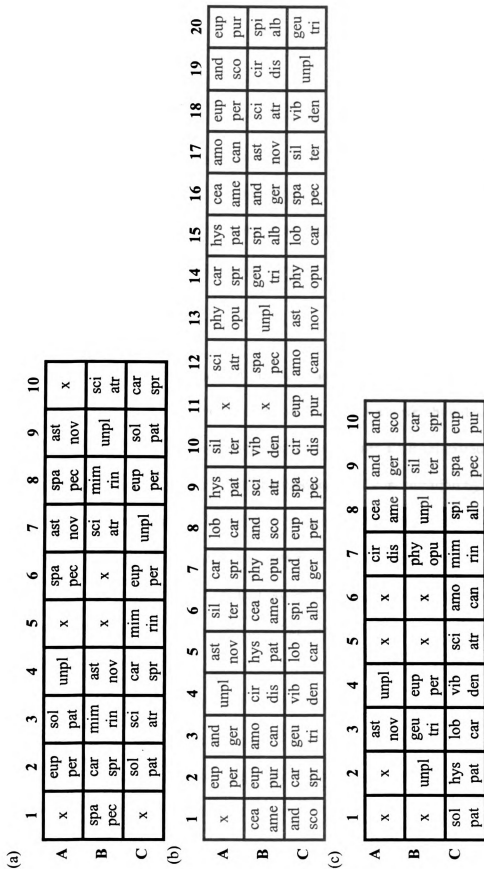


Figure 2.3. Phyto Demo Treatment Plots Layout schematic (a) Wetland Plot, (b) Upland Plot, (c) Control Plot. Each square represents a cell containing a plant species treatment or unplanted treatment. Refer to Table 2.1 for a list of plant species abbreviations. "Unpl" refers to Unplanted treatment. Cells marked with an "x" were not planted and not sampled.

refrigerator at the MSU Phyto laboratory. Soil samples were sieved using a stainless steel 8-inch diameter 2.36 mm sieve (Gilson Co.) to remove rocks, mulch or other debris before analysis. May soil samples were sieved immediately before each samples were extracted. July and September soil samples were sieved approximately one week after sampling and stored at 4°C until extraction. Plant leaf samples were obtained from 3 plants from each cell in July. Fresh soil samples, $\sim 5.0 \pm 0.1$ g, were placed in a drying oven for 48 hours at 105 °C for soil moisture and dry weight determination. Plant dry weight was determined after drying plant tissue samples in paper bags at 80 °C for 48 hours.

Extraction Protocol

Plant and soils were analyzed for PAH content by dichloromethane extraction. Plant tissue extractions were performed on $\sim 1.5 \pm 0.1$ g fresh weight unless there was insufficient tissue, in which case lesser amounts were used for PAH extraction and dry weight determination. Soil cores were analyzed for PAH concentration by dichloromethane extraction of 3.0 ± 0.1 g FW subsamples (1 subsample for May, 3 subsamples for July and Sept). Plant and soil PAHs were analyzed by phase extraction in 3 mL saturated potassium chloride solution and 10 mL of the organic extraction solvent, dichloromethane, in a 20 mL amber vials. The extract mixtures were vortexed for 20 seconds, sonicated for 10 minutes, and placed on a rotating shaker (~ 125 rpm) overnight. Sample extracts were filtered using 3 mL polypropylene sterile disposable (B&D, Fisher Scientific) syringes and 13 mm 0.45 μ m PTFE teflon syringe filters (SGE, DC Scientific) during transfer to 2 mL gas chromatography (GC) vials. Extraction vials were re-used

after being washed in soapy water, rinsed sequentially with acetone and pure water, and oven-dried.

The May soil samples and a portion of the July soil samples were shaken with the vials in the upright position. A test was performed to compare recovery rates when vials were placed in the upright versus the horizontal position. Results from this test indicated samples from vials placed horizontally on the shaker had 30-70% higher concentrations than samples from vials placed in an upright position (data not shown), therefore invalidating the results from the upright extracted samples. Compromised samples were re-extracted with the vials placed horizontally on the shaker using remaining soil from the jars stored at 4°C. For May samples, only one subsample from each jar was taken for [tPAH] determination due to the insufficient volume of the remaining soil for 3 subsamples.

Gas Chromatography-Flame Ionization Detector (GC-FID) Analyses

PAH analyses were performed on an Agilent 6890 Gas Chromatograph equipped with an Agilent 3396B/C Integrator and Agilent 7683ALS auto injector. An Alltech AT-5 capillary column with inside diameter of 0.53 mm, purchased length 30.0 m and film thickness 1.20 μm (Alltech: Deerfield, IL) was used for PAH compound separation. The column was cut to minimum length 15.0 m to exclude residue build up at the front of the column as it aged. The carrier gas was helium delivered at a rate of 5.4 mL/minute and fuel source for the FID was H_2 delivered at 40.0 mL/minute. The make-up gas consisted of N_2 (flow rate of 45 mL/minute) and 0.1 grade air (flow rate of 450 mL/minute). The capillary column oven was set with an initial isothermal period of 100° C for 1 minute followed by elevation at 100 °C/minute until 310° C was reached. The volume of the

injected sample was 5 μ L and the temperatures of the injection port and the detector were 270° C and 330° C, respectively.

The standards were made from EPA 610 PAH standard mix (Supelco, Bellefonte, PA), which includes the PAH compounds in Table 2.4. Calibration curves consisted of 3 to 8 points using 1% to 50% dilutions made of EPA 610 mix stock reagent. We assumed a standard curve through the origin. The determined concentrations of the first four compounds that eluted, naphthalene, acenaphthylene, acenaphthene, fluorene, were not reliable because the concentrations found in the samples were often lower than the lowest standard read by the GC-FID. However, these concentrations are included in the determination of total PAH concentration. Vials containing only 3mL KCl and 10mL dichloromethane or 10mL dichloromethane alone were analyzed as “blank” controls.

Calculations

1. GC-FID output (20.2 μ g/mL) sample extract concentration (mg/kg = μ g/mL) corrected by extraction volume and dilutions:

$$\text{e.g. } 20.2 \mu\text{g/mL} \times 10 \text{ ml} = 202 \mu\text{g in that sample}$$

2. μ g tPAH/g FW $\times$ g FW/g DW = μ g tPAH/g DW tissue or soil

$$\text{e.g. } 202 \mu\text{g PAH/g FW} \times 1.5 \text{ g FW/0.3 g DW} = 1010 \text{ mg/kg} \\ (\text{or } 1010 \mu\text{g/g DW})$$

The total PAH concentration [tPAH] was determined by taking the sum of the calculated concentrations of the compounds listed in Table 2.4. For ease of presentation and discussion, the full names of the PAH compounds analyzed were abbreviated (Table 2.4). Lowest calibration standard (1%) of EPA 610 standard mix for these compounds are displayed in (Table 2.4).

Table 2.4. Abbreviations and lowest calibration standard in 2001 for PAH compounds analyzed.

Compound	Abbreviation	Lowest standard (µg/mL)
Naphthalene	Naph	10
Acenaphthylene	Acny	20
Acenaphthene	Acne	10
Fluorene	Flre	2
Phenanthrene	Phen	1
Anthracene	Anth	1
Fluoranthene	Flra	2
Pyrene	Pyre	1
Benz(a)anthracene	Baan	1
Chrysene	Chry	1
Benzo(b)fluoranthene	Bbfl	2
Benzo(a)pyrene	Bapy	1
Dibenz(a,h)anthracene	Daha	2
Benzo(ghi)perylene	Bghp	2
Sum of concentrations for the above 14 PAH compounds	[tPAH]	56

Statistical Analyses

The upland soil [tPAH] data were tested for normality by analyzing stem-leaf plots, normal probability plots and residual plots. The upland May soil total PAH concentration ([tPAH]) data were analyzed using ANOVA and Students' *t*-test for significant differences between each pair of treatment means. The ANOVA test requires an equal number of subsamples per core. For May there was only one subsample per core and for July and September there were 3 subsamples per core. In order to compare May data to July and September data, two subsamples were arbitrarily excluded from the upland July and September soil [tPAH] data, and a two-way ANOVA statistical test was performed using May, July and September soil [tPAH] data. July and September soil [tPAH] data were analyzed using two-way ANOVA with all 3 subsamples. The Student's *t*-test was used to test for significant differences between pairs of treatment means at each sampling time and for significant differences between treatment means at different sampling times. A less conservative statistically significant level, $\alpha = 0.1$, as opposed to the conventional level $\alpha = 0.05$, was used to enhance the ability to detect differences between treatments and between sampling times. All statistical analyses were done using SAS version 8.01.

RESULTS

Plant species selection

The APGEN greenhouse screen of 40 species showed general trends in soil total PAH concentration ([tPAH]) reduction for most treatments (Appendix 1). Eighteen plant species treatments out of 36 achieved greater reduction in soil [tPAH] than the unplanted

pots. This is represented by calculation of treatment index, i.e. average planted soil [tPAH] divided by unplanted soil [tPAH] (Appendix 2).

Plant Mortality

Substantial over-wintering plant mortality was observed in May 2001 at the Phyto Demo site after the initial planting in September 2000 (Appendix 3). *A. scoparius*, *A. novae-angliae*, *C. americanus*, *C. discolor*, *P. opulifolius*, *S. teribinthinaceum*, and *S. patula* had mortality rates greater than 50% after over-wintering for the first year (Appendix 3). Plant mortality was greatly reduced in ensuing seasons (Appendix 3).

Wetland

Wetland plot mean (including all planted and unplanted control treatments) soil [tPAH] appeared to fluctuate over time. The mean total PAH concentration [tPAH] and standard error of the wetland plot for May, July, and September were: 98.9 ± 4.4 mg/kg, 122.1 ± 5.3 mg/kg, and 76.1 ± 2.3 mg/kg. Standard errors represent variability among all cells for the plot.

Upland

The upland plot (including all planted and unplanted treatments) soil [tPAH] means $\pm$ standard error of mean (SEM) for May, July and September were 108.80 ± 3.2 mg/kg, 100.3 ± 3.2 mg/kg, and 90.9 ± 3.2 mg/kg, respectively. Soil [tPAH] was observed to decrease over time for most upland treatments (Table 2.5). The overall decrease in soil [tPAH] of the upland plot is illustrated by color assignment to average cell soil [tPAH] ranges (Fig. 2.4 a – c). Cell color codes are easily observed to shift from an abundance of red-orange-yellow cells (i.e. high [tPAH]) to an increase in blue-green cells (i.e. low [tPAH]) from May to September. Most cells were observed to decrease in

Table 2.5. Soil total PAH concentration [tPAH] (mean $\pm$ SEM ^{γ}) for treatments in the upland plot from sampling times in May, July, and September 2001, $N = 3$ cells for each treatment.

Treatment	Soil Concentrations (mg/kg)		
	May	July	September
<i>Amorpha canescens</i>	101.3 $\pm$ 15.0	90.4 $\pm$ 0.5	81.9 $\pm$ 6.3
<i>Andropogon gerardii</i>	106.7 $\pm$ 19.7	106.4 $\pm$ 4.8	84.4 $\pm$ 5.3†
<i>Andropogon scoparius</i>	105.8 $\pm$ 7.4	79.2 $\pm$ 8.9*	80.3 $\pm$ 10.9
<i>Aster novae-angliae</i>	117.9 $\pm$ 16.6	104.0 $\pm$ 4.2	84.1 $\pm$ 1.0†
<i>Carex sprengelii</i>	106.1 $\pm$ 4.2	92.5 $\pm$ 14.9	91.8 $\pm$ 2.6
<i>Ceanothus americanus</i>	106.5 $\pm$ 4.5	103.9 $\pm$ 12.7	96.6 $\pm$ 4.3
<i>Cirsium discolor</i>	108.5 $\pm$ 6.4	92.3 $\pm$ 6.9	87.5 $\pm$ 5.8
<i>Eupatorium perfoliatum</i>	97.1 $\pm$ 7.6	79.1 $\pm$ 4.3*	84.3 $\pm$ 8.4
<i>Eupatorium purpureum</i>	110.8 $\pm$ 13.5	91.9 $\pm$ 7.5	72.1 $\pm$ 10.5*†
<i>Geum triflorum</i>	111.5 $\pm$ 15.2	93.9 $\pm$ 6.7	114.3 $\pm$ 2.3*†
<i>Hystrix patula</i>	101.1 $\pm$ 12.7	105.3 $\pm$ 8.7	105.4 $\pm$ 8.4
<i>Lobelia cardinalis</i>	109.5 $\pm$ 14.4	107.8 $\pm$ 7.4	93.2 $\pm$ 4.7
<i>Physocarpus opulifolius</i>	118.3 $\pm$ 6.9	84.5 $\pm$ 5.8	101.0 $\pm$ 8.7
<i>Scirpus atrovirens</i>	124.7 $\pm$ 14.1	95.7 $\pm$ 7.9	104.8 $\pm$ 19.4
<i>Silphium teribinthinaceum</i>	110.9 $\pm$ 20.0	118.6 $\pm$ 15.7	92.9 $\pm$ 3.7†
<i>Spartina pectinata</i>	100.2 $\pm$ 4.5	90.6 $\pm$ 5.4	82.7 $\pm$ 3.4
<i>Spirea alba</i>	127.3 $\pm$ 25.0	85.9 $\pm$ 2.3	95.65 $\pm$ 4.9
<i>Viburnum dentatum</i>	90.2 $\pm$ 6.8	113.4 $\pm$ 6.1	107.8 $\pm$ 14.4
Unplanted	112.9 $\pm$ 7.3	100.8 $\pm$ 5.1	93.7 $\pm$ 5.4
SEM (ANOVA mixed model) ^{δ}	13.07	8.09	8.09

^{γ} Standard errors represent variability between the averages of soil [tPAH] from each cell for a given treatment.

^{δ} Standard error of mean based on ANOVA mixed model.

* Significantly different from unplanted at that sampling time (t -test, $\alpha = 0.1$).

† Significant difference between July and September means (t -test, $\alpha = 0.1$).

Table 2.6. Statistical results from two-way ANOVA analyses for the upland plot.

Source	Df	MS	F	P-value
<u>May, July, Sept (1 subsample per core)</u>				
Treatment*	18	1153.8	0.7	0.78
Error (MST)	28.9	1644.5		
Time	2	13677.3	8.0	<0.01
Treatment x Time	38	1123.5	0.7	0.89
<u>July & Sept (3 subsamples per core)</u>				
Treatment	18	472.2	2.0	0.04
Error (MST)	38	242.1		
Time	1	526.7	3.5	0.07
Treatment x Time	18	248.0	1.6	0.10
Error (MSE)	38	150.9		

*Note: treatment includes plant species' and unplanted treatments.

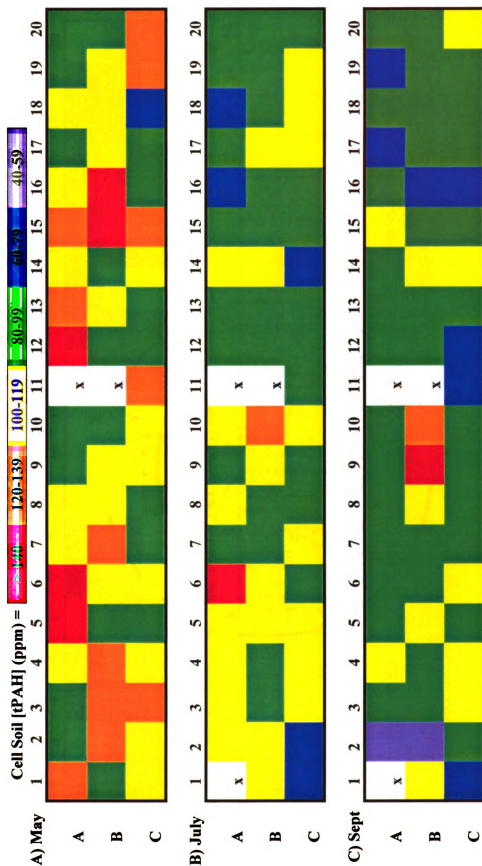
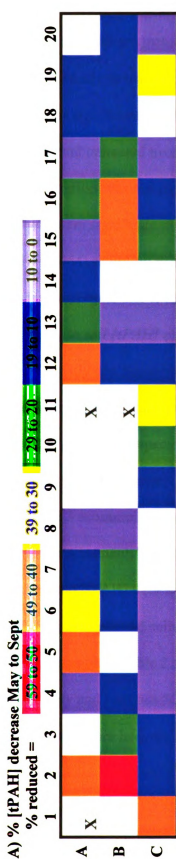


Figure 2.4. Upland Plot soil [rPAH] means (N=3). Ranges in ppm represented by colors (A) May, (B) July, (C) September. Cells marked with an "x" were not planted and not sampled. Images in this thesis are presented in color.



B) Coded treatments for cells in the upland plot – 2001 growth season

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
A	x	eup	per	ger	and	unpl	ast	nov	hys	sil	x	sci	phy	car	hys	cea	amo	eup	and	eup
B	cea	eup	amo	cir	hys	cea	phy	and	sci	vib	x	spa	unpl	geu	spi	and	ast	sci	cir	spi
C	ame	pur	can	dis	pat	ame	opu	sco	atr	den	eup	amo	ast	phy	lob	ger	nov	atr	dis	alb
	and	car	geu	vib	lob	spi	and	eup	spa	cir	pur	can	nov	opu	car	pec	sil	vib	unpl	geu
	sco	spr	tri	den	car	alb	ger	per	pec	dis	dis	can	nov	opu	car	pec	ter	den	unpl	tri

Figure 2.5. (A) Percentage change in mean cell soil total PAH concentration from May to September represented by colors in the upland plot (N=3). Cells marked with an “x” were not planted and not sampled. Cells without color had positive % change from May to September. **(B)** Treatment codes in cells. Refer to Table 2.1 for a list of plant species’ abbreviations. Note, “unpl” refers to the unplanted treatment. Images in this thesis are presented in color.

soil [tPAH] by 0-20 % (Fig. 2.5 a). For comparison of planted treatments to color codes, treatment ID codes are included in the adjacent figure (Fig. 2.5 b).

The analysis using one subsample showed the effect of sampling time on soil [tPAH] was significant (Table 2.6), the plant species' treatment effect (including unplanted and untreated treatments) was not significant, and the treatment x time interaction effect was not significant (Table 2.6). The one subsample analyses revealed that there were significant differences in mean soil [tPAH] between the May and July (t-test, $P = 0.064$) sampling times, July and September (t-test, $P = 0.045$) sampling times, and May and September (t-test, $P < 0.001$) sampling times.

Differences in soil [tPAH] over time for Upland Plot

For July and September sampling times the treatment effects were significant, the time effect was significant, and the treatment x time interaction effect was significant (Table 2.6). Four plant species (*A. gerardii*, *A. novae-angliae*, *E. purpureum*, *S. teribinthinaceum*) treatments showed significant decreases in soil [tPAH] from July to September (3 subsample analysis) (Table 2.5). Soil [tPAH] increased significantly in the *G. triflorum* treatment. The most effective phytoremediation treatment, *E. purpureum*, decreased soil [tPAH] 91.9 ± 7.5 to 72.1 ± 10.5 from July to September. By contrast, the soil [tPAH] in unplanted cells showed no significant difference between July and September samples (Table 2.5). Significant differences for specific treatments between May and July and between May and September could not be determined using 3 subsamples per core because May only had one subsample per core taken.

Differences in soil [tPAH] between treatments

There were no statistical differences among the 19 treatments (plant species and unplanted) for the May sampling time ($P = 0.95$; Table 2.5). Treatments *A. scoparius* and *E. perfoliatum* had soil [tPAH] significantly lower than the unplanted soil [tPAH] in July (Table 2.5). The September mean soil [tPAH] in cells planted with *E. purpureum* was significantly lower than that in the unplanted treatment (Table 2.5). By contrast, cells planted with *G. triflorum* had a significantly greater mean soil [tPAH] than the unplanted at the September sampling time (Table 2.5).

To compare APGEN data and Phyto Demo data, soil indices were calculated by dividing the soil [tPAH] for a planted treatment by the soil [tPAH] in the unplanted treatment for each sampling time, i.e. Planted soil [tPAH]/ Unplanted soil [tPAH]. An index of less than 1 indicates the planted treatment has reduced the soil [tPAH] to a greater extent than the unplanted treatment. An average soil index for each of the sampling times (APGEN, 4 sample times; Phyto Demo, 3 sample times) was calculated for each species common to both studies. In the Phyto Demo study, ten of the 18 plant species treatments had average soil indices less than one (Table 2.7). In addition, seven plant species treatments had soil indices less than one for all three sampling times. In both the APGEN and Phyto Demo studies *E. perfoliatum*, *A. scoparius*, *A. canescens*, *C. discolor*, *P. opulifolius* and *A. novae-angliae* treatments had average soil indices less than one (Table 2.7 and Appendix 2). Plant species treatments *S. alba*, *H. patula*, and *S. atrovirens* had average soil indices greater than one in both studies. Eleven out of the 17 plant treatments (71%) that were common to both studies have similar soil index values relative to the unplanted.

Table 2.7. Upland Phytoremediation Demonstration site 2001 soil indices ranked by "Avg" index. "Avg" is the average of May, July, and September soil indices.

Treatment	[Planted]/[Unplanted] Ratios				Each* indicates ratio < 1 at one sampling time
	May	July	Sept	Avg	
<i>E. perfoliatum</i>	0.86	0.78	0.90	0.85	***
<i>A. scoparius</i>	0.94	0.79	0.86	0.86	***
<i>E. purpureum</i>	0.98	0.91	0.77	0.89	***
<i>A. canescens</i>	0.90	0.90	0.87	0.89	***
<i>S. pectinata</i>	0.89	0.90	0.88	0.89	***
<i>C. discolor</i>	0.96	0.92	0.93	0.94	***
<i>C. sprengelii</i>	0.94	0.92	0.98	0.95	***
<i>A. gerardii</i>	0.94	1.06	0.90	0.97	**
<i>P. opulifolius</i>	1.05	0.84	1.08	0.99	*
<i>A. novae-angliae</i>	1.04	1.03	0.90	0.99	*
Unplanted	1.00	1.00	1.00	1.00	
<i>S. alba</i>	1.13	0.85	1.02	1.00	*
<i>C. americanus</i>	0.94	1.03	1.03	1.00	*
<i>L. cardinalis</i>	0.97	1.07	0.99	1.01	**
<i>H. patula</i>	0.89	1.04	1.12	1.02	*
<i>V. dentatum</i>	0.80	1.12	1.15	1.02	*
<i>G. triflorum</i>	0.99	0.93	1.22	1.05	**
<i>S. teribinthinaceum</i>	0.98	1.18	0.99	1.05	**
<i>S. atrovirens</i>	1.10	0.95	1.12	1.06	*

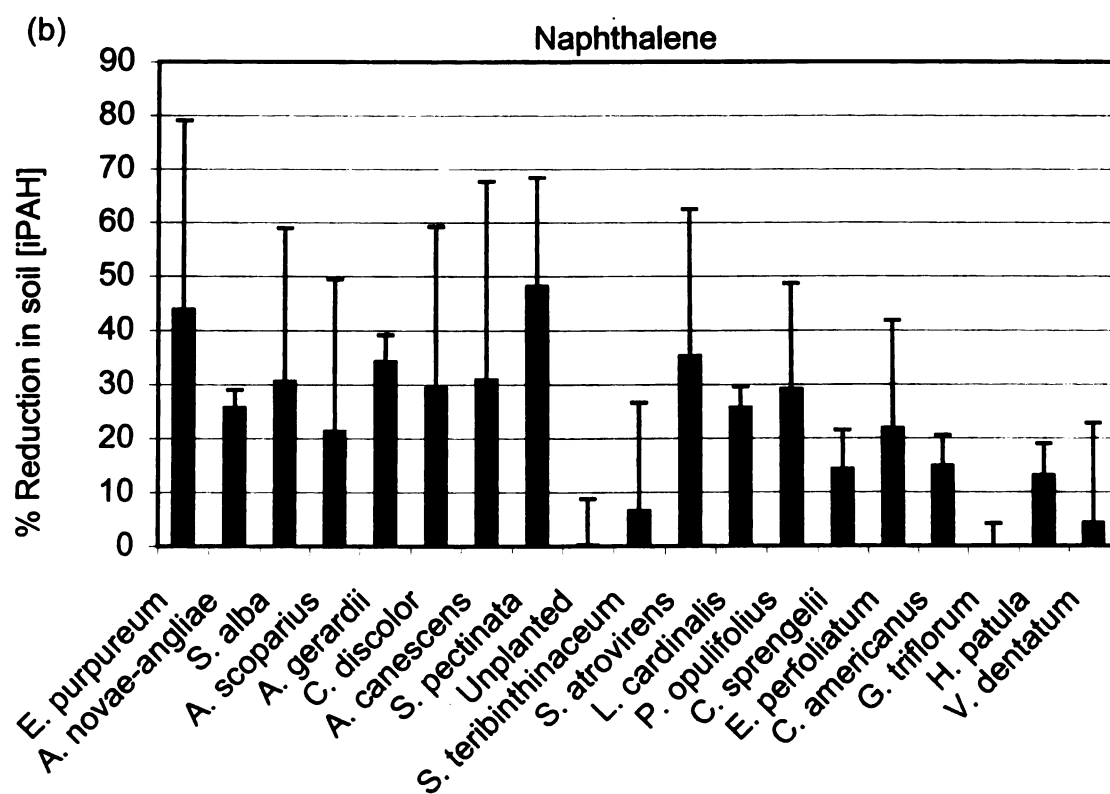
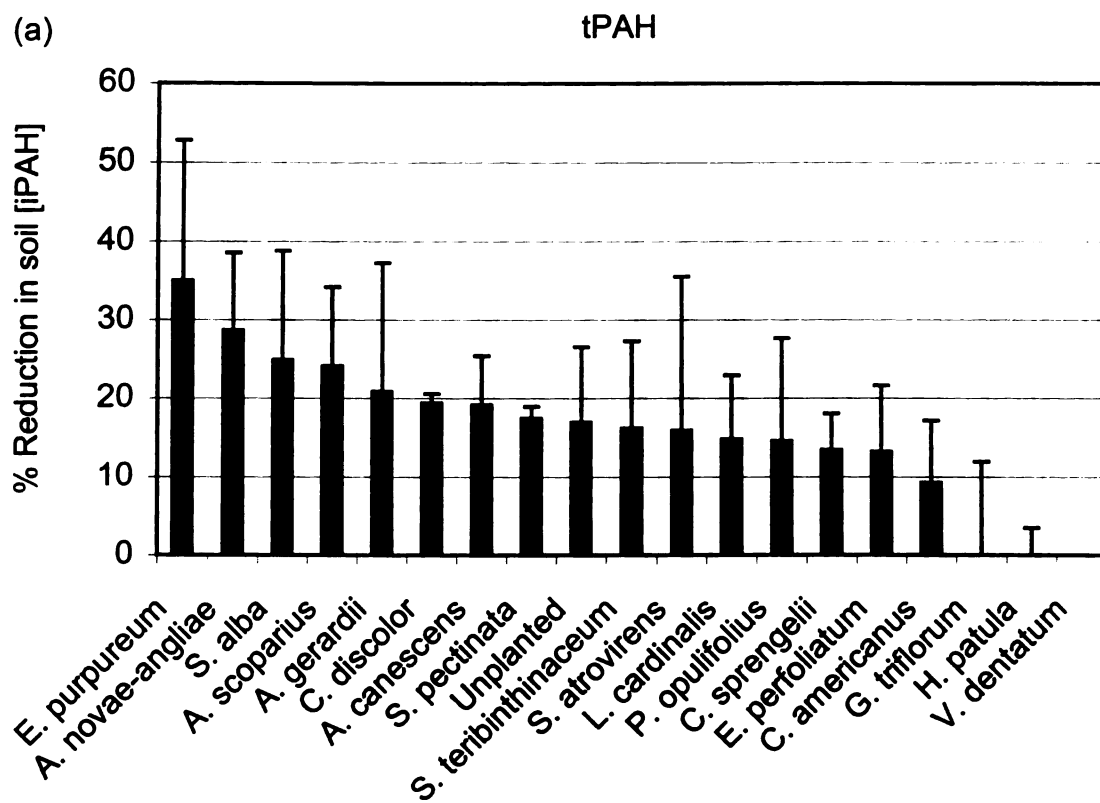
Soil [tPAH] percentage reduction

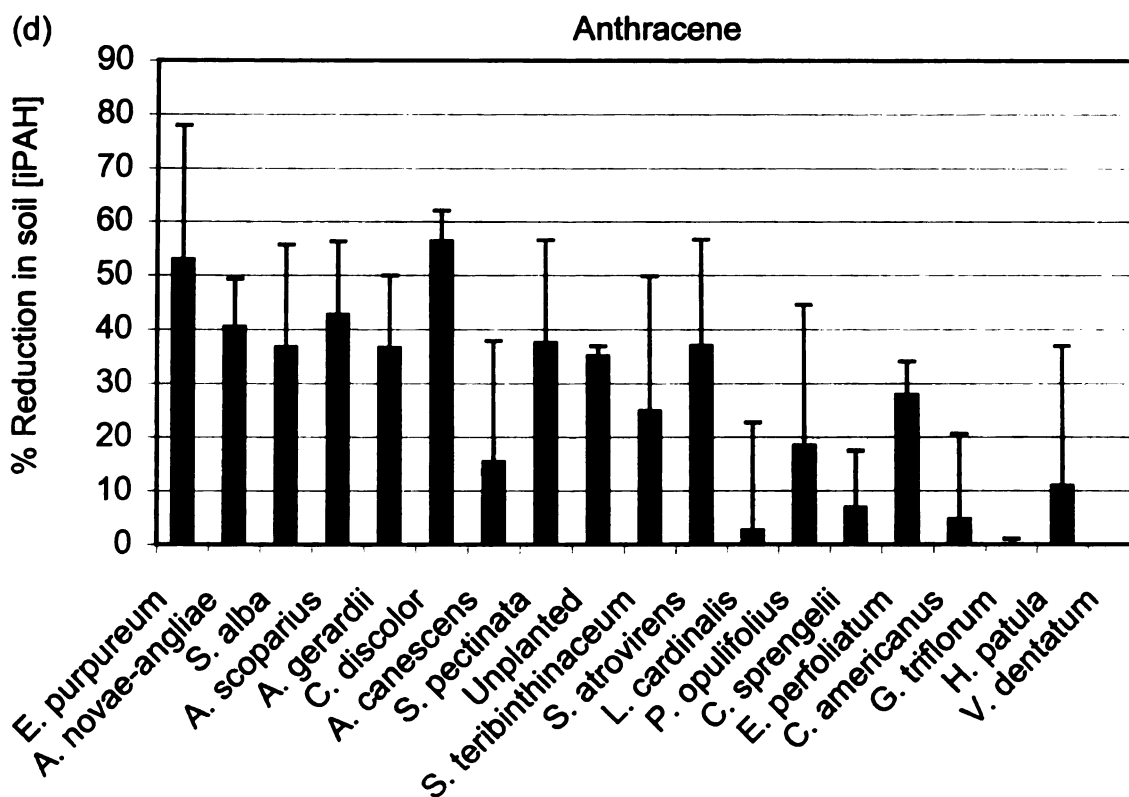
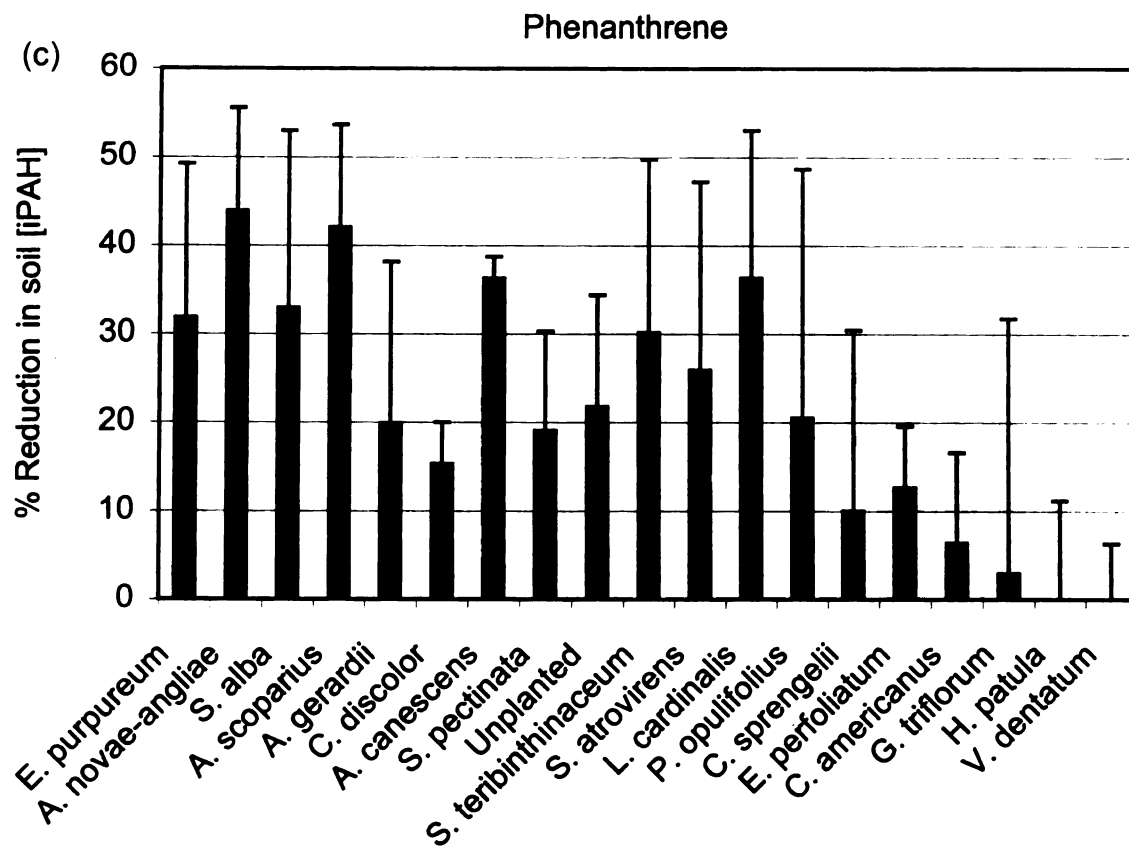
Planted treatments reduced soil [tPAH] 0–35 % from May to September 2001, while the unplanted control reduced soil [tPAH] by 17 % (Fig. 2.6 a). Treatments had different effects on a given PAH contaminant. For example, *E. purpureum* substantially decreased naphthalene, while the soil naphthalene concentration in the unplanted control did not change. The planted treatments that led to the greatest reduction in soil [tPAH] were *E. purpureum*, *A. novae-angliae*, *S. alba*, *A. scoparius*, and *A. gerardii* and these planted treatments had percentage reductions from 20–35 % from May to September 2001.

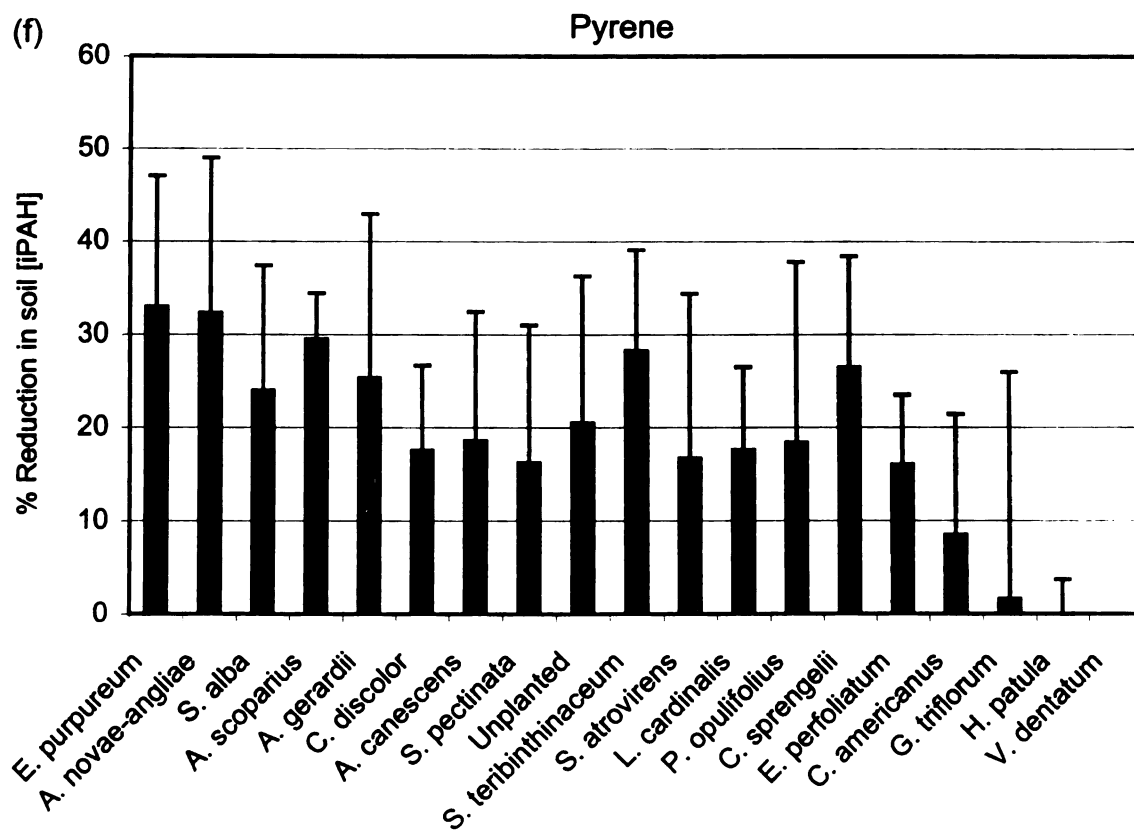
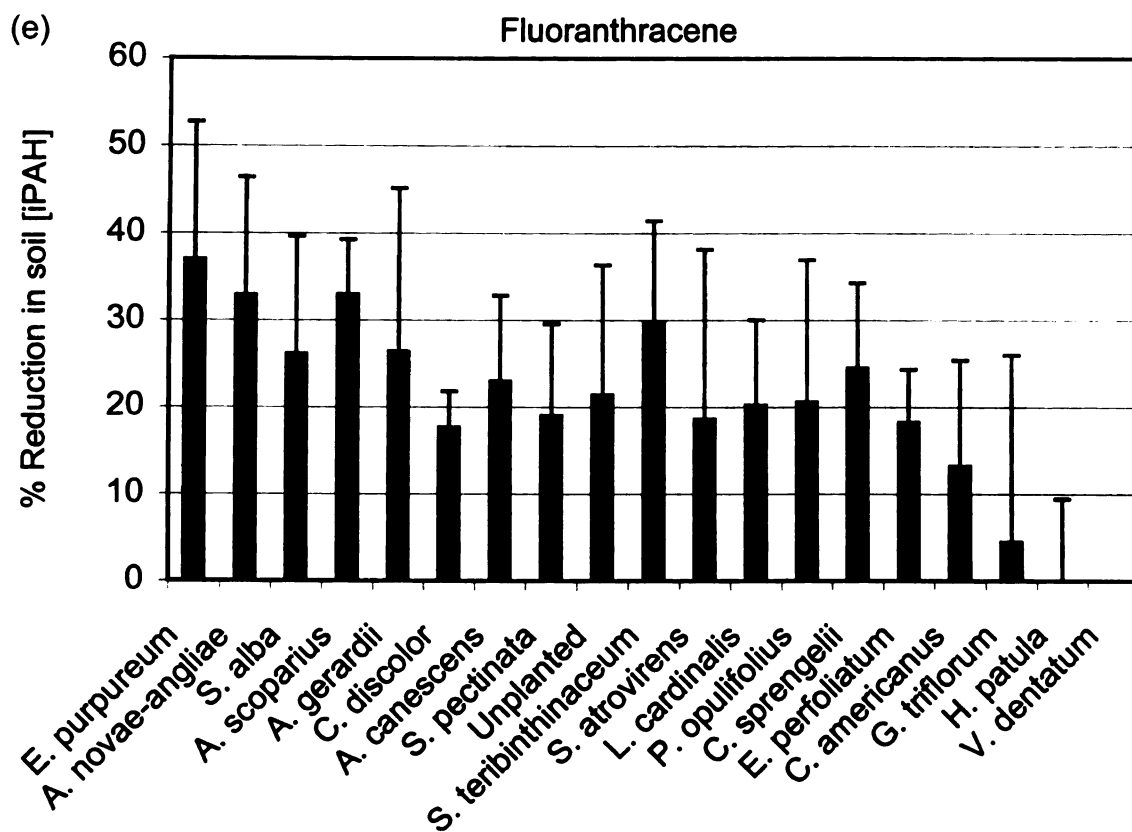
Individual PAH compounds percentage reduction

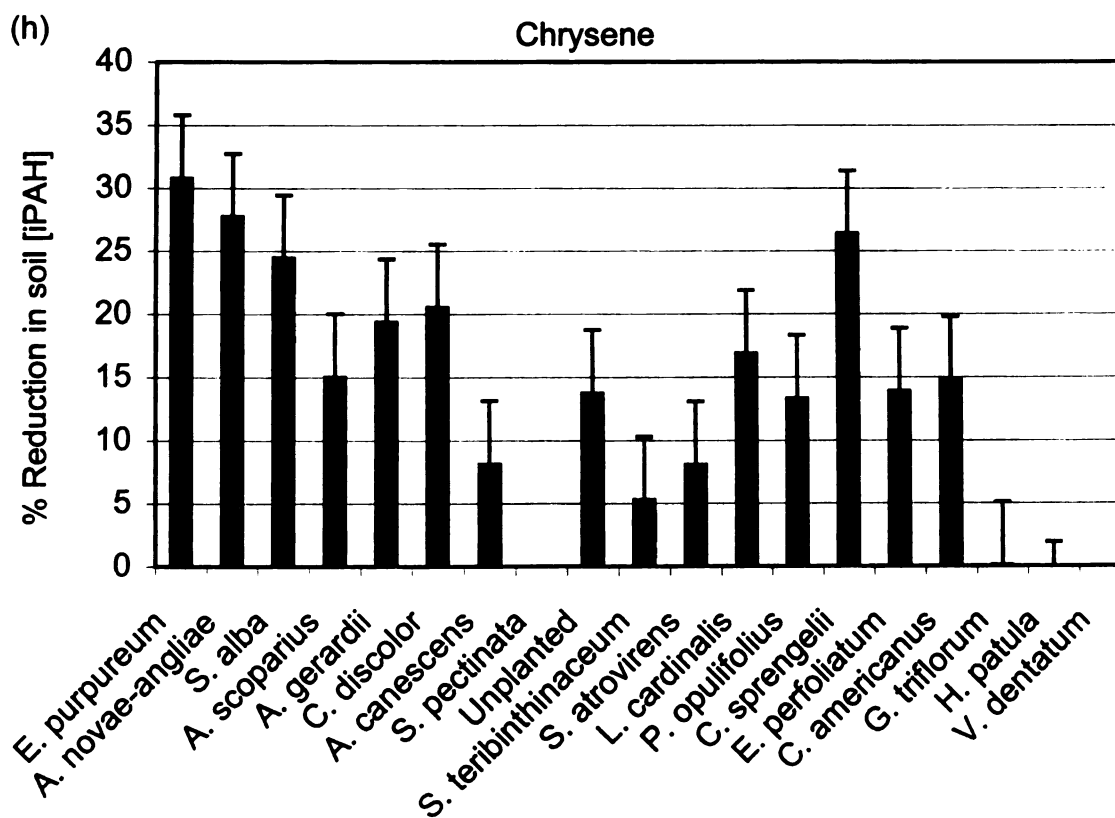
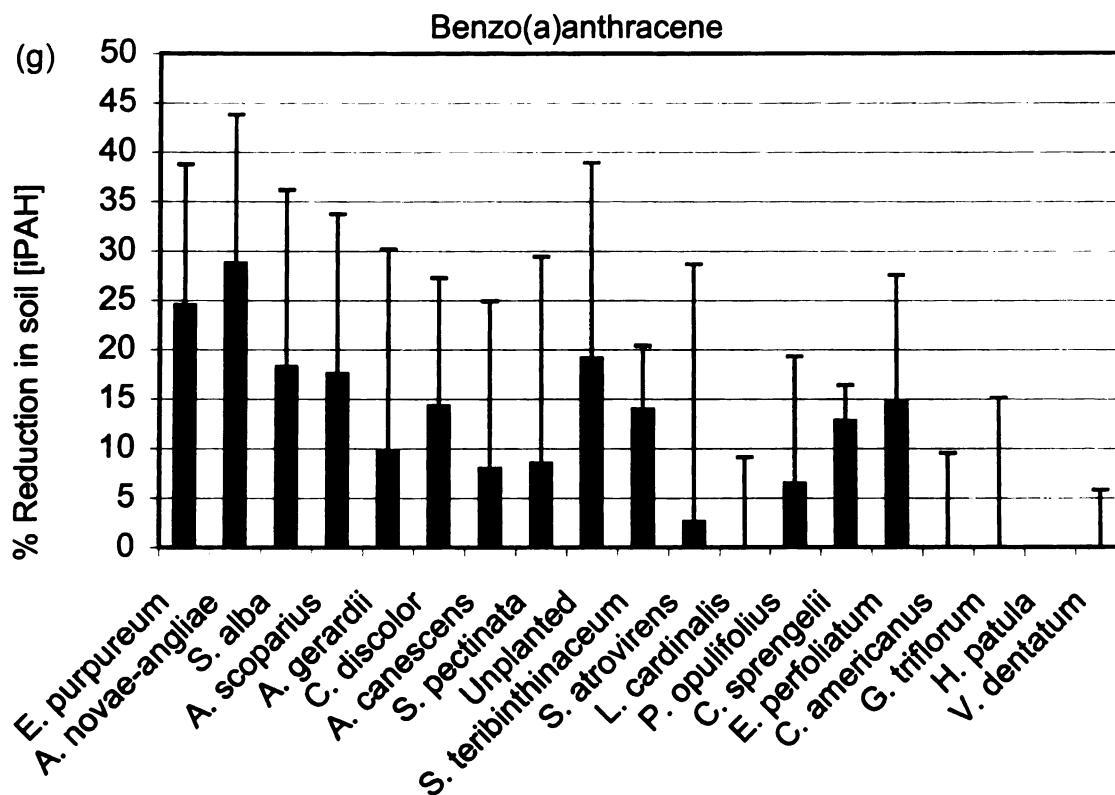
The percentage reductions of 11 individual PAH compounds and total PAH concentrations were not uniform among all treatments (Fig. 2.6 b-l). When each of the treatments are presented along the x-axes of graphs for individual PAH compounds in the ranked order of % [tPAH] reduction, apparent differences for % reduction for each given individual PAH compound are observed between the planted treatments (Fig. 2.6 b-l). In general, higher molecular weight PAHs (chrysene, benzo(b)fluoranthene, benzo(a)pyrene, dibenzo(ah)anthracene, and benzo(ghi)perylene) seemed to be reduced to a lesser extent (Fig. 2.6 g-l) than lower molecular weight PAHs (Fig. 2.6 b-f).

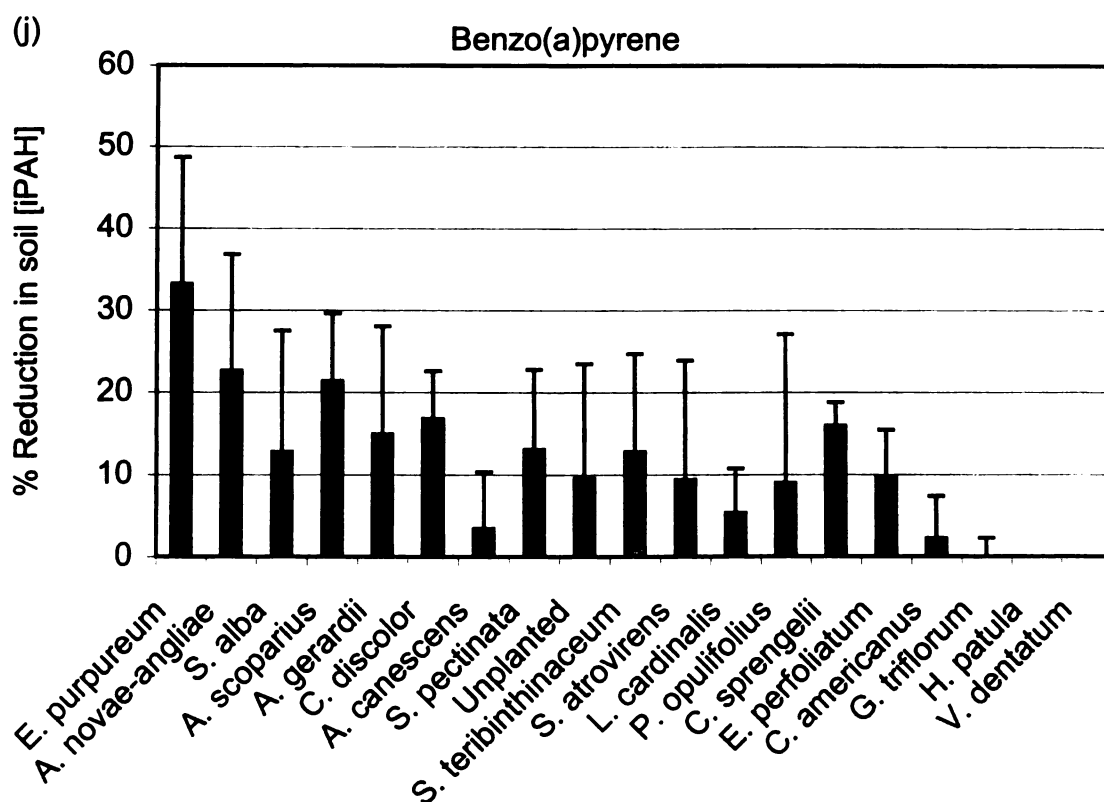
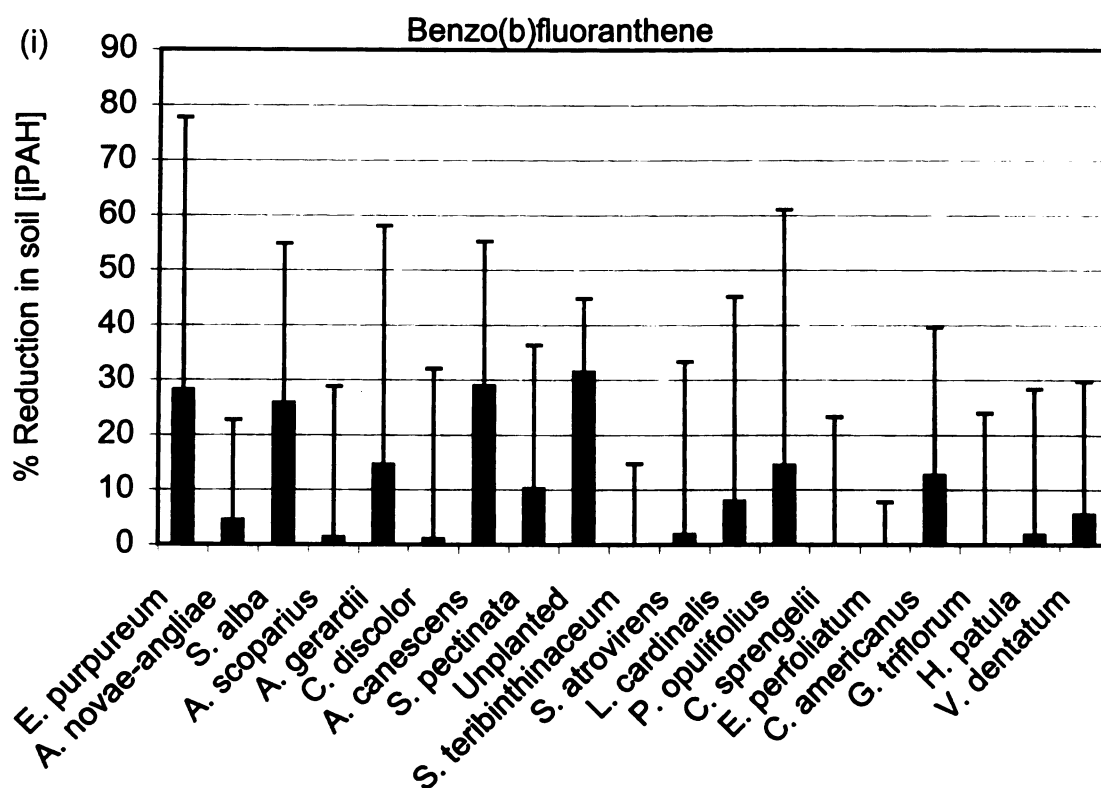
Figure 2.6 (a-l). Percentage reduction (mean $\pm$ SEM) in soil [tPAH] and individual PAH compounds for all treatments (May – September 2001) in Phyto Demo upland plot. Note naphthalene data may not be accurate because the concentrations in the samples were occasionally below the lowest standard of the calibration curve. Data for acenaphthene, acenaphthylene, and fluorene are not presented because the concentrations of samples were below the lowest calibration curve standard. The treatments are presented in order of decreasing % reduction in soil [tPAH] along the horizontal axes.



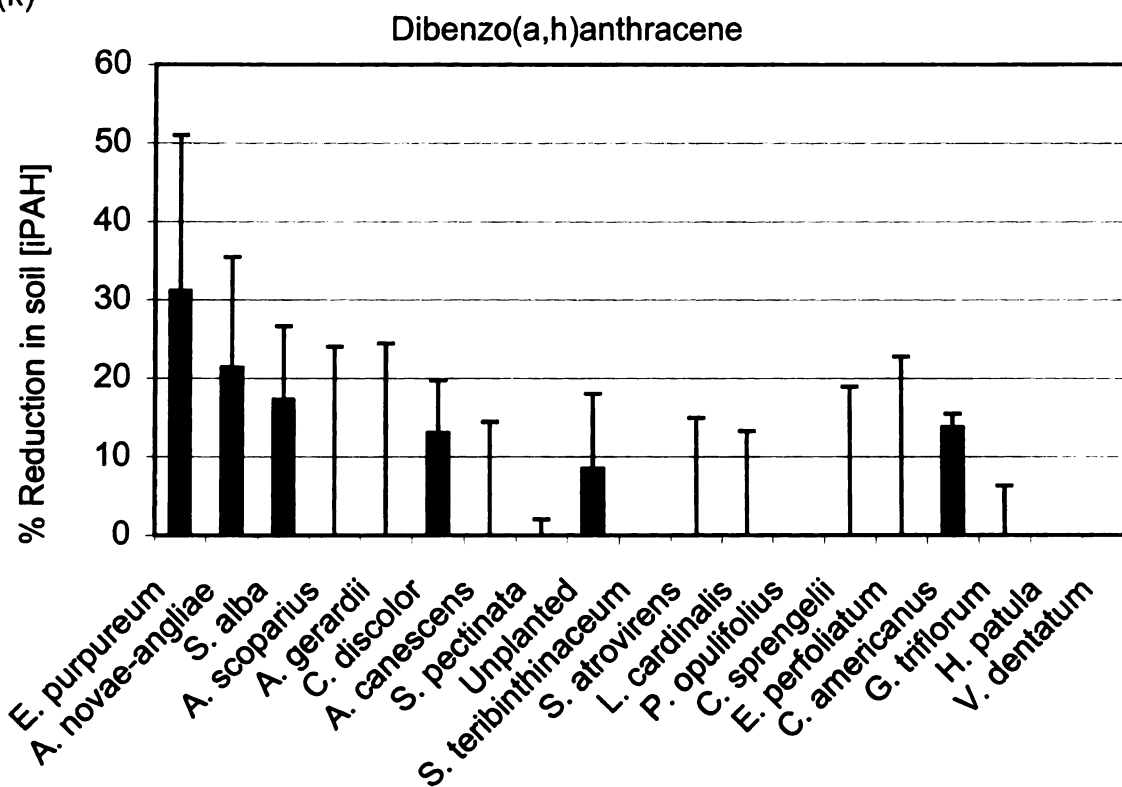




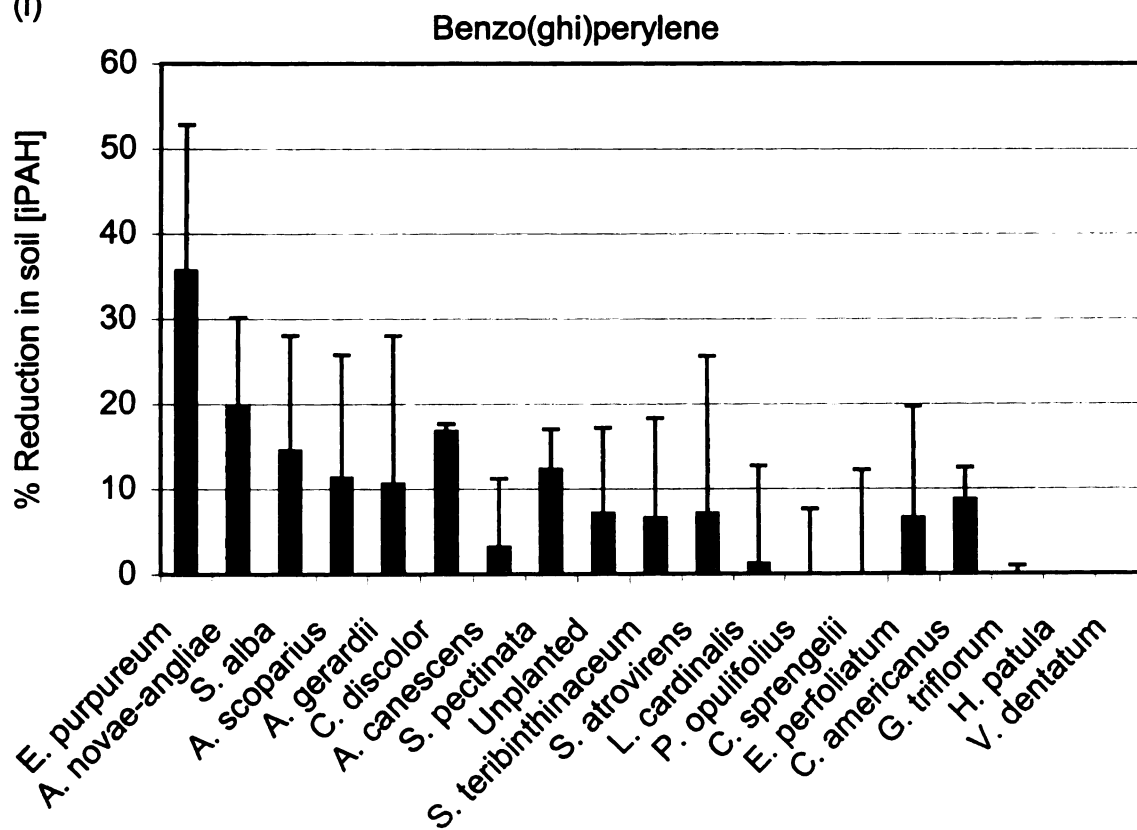




(k)



(l)



Plant PAH concentration

Plant leaf tissue tPAH concentrations were determined to be unusually high and variable, ranging in mean $\pm$ SEM from 260.3 ± 33.0 mg/kg for *C. americanus* to 7979.5 ± 2787.9 mg/kg for *E. purpureum* in the upland plot (Table 2.8). For the wetland plot, plant leaves had [tPAH] concentrations from 260.6 ± 30.0 mg/kg for *C. sprengelii* to 5292.9 ± 1771.4 mg/kg for *S. patula* (Table 2.8). Plant leaf tissue in the control plot had a concentration range from 72.6 mg/kg for *G. triflorum* to 9191.7 mg/kg for *S. patula* (Table 2.9). Since there was only one cell per plant species treatment, there were no standard errors of means in the control plot. The most abundant PAH compounds detected in plants and their respective range of concentration values were acenaphthylene (30-12000 mg/kg), benzo(a)anthracene (30-1000 mg/kg), chrysene (20- 7000 mg/kg), benzo(a)pyrene (30-4000 mg/kg), and benzo(ghi)perylene (40- 960 mg/kg). Individual PAH compound concentrations for plants are presented in Appendix 4.

Table 2.8. Plant total PAH concentrations in plant leaves (mean $\pm$ SEM) in mg/kg dry weight. Leaf tissues were collected from three plants per cell and pooled for [tPAH] analysis. Each mean represents the average concentration for three cells per plant treatment, with the exception of *C. americanus* treatment with only two cells sampled. Not all species in the Phyto Demo study were sampled due to low available biomass for some plants.

Treatment	[tPAH] (mg/kg)
Upland	
<i>Amorpha canescens</i>	1229.9 $\pm$ 70.6
<i>Andropogon gerardii</i>	834.6 $\pm$ 86.7
<i>Andropogon scoparius</i>	548.2 $\pm$ 123.4
<i>Aster novae-angliae</i>	901.0 $\pm$ 311.2
<i>Carex sprengelii</i>	310.2 $\pm$ 24.8
<i>Ceanothus americanus</i>	260.3 $\pm$ 33.0
<i>Cirsium discolor</i>	2649.4 $\pm$ 2294.2
<i>Eupatorium perfoliatum</i>	2111.0 $\pm$ 198.5
<i>Eupatorium purpureum</i>	7979.5 $\pm$ 2787.9
<i>Geum triflorum</i>	132.5 $\pm$ 19.9
<i>Hystrix patula</i>	1011.5 $\pm$ 119.0
<i>Lobelia cardinalis</i>	2579.2 $\pm$ 1120.5
<i>Physocarpus opulifolius</i>	549.6 $\pm$ 224.0
<i>Scirpus atrovirens</i>	304.2 $\pm$ 15.5
<i>Silphium teribinthinaceum</i>	1508.3 $\pm$ 681.5
<i>Spartina pectinata</i>	797.7 $\pm$ 38.9
<i>Spirea alba</i>	1139.7 $\pm$ 336.2
<i>Viburnum dentatum</i>	723.5 $\pm$ 172.9
Wetland	
<i>Aster novae-angliae</i>	775.6 $\pm$ 112.8
<i>Carex sprengelii</i>	260.6 $\pm$ 30.0
<i>Eupatorium perfoliatum</i>	1376.3 $\pm$ 156.5
<i>Mimulus ringens</i> *	452.0 $\pm$ 62.4
<i>Scirpus atrovirens</i>	274.6 $\pm$ 37.4
<i>Solidago patula</i> *	5292.9 $\pm$ 1771.4
<i>Spartina pectinata</i>	496.8 $\pm$ 78.8

Note: * means species was not in upland plot.

Table 2.9. Plant total PAH concentrations (mg/kg dry weight) in plant leaves from the control plot (uncontaminated soil). Samples were taken from three plants in each cell and pooled. The control plot contained only 1 cell per plant treatment. Not all species were sampled because of limited plant growth for some plants.

Cell	Treatment	[tPAH] (mg/kg)
C06C	<i>Amorpha canescens</i>	1256.8
C09A	<i>Andropogon gerardii</i>	638.0
C10A	<i>Andropogon scoparius</i>	803.5
C03A	<i>Aster novae-angliae</i>	1722.9
C10B	<i>Carex sprengelii</i>	340.2
C04B	<i>Eupatorium perfoliatum</i>	2125.9
C10C	<i>Eupatorium purpureum</i>	8563.9
C03B	<i>Geum triflorum</i>	72.6
C02C	<i>Hystrix patula</i>	779.5
C03C	<i>Lobelia cardinalis</i>	4933.1
C07C	<i>Mimulus ringens</i>	490.7
C07B	<i>Physocarpus opulifolius</i>	353.1
C05C	<i>Scirpus atrovirens</i>	151.9
C09B	<i>Silphium teribinthinaceum</i>	1283.1
C01C	<i>Solidago patula</i>	9191.7
C09C	<i>Spartina pectinata</i>	792.0
C08C	<i>Spirea alba</i>	1429.9
C04C	<i>Viburnum dentatum</i>	518.9

DISCUSSION

Plant species selection

The APGEN greenhouse study results (Appendix 1) indicate varying abilities among the tested plants for soil [tPAH] reduction. Anomalous points in the APGEN greenhouse study, such as for *Elymus virginicus*, week 6, may be the result of a small quantity of highly PAH-contaminated material in that particular sample. The variability in PAH concentration in the soil samples may be reflective of the heterogeneous distribution of organic matter since PAHs sorb strongly to organic matter. The goal of the APGEN screening experiment was to achieve rapid identification of candidate species for more extensive analysis under both field and greenhouse conditions. Consideration of both apparent reductions and consistent trends for soil [tPAH] reduction over time allowed selection of suitable species for future research.

Wetland Data

The wetland plot soil data is of concern due to extreme and unlikely fluctuations over the course of the season. The wetland soil [tPAH] values were perceived as initially low (May = 98.9 ± 4.4 mg/kg), then much higher (July = 122.1 ± 5.3 mg/kg), and then greatly reduced (September = 76.1 ± 2.3 mg/kg). The May data was obtained from samples stored under suboptimal conditions. It is suspected that there was a substantial loss of PAHs from the May soil samples due to a prolonged storage time in mostly empty jars having excessive unfilled headspace. September wetland data apparently misrepresent soil concentrations as unusually low, since a small number of grab samples taken in November 2001 and May 2002 had much higher soil [tPAH] of 110-120 mg/kg (Dr. Rugh, personal communication). Due to these inconsistencies and the likelihood

that the Wetland samples were inadvertently compromised, the wetland soil [tPAH] data is not considered further in this thesis.

Upland data

Phytoremediation and microbial degradation resulted in plot decreases in soil [tPAH] of ~10 mg/kg over 2 months. This is in accordance with previous studies, which have shown soil [tPAH] was reduced in planted treatments and the unplanted control (Pradhan *et al.*, 1998; Liste and Alexander, 2000; Aprill and Sims, 1990). As in previous studies, planted treatments reduced soil [tPAH] more than unplanted treatments (Qiu *et al.*, 1997; Schwab, 1994; Aprill and Sims, 1990; Nedunuri *et al.*, 2000; Pradhan *et al.*, 1998; Yateem *et al.*, 2000). Plant-assisted, aerobic bacterial degradation of PAHs was likely the primary mechanism for soil [tPAH] reduction in the upland plot over the May to September treatment period. Previous literature indicates bacterial aerobic degradation of PAHs contributes most to PAH reduction in soil compared to volatilization, leaching, or photolysis (Park *et al.*, 1990; Reilley *et al.*, 1996). PAH degradation by plant processes alone has not been clearly demonstrated as a means of effectively reducing soil [tPAH].

A. gerardii, *A. novae-angliae*, *E. purpureum*, and *S. teribinthinaceum* had significant declines in soil [tPAH] from July to September, indicating that these plant species are capable of phytostimulation of PAHs in soils. *A. scoparius* and *E. perfoliatum* (July) and *E. purpureum* (September) treatments had lower soil [tPAH] compared with unplanted, indicating that these taxa may also enhance soil [tPAH] reduction. Soils grown with *G. triflorum* had significantly higher soil [tPAH] compared to the unplanted control in September and the soil [tPAH] increased significantly from July to September. The low July concentration, 93.9 ± 6.7 mg/kg, was not significantly different from the May

concentration 111.5 ± 15.2 mg/kg. *G. triflorum* soil data may be indicative of sample variability than actual plant-soil phenomena. In this study, most plants were generally observed to enhance biodegradation of PAHs, which is consistent with previous studies (Aprill and Sims, 1990; Liste and Alexander, 2000; Pradhan *et al.*, 1998; Reilley *et al.*, 1996; Yateem *et al.*, 2000).

Variable plant effects on soil PAH concentrations are possibly the result of differences in plant exudation. Plant taxa secrete different amounts and types of exudates (Fletcher and Hegde, 1995; Siciliano and Germida, 1998). Some of these chemical exudates have been shown to promote or inhibit microbial PAH-degraders (Leigh *et al.*, 2002; Schwab, 1994). The composition and amount of root exudate can change at different growth stages of a plant. During senescence, the amount of phenols that support PCB-degrading bacteria and possibly PAH-degrading bacteria released were observed to increase in mulberry plants (Hegde and Fletcher, 1996). In addition, greater root volume or biomass can result in more exudation, enhance the stress tolerance of a plant, or increase oxygen in the soil and thereby facilitate the first step in PAH metabolism, all of which promote PAH phytoremediation. Thus, in this study, the variable plant effects on the concentrations of PAHs may have resulted from the combined effects of taxa-specific root exudation, plant age, or root biomass.

Other published research that used the same plant species as this study had different results with regard to PAH reduction. Specifically, the *A. scoparius* treatment was not significantly different from the unplanted control in September. Yet in another study, *A. scoparius* enhanced PAH reduction by 8% to 50%, in contrast to unplanted controls (0% and 26%) in two soils over 6 months (Pradhan *et al.*, 1998). Likewise, the

A. gerardii treatment was not significantly different from unplanted control in September, yet it has been shown to decrease PAH concentration (Aprill and Sims, 1990).

Benz(a)anthracene, chrysene, benzo(a)pyrene, and dibenz(ah)anthracene biodegradation was reportedly enhanced by a mixture of plant species that included *A. scoparius*, *A. gerardii* and other prairie grasses after 151 days (Aprill and Sims, 1990). The discrepancies between these plant species effects on soil PAH reduction presented here and previous literature may be due to insufficient treatment time and high variation among soil analytes, rather than ineffectiveness of the *Andropogon* species.

The use of single subsamples for May soil core analysis restricted the ability to statistically analyze differences within the May data and compared to the other sampling times. The lack of significance of treatment and the treatment x time interaction effects in the ANOVA test using one subsample indicated that the use of one subsample was not sufficient to distinguish significant differences in soil [tPAH] between treatments. Pair-wise least significant differences between treatments are not considered significant unless the ANOVA F-test for treatment is significant (Carmer and Swanson, 1973; Montgomery, 1997). In contrast, the two-way ANOVA analysis using 3 subsamples showed that treatment effect was significant. The treatment x time interaction term was also significant for July and September, which means there was a treatment effect that varied by sampling date.

The limited statistical power of single soil sample analysis was exacerbated by wide sample variation in observed soil [tPAH] levels among subsamples. It has been suggested that better soil homogenization be achieved by pulverization, use of finer

sieves, or use of greater soil volumes for extraction in future studies (Dr. G. Phil Robertson, MSU; personal communication).

Comparison of field results to greenhouse results

The results from the Phyto Demo field plots are consistent with those obtained from the pilot greenhouse study conducted by APGEN (Carreira and Rugh, unpublished). The species with the greatest potential to decrease soil [tPAH] in the Phyto Demo upland plot as indicated by significant differences in soil [tPAH] compared to the unplanted control or over time were *A. gerardii*, *A. scoparius*, *A. novae-angliae*, *E. perfoliatum*, *E. purpureum*, and *S. teribinthinaceum* (Table 2.5). These 6 plant species had soil [tPAH] lower than the unplanted control either 4 out of the 4 sampling times or 3 out of the 4 sampling times in the APGEN study (Appendix 2). Out of 38 species in the APGEN study, *A. novae-angliae*, *E. perfoliatum* and *S. teribinthinaceum* were 3 species treatments that had lower soil [tPAH] than the unplanted control 4 out of the 4 sampling times (Appendices 1 and 2). Similarly, *A. gerardii*, *A. scoparius*, and *E. purpureum* were 3 treatments of 15 plant species treatments that had lower soil [tPAH] compared with the unplanted control three out of the four sampling times in the APGEN study. Eleven out of the 17 treatments common to both studies have soil indices (average of planted soil [tPAH] divided by unplanted soil [tPAH]) that rank the same relative to the unplanted soil (Table 2.7 and Appendix 2). The agreement between these two studies indicates greenhouse studies can provide useful information for environmental field studies.

Individual PAH compounds in soil

Different individual PAH compounds vary in biodegradability and respond differently to a given treatment. Although soil concentrations decreased for

benzo(a)pyrene, dibenz(ah)anthracene, and benzo(ghi)perylene in most treatments, these high molecular weight PAH compounds (Fig. 2.6 g – k) showed smaller decreases compared with lower molecular weight PAHs (Fig. 2.6 b - f). This trend is likely a reflection of the physiochemical properties of PAHs (Park *et al.*, 1990). PAHs with higher molecular weight are more recalcitrant to soil desorption and biodegradation compared to lower molecular weight PAHs (Trapp, 2000; Cerniglia, 1992). In this study, we observed that phytoremediation was more effective for reduction of lower molecular weight PAHs compared with those of higher molecular weight.

Plant [tPAH]

The concentrations of PAHs observed in leaf tissue from plants in the Phyto Demo study (Appendix 4) were higher than values reported in published literature. We are currently uncertain of the reason(s) for the high PAH concentrations detected in plant leaf tissues in this study and suspect that there may be analytical problems. Data from previous literature indicate that the plant concentration values obtained in this study may be erroneous since leaf PAH concentrations are typically detected in only parts per billion (ppb) and in this study the levels are in hundreds and thousands of parts per million (ppm).

PAHs in plant tissue may occur by plant uptake, plant biosynthesis, biomagnification, atmospheric deposition or wind-blown dust, but the literature has not reported values in the hundreds to thousands of parts per million in plants from these sources. Natural background levels of PAHs can occur in plants with concentrations from 10-90 µg/kg dry weight for each PAH compound (Sims and Overcash, 1983), which are 1000 times lower than levels detected in plants in this study. Previous studies indicated

plants accumulate PAHs at levels ranging from undetectable to low natural background concentrations (Sims and Overcash, 1983). Very low to undetectable PAH levels have been observed in grasses (Qiu *et al.*, 1997) alfalfa (Schwab, 1994) and carrot foliage (Wild and Jones, 1992). These studies indicate these plants do not readily uptake and accumulate PAHs, and that background PAH concentrations are typically undetectable to low.

Biomagnification, which can be defined as plant to soil concentration ratios greater than one, of PAHs is one possible explanation for the high concentrations detected in plant shoot tissues in this study, though has not been shown to result in the high PAH concentration values detected here. In this study the plant-to-soil (July) benzo(b)fluoranthene concentration ratios for grasses were: *A. gerardii* 1.8 (20.0 mg/kg dry weight in plant; 10.9 mg/kg dry weight in soil), *A. scoparius* 2.8 (21.3 mg/kg dry weight in plant; 7.4 mg/kg dry weight in soil), *H. patula* 7.7 (83.0 mg/kg dry weight in plant; 10.8 mg/kg dry weight in soil), and *S. pectinata* 11.1 (103.0 mg/kg dry weight in plant; 9.3 mg/kg dry weight in soil). These values are not the highest plant to soil concentration values reported in the literature. Crop to soil concentration ratios for benzo(b)fluoranthene in wheat can be as high as 59.4 (119 µg/kg dry weight in plant; 2 µg/kg dry weight in soil; yield = 44.9 g dry weight) (Sims and Overcash, 1983). Previous literature, however, may not provide an equivalent comparison because previous reports of plant PAH concentrations did not have soil PAH concentrations as high as in this study. In addition, high plant PAH concentrations were seen in leaf tissues obtained from plants grown in uncontaminated soils, which would be indicative of extremely high and implausible biomagnification from low soil [PAH] (Table 2.9).

Alternatively, PAHs detected in plant tissue in this study may have resulted from atmospheric deposition (Lodovici *et al.*, 1994; Sims and Overcash, 1983), but again this source of PAHs has not been shown to result in hundreds or thousands of parts per million in plants. Lodovici (1994) detected PAH concentrations on the level of hundreds of ppb in leaves of evergreen tree *Laurus nobilis*.

It is also possible that PAH-enriched dust contributed to the high levels of PAH concentration in our study, though high concentrations of PAHs were also detected in greenhouse grown plants prior to exposure to PAH-contaminated soils (Appendix 5). These findings indicate that dust was not a source of high leaf PAH concentrations in this study. To minimize the possibility of wind-blown PAH-enriched dust contribution to detected concentrations in future studies, it is recommended to rinse settled dust from plant tissues prior to extraction.

It is possible that our analytical methods are the source of the elevated leaf PAH concentrations. The GC-FID may have detected plant compounds with similar chromatographic properties to PAHs, however, previous literature has not reported such problems. The compounds we identified as PAHs should be verified using other analytical detection methods such as gas chromatography–mass spectrometry (GC-MS). In addition, our plant extraction protocol should be compared to other published methods or modified to use larger quantities of tissue to avoid magnification of “noise” from residual PAHs in extraction glassware or the GC column.

SUMMARY AND CONCLUSION

Phytoremediation using native plants has potential to become an effective remediation strategy for PAH-contaminated soils. Soil [tPAH] in the upland plot was reduced 10 mg/kg over each 2 month period between May to September of the 2001 growing season. This study has also shown that the selection of plant species is an important consideration for implementation of phytoremediation. Certain plant species treatments showed enhanced soil [tPAH] reduction compared to the unplanted control. Unplanted control soils decreased soil [tPAH] by 17%, while other plant species treatments, notably *E. purpureum*, resulted in accelerated soil [tPAH] reduction of up to 35%. The species best able to achieve soil remediation over the first growing season were *A. gerardii*, *A. novae-angliae*, *E. purpureum*, and *S. teribinthinaceum*, which decreased soil [tPAH] significantly from July to September sampling times. By contrast, the unplanted control soil [tPAH] did not significantly decrease. These results indicate that these plant species enhanced soil [tPAH] reduction, possibly by stimulation of microbial metabolic processes for PAH degradation.

The effect of a plant species on a given contaminant was not uniform across all individual PAH compounds for all treatments. Different PAHs varied in biodegradability; PAHs with 5 or 6-rings did not change in soil concentration or increased in relative percentage from May to September. The results of this study are in accordance with previous reports which demonstrated higher molecular weight PAHs are generally more environmentally persistent than those of lower molecular weight.

Phytoremediation has advantages over engineered remediation approaches. Other remediation methods that are commonly used, such as solidification, excavation and

landfilling, do not lead to decreases in contaminant concentration, are expensive, and highly disruptive to a site. Phytoremediation is substantially less expensive, is minimally disruptive during site rehabilitation, and can be used as an alternative or complement to other remediation strategies. Identifying plant taxa and the cultivation conditions that promote PAH-biodegradation will lead to improved PAH-phytoremediation technology.

Persistent organic pollutants (POPs), which include PAHs, are toxic chemicals that are widespread in the environment. They do not readily undergo biogeochemical reactions, remain in soils for a long time, and are prone to biomagnify through the food chain. The rise in contaminated areas with POPs associated with anthropogenic activities such as fossil fuel burning, and pesticide use since the industrial revolution has presented the world with major environmental crises. Environmental clean-up of these contaminants is needed globally. Phytoremediation is an emerging field in environmental rehabilitation. The major barrier for phytoremediation of organic pollutants is their lack of bioavailability as a result of their strong sorption to organic material. In this study, we evaluated the effectiveness of 18 native plant species to phytoremediate soil contaminated with PAHs in field demonstration plots. PAH phytoremediation capabilities are variable among plants and preliminary pilot greenhouse studies can provide good estimates of plant effectiveness in the field. We have identified a group of plant species that accelerate biodegradation of PAHs after one growing season that can be used for future field and laboratory phytoremediation applications.

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APPENDICES

Appendix 1. Soil iPAH concentration (mg/kg) by dichloromethane extraction and detected by GC-FID from the APGEN greenhouse study (Athens, GA), (N=1).

APGEN analyses (Aug-Oct 2000)

Treatment	Common Name	Family	Week 4	Week 6	Week 10	Week 14
Untreated Control			1102.5	843.9	426.1	398.9
Unplanted Control			424.5	279.1	219.5	196.2
<i>Amorpha canescens</i>	Leadplant	Fabaceae	264.5	112.9	129.2	138.4
<i>Andropogon gerardii</i>	Big Bluestem	Poaceae	245.9	60.9	934.0	71.6
<i>Andropogon scoparius</i>	Little Bluestem	Poaceae	296.9	383.3	94.0	99.9
<i>Aristida purpurens</i>	Three Awned Grass	Poaceae	571.5	256.4	203.3	123.0
<i>Asclepias incarnata</i>	Swamp Milkweed	Asclepiadaceae	344.1	219.2	550.1	294.0
<i>Asclepias tuberosa</i>	Butterfly Weed	Asclepiadaceae	534.0	370.6	87.7	306.2
<i>Aster novae-angliae</i>	New England Aster	Asteraceae	115.5	172.6	135.0	132.3
<i>Carex sprengei</i>	Sedge	Cyperaceae	311.3	866.9	872.0	306.5
<i>Ceanothus americanus</i>	New Jersey Tea	Rhamnaceae	122.2	248.6	162.3	162.3
<i>Cirsium discolor</i>	Pasture Thistle	Asteraceae	113.2	20.5	252.4	137.5
<i>Coreopsis tripteris</i>	Tall Tickseed	Asteraceae	427.9	68.9	355.1	230.4
<i>Corylus americana</i>	American Hazelnut	Betulaceae	951.4	343.6	821.6	150.1
<i>Elymus virginicus</i>	Virginia Wild Rye	Poaceae	804.1	3723.7	269.2	205.7
<i>Eupatorium perfoliatum</i>	Boneset	Asteraceae	253.1	229.8	132.8	131.3
<i>Eupatorium purpureum</i>	Joe-Pye Weed	Asteraceae	1828.0	51.7	167.3	73.3
<i>Fraxinus pennsylvanica</i>	Ash	Oleaceae	275.4	260.6	644.5	7.3

Appendix 1. cont'd

Treatment	Common Name	Family	Week 4	Week 6	Week 10	Week 14
<i>Hystrix patula</i>	Bottlebrush Grass	Poaceae	288.7	363.1	162.2	259.6
<i>Koeleria macrantha</i>	June Grass	Poaceae	4455.2	367.5	246.4	115.1
<i>Liatris aspera</i>	Rough Blazing Star	Asteraceae	640.1	742.7	742.7	290.2
<i>Liquidambar styraciflua</i>	Sweetgum	Hamamelidaceae	430.0	164.1	276.1	143.5
<i>Malus coronaria</i>	Wild Crab Apple	Rosaceae	381.1	213.3	177.5	266.6
<i>Mimulus ringens</i>	Monkey-Flower	Scrophulariaceae	520.7	126.6	78.3	47.7
<i>Nyssa sylvatica</i>	Blackgum	Nyssaceae	256.0	206.9	381.6	190.5
<i>Physocarpus opulifolius</i>	Common Ninebark	Rosaceae	292.3	145.8	324.7	168.9
<i>Quercus nigra</i>	Water Oak	Fagaceae	305.3	209.1	261.5	114.0
<i>Rudbeckia laciniata</i>	Green-Headed Coneflower	Asteraceae	867.1	255.9	370.2	125.3
<i>Scirpus atrovirens</i>	Bulrush	Cyperaceae	526.1	552.8	225.5	118.6
<i>Silphium terbinthinaceum</i>	Prairie-dock	Asteraceae	310.8	163.5	185.0	123.3
<i>Solidago patula</i>	Swamp Goldenrod	Asteraceae	203.5	150.9	103.2	88.7
<i>Spartina pectinata</i>	Prairie Cordgrass	Poaceae	271.3	205.9	758.2	134.2
<i>Spirea alba</i>	Meadowsweet	Rosaceae	270.3	1859.0	152.1	ND
<i>Verbena hastata</i>	Blue Vervain	Verbenaceae	436.4	138.4	279.3	328.5
<i>Viburnum dentatum</i>	Arrowhead Viburnum	Caprifoliaceae	290.5	169.2	135.5	187.2

Note: ND, not detectable.

Appendix 2. APGEN greenhouse study results - soil [tPAH] ratios. Each * indicates a soil index value <1.

	Planted [tPAH]/ Unplanted [tPAH]				
Plant species	6 weeks	10 weeks	14 weeks	AVG	Trend
<i>Mimulus ringens</i>	0.45	0.36	0.24	0.35	***
<i>Eupatorium purpureum</i>	0.19	0.76	0.37	0.44	***
<i>Solidago patula</i>	0.54	0.47	0.45	0.49	***
<i>Amorpha canescens</i>	0.40	0.59	0.71	0.57	***
<i>Aster novae-angliae</i>	0.62	0.62	0.67	0.64	***
<i>Cirsium discolor</i>	0.07	1.15	0.70	0.64	**
<i>Silphium teribinthinaceum</i>	0.59	0.84	0.63	0.69	***
<i>Eupatorium perfoliatum</i>	0.82	0.61	0.67	0.70	***
<i>Viburnum dentatum</i>	0.61	0.62	0.95	0.73	***
<i>Andropogon scoparius</i>	1.37	0.43	0.51	0.77	**
<i>Ceanothus americanus</i>	0.89	0.74		0.82	**
<i>Aristida purpurea</i>	0.92	0.93	0.63	0.82	***
<i>Quercus nigra</i>	0.75	1.19	0.58	0.84	**
<i>Liquidambar styraciflua</i>	0.59	1.26	0.73	0.86	**
<i>Physocarpus opulifolius</i>	0.52	1.48	0.86	0.95	**
<i>Malus coronaria</i>	0.76	0.81	1.36	0.98	**
unplanted control	1.00	1.00	1.00	1.00	
<i>Koeleria macrantha</i>	1.32	1.12	0.59	1.01	*
<i>Coreopsis tripteris</i>	0.25	1.62	1.17	1.01	*
<i>Aesculus glabra</i>	1.02	0.59	1.52	1.04	*
<i>Rudbeckia lacinata</i>	0.92	1.69	0.64	1.08	*
<i>Asclepias tuberosa</i>	1.33	0.40	1.56	1.10	*
<i>Hystrix patula</i>	1.30	0.74	1.32	1.12	*
<i>Verbena hastata</i>	0.50	1.27	1.67	1.15	*
<i>Nyssa sylvatica</i>	0.74	1.74	0.97	1.15	**
<i>Scirpus atrovirens</i>	1.98	1.03	0.60	1.20	*
<i>Panicum sp.</i>	0.88	2.22	0.51	1.21	**
<i>Fraxinus pennsylvanica</i>	0.93	2.94	0.04	1.30	**
<i>Asclepias incarnata</i>	0.79	2.51	1.50	1.60	*
<i>Andropogon gerardii</i>	0.22	4.26	0.36	1.61	**
<i>Spartina pectinata</i>	0.74	3.45	0.68	1.63	**
<i>Corylus americana</i>	1.23	3.74	0.77	1.91	*
<i>Liatris aspera</i>	2.66		1.48	2.07	
<i>Spirea alba</i>	6.66	0.69	0.00	2.45	**
<i>Carex sprengei</i>	3.11	3.97	1.56	2.88	
<i>Elymus virginicus</i>	13.34	1.23	1.05	5.21	

Appendix 3. Phytoremediation Demonstration site plant mortality inventory. "M.R." is mortality rate.

	Overwinter	Season 1	Season 2
Scientific Name	M. R.% (May01)	M.R.% (July01)	M.R.% (June02)
<i>Amorpha canescens</i>	37.5	27.3	2.8
<i>Andropogon gerardii</i>	31.3	23.8	0.0
<i>Andropogon scoparius</i>	77.1	10.6	11.1
<i>Aster novae-angliae</i>	91.7	47.8	1.4
<i>Carex spengelii</i>	33.3	17.0	0.0
<i>Ceanothus americanus</i>	100.0	62.5	50.0
<i>Cirsium discolor</i>	100.0	51.0	2.8
<i>Eupatorium perfoliatum</i>	44.0	38.8	9.7
<i>Eupatorium purpureum</i>	not planted	0.0	8.3
<i>Geum triflorum</i>	not planted	6.3	16.7
<i>Hystrix patula</i>	not planted	0.0	2.8
<i>Lobelia cardinalis</i>	not planted	4.2	13.9
<i>Mimulus ringens</i>	not planted	0.0	0.0
<i>Physocarpus opulifolius</i>	95.8	48.9	0.0
<i>Scirpus atrovirens</i>	not planted	0.0	0.0
<i>Silphium teribinthinaceum</i>	85.4	48.3	0.0
<i>Solidago patula</i>	100.0	50.0	11.1
<i>Spartina pectinata</i>	4.2	2.1	0.0
<i>Spirea alba</i>	not planted	0.0	0.0
<i>Viburnum dentatum</i>	4.2	2.1	0.0
Average M.R. (%) =	61.9	22.0	6.5

Appendix 4. Phyto Demo plant [tPAH] data harvested July 2001. Each value represents PAH concentrations (ppm) of pooled leaf tissues of 3 plants per cell. Refer to Table 2.1 for full plant names and Table 2.4 for full compound names.																
Cell	Plant	Naph	Acny	Acne	Flre	Phen	Anth	Flra	Pyre	Baan	Chry	Bbfl	Bapy	Daha	Bghp	[tPAH]
U17A	amocan	0	16	84	89	0	0	0	0	0	0	51	749	104	90	1182
U12C	amocan	0	24	134	135	0	0	0	0	21	17	58	848	21	110	1369
U03B	amocan	0	14	91	97	0	0	0	0	16	14	50	726	12	118	1139
C06C	amocan	0	27	163	14	0	0	0	0	27	23	57	748	110	88	1257
U16B	andger	0	0	0	0	0	0	0	0	0	0	25	162	0	496	683
U07C	andger	0	0	0	0	0	0	0	0	27	0	18	233	42	663	984
U03A	andger	0	0	0	0	0	0	0	0	45	0	17	132	33	610	837
C09A	andger	0	0	0	0	0	0	0	0	20	23	13	241	0	341	638
U19A	andsco	0	0	0	0	0	0	0	0	0	0	16	65	0	234	315
U08B	andsco	0	0	0	0	0	0	0	0	0	0	30	170	0	395	596
U01C	andsco	0	0	0	0	0	0	0	0	47	21	18	237	0	411	734
C10A	andsco	0	0	0	0	0	0	0	0	0	27	23	182	153	418	803
W09A	astnov	0	502	28	0	0	0	0	0	0	0	31	240	0	0	801
W07A	astnov	0	245	0	0	0	0	0	0	0	0	40	284	0	0	569
W04B	astnov	0	551	0	0	0	0	0	0	0	0	52	354	0	0	957
U17B	astnov	0	1061	0	0	0	0	0	0	27	0	44	367	0	0	1500
U13C	astnov	0	491	0	0	0	0	0	0	89	0	87	81	0	0	748
U05A	astnov	0	387	0	0	0	0	0	0	0	0	43	25	0	0	455
C03A	astnov	0	26	1087	0	0	0	0	0	41	0	62	506	0	0	1723

Cell	Plant	Naph	Acny	Acne	Fire	Phen	Anth	Fira	Pyre	Baan	Chry	Bbfl	Bapy	Daha	Bghp	[PAH]
W10C	carspr	0	0	0	0	25	0	0	0	38	0	109	31	0	0	203
W04C	carspr	0	0	0	0	0	0	0	0	0	0	113	62	0	98	274
W02B	carspr	0	0	0	0	16	0	0	0	16	0	140	45	0	88	305
U14A	carspr	0	0	0	0	0	0	0	0	21	0	116	43	0	98	278
U07A	carspr	0	0	0	0	23	0	0	0	53	0	136	51	0	97	359
U02C	carspr	0	0	0	0	0	0	0	0	33	0	120	41	0	100	294
C10B	carspr	0	0	0	0	0	0	0	0	26	0	188	48	0	78	340
U16A	ceaame	0	0	0	0	0	0	0	0	0	22	191	51	29	0	293
U01B	ceaame	0	0	0	0	0	0	0	0	0	22	121	85	0	0	227
U19B	cirdis	0	0	0	0	0	0	0	0	40	0	141	238	0	0	419
U10C	cirdis	0	0	0	0	0	0	0	0	0	0	1125	6112	0	0	7237
U04B	cirdis	0	0	0	0	0	0	0	0	0	0	262	30	0	0	292
W08C	euppr	0	323	0	35	53	0	49	49	369	14	362	40	0	134	1428
W06C	euppr	0	294	0	46	61	0	59	19	332	9	568	99	0	130	1618
W02A	euppr	0	250	0	25	84	0	49	62	158	70	229	92	0	64	1083
U18A	euppr	0	140	0	33	73	0	62	34	192	97	572	118	75	318	1714
U08C	euppr	0	468	83	63	72	0	118	39	501	93	583	101	100	75	2297
U02A	euppr	0	39	801	60	103	0	37	25	343	99	640	81	93	0	2322
C04B	euppr	0	485	0	65	80	0	35	41	504	71	599	133	0	113	2126
U20A	euppr	0	5270	176	0	27	0	0	0	73	24	61	46	53	0	5730
U11C	euppr	0	12991	172	0	32	0	0	0	31	50	62	42	70	72	13523
U02B	euppr	0	4246	229	0	0	0	0	0	54	42	29	85	0	0	4686
C10C	euppr	0	8046	399	0	0	0	0	0	39	0	46	34	0	0	8564
U20C	geutri	0	0	0	0	0	0	0	0	17	0	73	23	0	0	114
U14B	geutri	0	0	0	0	0	0	0	0	39	0	85	48	0	0	172
U03C	geutri	0	0	0	0	0	0	0	0	20	0	63	28	0	0	112
C03B	geutri	0	0	0	0	0	0	0	0	29	0	44	0	0	0	73

Cell	Plant	Naph	Acny	Acne	Flre	Phen	Anth	Flra	Pyre	Baan	Chry	Bbfl	Bapy	Daha	Bghp	[tPAH]
U15A	hyspat	0	0	0	0	0	0	0	0	0	25	67	787	0	146	1025
U09C	hyspat	0	0	0	0	0	0	23	0	25	51	86	344	27	315	870
U09A	hyspat	0	0	0	0	30	0	0	0	19	33	90	520	0	108	799
U05B	hyspat	0	0	0	0	0	0	0	0	26	38	92	913	0	142	1210
C02C	hyspat	0	0	0	0	0	0	0	0	38	27	38	603	0	74	780
U15C	lobcar	0	0	0	0	0	0	0	0	0	0	146	193	0	0	339
U08A	lobcar	0	0	0	0	0	0	0	0	38	45	633	3033	0	0	3749
U05C	lobcar	0	0	0	0	0	0	0	0	46	33	529	3041	0	0	3649
C03C	lobcar	0	0	0	0	0	0	0	0	0	31	364	4538	0	0	4933
W08B	mimir	0	42	73	0	0	0	0	0	31	0	163	52	0	0	360
W05C	mimir	0	102	227	0	0	0	0	0	47	0	149	46	0	0	571
W03B	mimir	0	96	166	0	0	0	0	0	0	0	126	37	0	0	424
C07C	mimir	0	55	127	0	0	0	0	0	50	0	125	134	0	0	491
U14C	phyopu	0	0	0	0	0	0	0	0	0	0	842	98	0	0	939
U13A	phyopu	0	0	0	0	0	0	0	0	15	0	360	76	39	55	546
U07B	phyopu	0	0	0	0	0	0	0	0	24	0	21	64	0	55	163
C07B	phyopu	0	0	0	0	0	0	0	0	19	0	183	43	45	63	353
W10B	sciutr	0	0	0	0	0	0	0	0	70	20	67	139	0	0	297
W07B	sciutr	0	0	0	0	0	0	0	0	0	0	63	138	0	0	202
W03C	sciutr	0	0	0	0	0	0	0	0	35	0	74	158	0	58	325
U18B	sciutr	0	0	0	0	0	0	0	0	56	0	87	187	0	0	329
U12A	sciutr	0	0	0	0	0	0	0	0	97	21	54	135	0	0	307
U09B	sciutr	0	0	0	0	0	0	0	0	38	0	74	164	0	0	276
C05C	sciutr	0	0	0	0	0	0	0	0	0	0	52	100	0	0	152

Cell	Plant	Naph	Acny	Acne	Flre	Phen	Anth	Flra	Pyre	Baan	Chry	Bbfl	Bapy	Daha	Bghp	[PAH]
U17C	siler	0	169	79	46	0	0	0	92	77	33	68	0	0	111	676
U10A	siler	0	91	40	0	0	0	0	52	22	0	42	0	0	744	990
U06A	siler	0	70	1939	0	0	0	0	0	30	31	66	0	0	724	2859
C09B	siler	0	132	91	41	0	0	0	32	0	0	25	0	0	962	1283
W09C	solpat	0	172	0	0	0	0	21	72	1000	6536	59	28	0	72	7961
W03A	solpat	0	130	0	0	0	0	25	71	440	5216	64	31	0	0	5978
W02C	solpat	0	341	0	0	0	0	89	41	968	317	63	29	0	92	1940
C01C	solpat	0	287	0	0	0	0	33	86	1072	7544	56	28	0	86	9192
W08A	spapec	0	0	0	0	0	0	0	0	0	0	109	104	0	139	351
W06A	spapec	0	0	0	0	0	0	0	0	0	0	90	126	0	301	517
W01B	spapec	0	0	0	0	32	0	0	0	41	24	115	215	0	195	622
U16C	spapec	0	0	0	0	28	0	27	0	0	22	114	274	0	320	786
U12B	spapec	0	0	0	0	26	16	23	0	0	0	109	139	0	424	737
C09C	spapec	0	0	0	0	46	0	0	0	22	27	115	367	0	215	792
U20B	spialb	0	0	21	0	288	120	0	0	52	32	805	206	71	93	1688
U15B	spialb	0	0	0	0	120	20	0	0	0	43	18	184	48	95	528
U06C	spialb	0	0	0	0	116	28	0	0	0	32	726	188	21	92	1203
C08C	spialb	0	0	0	0	195	93	0	0	0	29	738	201	41	135	1430
U18C	vibden	20	0	26	0	27	0	0	47	19	26	352	71	0	96	682
U10B	vibden	29	0	19	0	37	0	0	54	22	29	23	190	44	0	447
U04C	vibden	18	0	0	0	0	0	0	27	0	32	823	104	37	0	1042
C04C	vibden	32	0	22	0	43	0	0	39	0	23	246	74	0	40	519

Appendix 5

Greenhouse Study of Effects of Plant Species and Compost on PAH Phytoremediation

INTRODUCTION

Phytoremediation and microbial bioremediation are both biological methods of environmental clean-up. Bioremediation often involves the construction of injection wells for adding microorganisms, food sources, or chemicals to the site of contamination in order to enhance remediation. Some bioremediation efforts include removing the soil and placing it in bioreactors. Biological reactors (bioreactors) are chambers that are used to mix contaminated soil, microorganisms and sometimes amendments for the purpose of bioremediation. The use of reactors involves disruptive excavation and is expensive. Phytoremediation uses plants to degrade, or detoxify environmental contaminants. Phytostimulation is a phytoremediation strategy, which involves the stimulation of microbial degradation of a contaminant by plants and exudates. Plants and microorganisms rely on soil nutrients and water for survival, and for degradation environmental contaminants. The availability of soil nutrients and water are dependent upon the soil structure and properties. Addition of compost to contaminated soils can improve soil structure, enhance soil nutrients, increase organic matter, increase porosity, and increase soil cation exchange capacity. These changes could be favorable for plants and microorganisms and enhance biological methods of remediation. This experiment was conducted to evaluate the use of plants in combination with compost amendments for biological degradation of PAHs in contaminated soils.

Composts have been shown to be beneficial to remediation of organic and inorganic contaminants. Compost can increase the microbial activity and perhaps the

number of microbial degraders in contaminated soils. Using compost for bioremediation of PAHs has been demonstrated to be an effective method of reducing PAH concentration in bioreactors (Lilja *et al.*, 1996; Civilini and Sebastianutto, 1996). Addition of steer manure compost was shown to enhance degradation of 1, 3-dichloropropene after 8 weeks (Ibekwe, 2001). Bacterial and fungal cell densities were greater in yard compost-amended pesticide-contaminated soil when compared to unamended contaminated soil (Cole, 1994). Poultry litter and peat moss amendments have been demonstrated to increase plant biomass and percentage accumulation of radioactive contaminants 137 Cesium and 90 Strontium compared to controls with no amendments (Entry *et al.*, 2001). Compost, therefore, has previously been beneficial to environmental rehabilitation processes. In this study, the effects of plants in soils amended with composts on the phytoremediation of PAHs were evaluated using three plant species and an unplanted control.

MATERIALS AND METHODS

The experiment involved 3 different plant species treatments, 6 soil mixtures, and 3 sampling times. The 3 plant species were: *Andropogon gerardii* (Big bluestem), *Eupatorium perfoliatum* (Boneset), *Lobelia cardinalis* (Cardinal flower). For ease of presentation and communication, abbreviations were developed for each treatment. The plant species was abbreviated as the first letter of its genus (Table A5.1). In addition, the experiment had an unplanted soil control (coded “U”), and an untreated soil control. The unplanted soil control was watered and fertilized in the greenhouse along with the other planted species’ treatments and was used as a control to assess the effects of plant species. The untreated control treatment soil was stored in amber jars with teflon caps at

4 °C and was used as a control to assess the effects of abiotic (volatilization, leaching and photolysis) and microbial processes on soil total PAH concentration [tPAH] that could have occurred in the pots. This untreated control was also used to estimate an acceptable storage time for soil samples at 4 °C.

The 6 soil mixtures were designated as treatments A, B, C, D, E, and F (Table A5.1). Treatments B and E were soils amended with 15% yard compost (Charter Township of Ypsilanti, MI) by volume, and soil treatments C and F were amended with 10% yard compost (sieved ≤ 2.36 mm) and 5% poultry manure (sieved ≤ 2.36 mm) (Herbruck's Poultry Ranch, Saranac, MI) by volume.

Table A5.1. Treatment Codes.

<u>Plant treatment code</u>	<u>Treatment</u>
A	<i>Andropogon gerardii</i>
E	<i>Eupatorium perfoliatum</i>
L	<i>Lobelia cardinalis</i>
U	unplanted
<u>Soil treatment code</u>	
A	contaminated soil
B	contaminated soil + 15% yard compost (v/v)
C	contaminated soil + 10% yard compost (v/v) + 5% poultry manure (v/v)
D	uncontaminated soil
E	uncontaminated soil + 15% yard compost (v/v)
F	uncontaminated soil + 10% yard compost (v/v) + 5% poultry manure (v/v)

Samples were taken at weeks zero, four and eight. The total number of pots in the experimental set up were calculated as follows: 4 plant species treatments (including unplanted) x 6 soil treatments x 6 replications x 2 sampling times, which amounted to 288 pots. The actual replication number sampled at each sampling time in the experiment was four, allowing for two extra replications per sampling time in case of plant mortality or accidents.

Soil

Uncontaminated soils

The uncontaminated soil consisted of a mixture of sandy loam and 2-NS sand (sieved ≤ 4.75 mm). 2-NS sand as classified by the Michigan Department of Transportation typically possesses a high carbonate concentration (Dr. Delbert Mokma, MSU, personal communication). The uncontaminated soil was made by mixing 1/3 sieved 2-NS sand (40 L) and 2/3 sieved sandy loam (80 L) by volume using a cement mixer.

Contaminated soils

Contaminated field soil was obtained from the coking oven area of the Rouge Manufacturing Complex in Dearborn, MI and stored at 4° C until use in the greenhouse experiment. The PAH-contaminated soil was mixed in a 1:1 (v/v) ratio with the uncontaminated soil mix (1/3 of sandy loam + 2/3 2-NS sand), all sieved (≤ 4.75 mm) prior to mixing.

Soil characteristics

Nutrient and structural characteristics for each soil treatment were determined by MDS Harris (Lincoln, NE) at weeks zero and eight (Tables A5.2, A5.3). The Rouge

PAH-contaminated coke oven soil had a pH range from 8.0 - 8.1 and was classified as loamy sand or sand (MDS Harris, Lincoln, NE). The pH of the contaminated soil was maintained to favor indigenous microbial PAH degraders. The soil pH was measured weekly from pot leachate with pH paper strips.

In general, contaminated soils had higher soluble salts, nutrients, sodium, and cation exchange capacities than uncontaminated soils (Tables A5.2, A5.3). Amended soils had apparently higher concentrations of nutrients compared with unamended soils (Tables A5.3). Poultry manure treatments seemed to have higher phosphorus and potassium than the other treatments. Yard compost treatments had the lowest pH values (Table A5.2).

Plants

Plant species included in this study, *A. gerardii* (Big bluestem), *E. perfoliatum* (Boneset), and *L. cardinalis* (Cardinal flower), were chosen based on Chapter 2 field results and seed availability. The seed germination rates for *A. gerardii*, *E. perfoliatum*, and *L. cardinalis* were roughly 44.9 %, 68.1 % and 12.2 %, respectively. Seeds for *A. gerardii* were donated by North American Prairies (Annandale, MN) and the seeds for *E. perfoliatum* and *L. cardinalis* were obtained from Wildtype Nurseries (Mason, MI). Seeds were germinated in potting soil in plastic germination trays.

Table A5.2. Soil properties at week 0 and week 8. Each value represents the mean of two samples. Soil and Plant codes described in Table A5.1. For Texture: L.S. = loamy sand, S. = sand, S.* = loamy sand (one replicate) or sand (one replicate).

Soil	Trmt Wks	Plant	Soluble				% of total CEC					Soil Composition (%)					Density (g/cc)
			pH	Salts (mmhos/cm)	Na ppm	H	K	Mg	Ca	Na	CEC (cmol/kg)	OM	Sand	Silt	Clay	Texture	
A	0	none	8.1	4	132	0	1	12	85	2	33	2.0	86	9	5	L.S.	1.40
A	8	A	8.3	3	49	0	1	12	86	1	26	2.3	84	12	4	L.S.	1.50
A	8	E	8.4	1	31	0	1	14	84	1	18	2.2	85	11	4	L.S.	1.40
A	8	L	8.3	3	45	0	1	13	86	1	26	2.3	84	13	3	L.S.	1.45
A	8	U	8.4	1	31	0	1	12	86	1	21	2.3	84	12	4	L.S.	1.45
B	0	none	8.0	4	121	0	2	13	83	2	31	2.6	86	9	5	L.S.	1.40
B	8	A	8.4	2	30	0	1	13	85	1	23	2.9	85	11	4	L.S.	1.40
B	8	E	8.5	0	31	0	1	14	84	1	19	2.7	84	12	4	L.S.	1.40
B	8	L	8.4	2	39	0	2	13	84	1	25	2.8	82	14	4	L.S.	1.50
B	8	U	8.6	1	26	0	1	14	85	1	20	2.6	85	12	3	S.*	1.30
C	0	none	8.1	4	240	0	7	15	75	3	34	2.8	90	4	6	S.	1.40
C	8	A	8.6	3	52	0	4	14	81	1	24	3.0	87	9	4	S.*	1.45
C	8	E	8.5	1	31	0	1	13	85	1	19	2.9	92	4	4	S.	1.40
C	8	L	8.6	2	44	0	4	15	80	1	23	2.8	85	10	5	L.S.	1.35
C	8	U	8.7	1	29	0	3	15	81	1	20	3.1	87	9	4	S.*	1.35

Table A5.2 continued

Table A5.2 continued				Soluble		% of total CEC						Soil Composition (%)						
Soil	Trmt Wks	Plant	pH	Salts (mmhos/cm)	Na ppm	H	K	Mg	Ca	Na	CEC (cmol/kg)	OM	Sand	Silt	Clay	Texture	Density (g/cc)	
D	0	none	7.7	1	20	0	1	7	92	1	15	1.4	89	7	4	S.	1.60	
D	8	A	8.3	0	17	0	0	10	89	1	12	1.5	89	7	4	S.	1.55	
D	8	E	8.4	0	20	0	0	12	87	1	12	1.5	88	8	4	S.	1.60	
D	8	L	8.4	0	16	0	0	10	89	1	12	1.5	88	8	4	S*	1.55	
D	8	U	8.3	0	15	0	0	11	88	1	13	1.5	88	7	5	S*	1.60	
E	0	none	7.5	1	25	0	2	9	88	1	15	2.2	89	6	5	S.	1.60	
E	8	A	8.3	0	17	0	1	12	87	1	13	2.1	87	9	4	S*	1.50	
E	8	E	8.4	0	22	0	0	14	85	1	14	2.2	84	12	4	L.S.	1.50	
E	8	L	8.3	0	18	0	1	12	87	1	14	2.2	87	9	4	S*	1.45	
E	8	U	8.3	0	16	0	1	14	85	1	13	2.2	86	10	4	S*	1.50	
F	0	none	7.8	1	100	0	10	12	75	3	15	2.2	92	4	4	S.	1.50	
F	8	A	8.3	0	24	0	2	17	80	1	14	2.4	87	9	4	S*	1.55	
F	8	E	8.5	0	24	0	1	16	83	1	14	2.4	88	7	5	S*	1.50	
F	8	L	8.1	0	33	0	5	17	77	1	15	2.4	86	10	4	S*	1.55	
F	8	U	8.3	0	21	0	1	17	81	1	13	2.2	87	8	5	L.S.	1.45	

Table A5.3. Soil nutrient concentrations at week 0 and week 8. Each value represents the mean of two samples. Soil and plant codes defined in Table A5.1.

			Nutrients (ppm)										
Soil	Trmt Wks	Plant	N	P	K	Mg	Ca	S	Zn	Mn	Cu	Fe	B
A	0	none	77	15	129	479	5571	999	10	10	3	20	3
A	8	A	14	30	100	384	4435	879	12	11	3	20	2
A	8	E	6	65	38	303	2960	84	13	11	3	22	1
A	8	L	15	39	116	397	4481	930	12	11	3	21	2
A	8	U	3	63	96	316	3640	408	13	28	3	22	2
B	0	none	119	25	253	483	5129	999	11	9	2	70	3
B	8	A	15	51	123	348	3887	478	14	14	3	49	2
B	8	E	5	53	49	321	3134	80	15	13	3	40	1
B	8	L	20	52	177	398	4173	656	14	12	2	44	2
B	8	U	6	67	106	327	3395	204	15	37	3	48	2
C	0	none	114	88	878	608	5034	999	16	25	3	36	3
C	8	A	24	147	394	409	3888	711	19	28	3	52	3
C	8	E	15	174	82	296	3202	139	20	17	2	41	2
C	8	L	27	179	369	405	3713	595	20	17	3	45	3
C	8	U	4	201	228	376	3316	290	21	77	4	45	2
D	0	none	14	4	34	119	2659	97	1	4	1	31	0
D	8	A	7	21	9	144	2124	19	2	4	1	29	0
D	8	E	3	29	11	179	2136	17	2	4	1	30	1
D	8	L	6	23	18	143	2156	18	2	4	1	30	0
D	8	U	4	26	14	173	2204	24	3	5	1	32	1
E	0	none	52	11	116	168	2682	82	3	4	1	44	1
E	8	A	7	28	25	196	2273	18	5	5	1	46	1
E	8	E	3	43	13	235	2357	14	5	5	1	44	1
E	8	L	7	33	36	203	2416	23	5	5	1	52	1
E	8	U	6	47	31	210	2198	22	4	5	1	46	1
F	0	none	25	83	603	220	2263	140	7	15	1	89	1
F	8	A	10	128	82	284	2200	31	10	19	2	52	1
F	8	E	9	117	28	269	2282	27	10	8	1	43	1
F	8	L	22	117	281	308	2318	40	13	9	1	111	1
F	8	U	4	124	70	275	2158	25	9	29	2	46	1

Experimental Set-up

A coffee filter was placed in each labeled plastic pot (10 cm x 10 cm x 4") prior to filling the pot with soil to minimize loss of soil. Contaminated soils were put in pots in chemical safety fume hood. *E. perfoliatum*, *L. cardinalis* plantlets (each 6 weeks old), and *A. gerardii* plantlets (3 weeks old) were transplanted into the pots containing the six soil mixtures. *E. perfoliatum* was severely wilted the second day after transplanting and a total of 18 plants were replaced. To ease transplanting stress, the plants were covered with Ziploc bags for 1 day. Plant viability was recorded weekly and photos of the planted treatments were taken every two weeks. Each pot had been assigned a number from a random number table (Moore and McCabe 1999) for placement in staggered randomized rectangular grids (6 pots x 16 pots) spaced one pot width apart on greenhouse benches.

Greenhouse conditions

The experiment was conducted from February to April 2002, during which time daylight hours increased from 9 hours to 11 hours. A LI-189 photometer (LI-COR, Lincoln, NE) was used to measure photosynthetically active radiation (PAR) at pot height. At noon on a relatively sunny day PAR levels were 350 – 700 $\mu\text{E}\cdot\text{s}^{-1}\text{m}^{-2}$; on partly cloudy days, PAR levels were 300 – 600 $\mu\text{E}\cdot\text{s}^{-1}\text{m}^{-2}$. Although daylight hours were 9 to 11 hours per day, artificial lights (400 Watts, high pressure sodium, intensity 970 candles, General Electric) were on 16 hours per day. PAR levels at night when artificial lights were illuminated were 40 – 75 $\mu\text{E}\cdot\text{s}^{-1}\text{m}^{-2}$. Greenhouse temperature was typically between 20 °C to 30 °C except for a few days near the end of March when the greenhouse temperature was ~38 °C due to temperature control malfunction.

Watering & Fertilization

Plants were watered as required and unplanted treatments were watered daily to field capacity. When the weather was cloudy, plants typically required watering once a day and sometimes every two days. When the weather was sunny, plants required watering at least three times a day to up to seven times a day for *E. perfoliatum* between weeks four and eight. All treatments were watered with N-P-K fertilizer solution (20-20-20, ~475 ppm) once a week.

Sample handling

Time zero samples were extracted and stored in amber jars with teflon lids at 4 °C and used as untreated control samples for weeks 4 and 8. A representative 4 samples from each plant species were used for extraction and a representative four plants were used for determination of dry weights. Plant tissue dry weights were determined by oven drying at ~80 °C for at least 48 hours.

Plants and soil were destructively sampled at weeks 4 and 8. For each pot, the top ~1 cm of soil was discarded using a metal spatula. The root portion of the plant was removed from the pot by gently prying into the soil around the roots with a metal spatula and collecting the soil off the roots. Soil was rinsed off roots in a bucket of water and the roots were blotted dry and weighed using an electronic balance. After the fresh weights were recorded, the plants were dried in an oven for at least 48 hours at ~ 80 °C for determination of dry weight.

At weeks 4 and 8, the soil from the center of the pot was collected and stored in 150 mL amber jars with a teflon lid at 4°C and extracted the day after harvesting. For uncontaminated pots, one subsample was taken from each plant x soil treatment. Fewer

samples were taken from the uncontaminated pots because it was hypothesized soil [tPAH] would be minimal, therefore, the data would not be useful and devoting more resources to this would have been wasteful. Uncontaminated soil and plant treatment samples were used for determination of background soil [tPAH] levels.

Analyses of samples

Extractions of PAHs from samples, and analyses for PAH concentration were done as stated previously in Chapter 2 Materials and Methods section.

The total PAH concentration [tPAH] was determined by taking the sum of the concentrations of the following compounds: naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, and benzo(g,h,i)perylene. Refer to Table 2.4 for abbreviations and lowest calibration standards.

RESULTS

Soil total PAH concentration ([tPAH])

There were no obvious differences in soil [tPAH] among plant treatments (including unplanted and untreated treatments (4°C). In addition, there were no differences among soil treatments at each sampling time (Table A5.4) during this 8-week greenhouse study and no trends in soil [tPAH] over time. Soil [tPAH] was highly variable in all planted, unplanted, and untreated treatments at each sampling time (Table A5.5).

Table A5.4. Soil total PAH concentration (mean $\pm$ SEM) in pots containing PAH-contaminated soil over time (N = 4 pots, N = 1 as noted by “*” due to mortality). Subsamples from each pot were averaged and standard errors represent the variability of the average [tPAH] among pots for a given treatment.

Treatment	Soil Treatment	Soil Concentrations (mg/kg)		
		Week 0	Week 4	Week 8
<i>A. gerardii</i>	A	226.3 $\pm$ 20.7	364.3 $\pm$ 16.3	311.3 $\pm$ 34.8
<i>A. gerardii</i>	B	233.9 $\pm$ 27.5	251.6 $\pm$ 24.8	331.2 $\pm$ 63.9
<i>A. gerardii</i>	C	256.9 $\pm$ 34.4	370.9 $\pm$ 78.3 *	250.3 $\pm$ 12.7
<i>E. perfoliatum</i>	A	226.3 $\pm$ 20.7	283.5 $\pm$ 26.6	384.7 $\pm$ 62.9
<i>E. perfoliatum</i>	B	233.9 $\pm$ 27.5	222.0 $\pm$ 19.8	269.7 $\pm$ 25.1
<i>E. perfoliatum</i>	C	256.9 $\pm$ 34.4	193.1 $\pm$ 11.6	345.5 $\pm$ 70.5
<i>L. cardinalis</i>	A	226.3 $\pm$ 20.7	321.9 $\pm$ 49.3	308.6 $\pm$ 40.8
<i>L. cardinalis</i>	B	233.9 $\pm$ 27.5	418.8 $\pm$ 100.1	296.1 $\pm$ 56.2
<i>L. cardinalis</i>	C	256.9 $\pm$ 34.4	346.9 $\pm$ 39.0	268.5 $\pm$ 32.1 *
Unplanted	A	226.3 $\pm$ 20.7	420.0 $\pm$ 34.0	247.3 $\pm$ 22.7
Unplanted	B	233.9 $\pm$ 27.5	223.6 $\pm$ 19.3	210.2 $\pm$ 11.8
Unplanted	C	256.9 $\pm$ 34.4	334.3 $\pm$ 63.4	261.7 $\pm$ 40.3
Untreated	A	226.3 $\pm$ 20.7	322.1 $\pm$ 61.7	337.9 $\pm$ 105.1
Untreated	B	233.9 $\pm$ 27.5	196.7 $\pm$ 6.3	281.8 $\pm$ 29.6
Untreated	C	256.9 $\pm$ 34.4	283.5 $\pm$ 38.6	447.5 $\pm$ 88.9

Table A5.5. Soil [tPAH] maximum pot ranges determined from 3 subsamples per pot per treatment and treatment soil [tPAH] ranges determined from means of subsamples from 4 individual pots at week 8.

Soil [tPAH] (mg/kg)			
Treatment	Soil Treatment	Pot Range	Treatment Range
<i>A. gerardii</i>	A	220 - 522	237 – 376
<i>A. gerardii</i>	B	194 - 986	222 – 484
<i>A. gerardii</i>	C	140 - 335	218 – 275
<i>E. perfoliatum</i>	A	258 - 692	270 – 542
<i>E. perfoliatum</i>	B	180 - 430	239 – 320
<i>E. perfoliatum</i>	C	195 - 1031	166 – 485
<i>L. cardinalis</i>	A	207 - 526	230 – 416
<i>L. cardinalis</i>	B	146 - 1081	223 – 464
<i>L. cardinalis</i>	C	141 - 433	199 – 354
Unplanted	A	292 - 401	191 – 301
Unplanted	B	104 - 287	191 – 243
Unplanted	C	178 - 608	150 – 331
Untreated	A	157 - 1597	231 – 653
Untreated	B	220 - 544	206 – 347
Untreated	C	396 - 938	313 – 709

Plant [tPAH]

The [tPAH] in plant tissues were high (Table A5.6). Individual PAH compounds detected in plants were predominantly benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(a)pyrene, and benzo(ghi)perylene, though *Eupatorium* leaves were also observed to contain elevated levels of acenaphthylene and acenaphthene (data not shown).

Plant Dry Biomass

Plants had varied initial fresh weight at time 0 and were randomly assigned to treatments. Further analyses of these data should utilize Analysis of Covariance with initial plant fresh weight as a covariate. Contrasts should be utilized for treatment comparisons.

Shoot Dry Weight

For each species, shoot dry weight increased over time (Fig A5.1). *E. perfoliatum* plants had greater shoot dry weight than *A. gerardii* or *L. cardinalis*. For *A. gerardii* and *E. perfoliatum*, plants grown in soil F (uncontaminated soil + yard compost + poultry manure) showed substantially greater shoot dry weight than plants grown in soils A through E at week 8. For *A. gerardii* there was a trend of increasing shoot dry weight in soils $D < E < F$ at week 8.

Root Dry Weight

Root dry weight increased over time from week 0 to week 8 in all soil treatments (Fig. A5.2). In most cases, root dry weight was lower in contaminated soil treatments than uncontaminated treatments.

It should be noted that that certain treatments showed higher mortality than others. Treatments amended with poultry manure (treatments C and F) had plant

mortality. Shoot and root dry weights were low for treatments C and F at week 4 for all species.

Table A5.6. PAH concentration (mean $\pm$ SEM) mg/kg dry weight in plant shoot tissue from plants grown in pots in the greenhouse. Standard errors represent the variability among plants for a given treatment. N = 1, N = 3 as noted (*) due to mortality, and N = 1 as indicated by †.

Plant Species	Soil	Plant [PAH] (mg/kg)		
		Week 0*	Week 4	Week 8
<i>A. gerardii</i>	A	1200.6 $\pm$ 234.5	1593.8 $\pm$ 412.7	490.6 $\pm$ 78.1
<i>A. gerardii</i>	B	1200.6 $\pm$ 234.5	562.1 $\pm$ 81.0	605.3 $\pm$ 86.7
<i>A. gerardii</i>	C	1200.6 $\pm$ 234.5	2263.9 $\pm$ N/A†	1666.1 $\pm$ 892.9*
<i>A. gerardii</i>	D	1200.6 $\pm$ 234.5	425.7 $\pm$ 37.6	546.0 $\pm$ 24.3
<i>A. gerardii</i>	E	1200.6 $\pm$ 234.5	703.5 $\pm$ 81.2	581.6 $\pm$ 124.5
<i>A. gerardii</i>	F	1200.6 $\pm$ 234.5	589.8 $\pm$ 54.3	563.1 $\pm$ 90.4
<i>E. perfoliatum</i>	A	1246.0 $\pm$ 272.7	1369.4 $\pm$ 230.7	569.7 $\pm$ 88.3
<i>E. perfoliatum</i>	B	1246.0 $\pm$ 272.7	1024.5 $\pm$ 210.2	597.2 $\pm$ 79.8
<i>E. perfoliatum</i>	C	1246.0 $\pm$ 272.7	1683.9 $\pm$ 320.3	448.5 $\pm$ 28.5
<i>E. perfoliatum</i>	D	1246.0 $\pm$ 272.7	1181.1 $\pm$ 300.8	444.3 $\pm$ 92.6
<i>E. perfoliatum</i>	E	1246.0 $\pm$ 272.7	800.0 $\pm$ 132.6	543.5 $\pm$ 178.8
<i>E. perfoliatum</i>	F	1246.0 $\pm$ 272.7	2148.5 $\pm$ 1336.5	445.6 $\pm$ 63.3
<i>L. cardinalis</i>	A	1198.1 $\pm$ 330.0	322.1 $\pm$ 57.1	3422.3 $\pm$ 1802.3
<i>L. cardinalis</i>	B	1198.1 $\pm$ 330.0	316.4 $\pm$ 63.0	402.9 $\pm$ 64.2
<i>L. cardinalis</i>	C	1198.1 $\pm$ 330.0	345.8 $\pm$ 105.1*	228.1 $\pm$ N/A†
<i>L. cardinalis</i>	D	1198.1 $\pm$ 330.0	272.4 $\pm$ 76.3	413.4 $\pm$ 171.2
<i>L. cardinalis</i>	E	1198.1 $\pm$ 330.0	299.5 $\pm$ 64.0	278.8 $\pm$ 59.2
<i>L. cardinalis</i>	F	1198.1 $\pm$ 330.0	267.2 $\pm$ 25.2*	239.5 $\pm$ N/A†

* At time zero, a representative 4 plants were sampled per species.

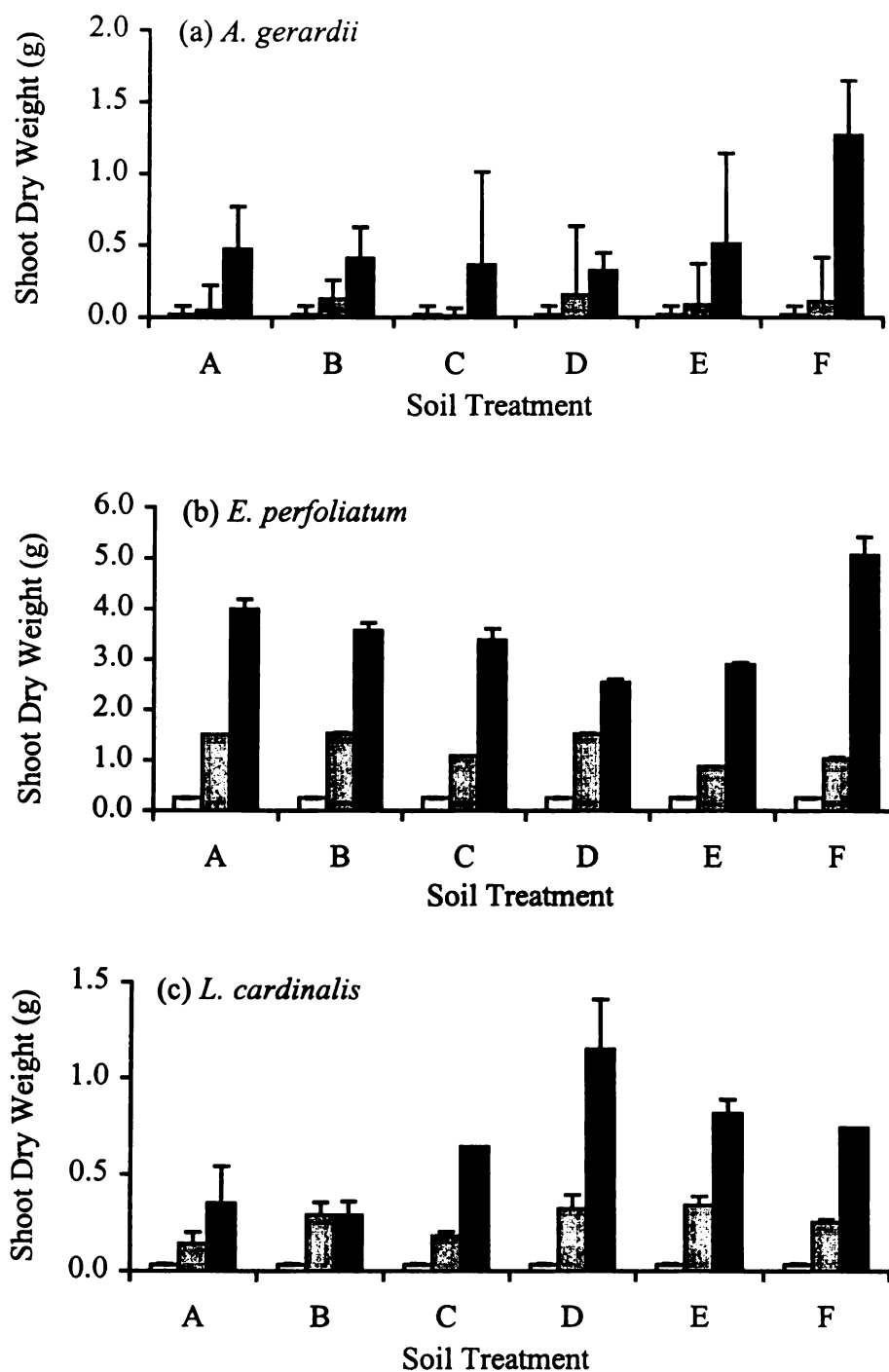


Figure A5.1 (a-c). Plant shoot dry weights (mean + SE) for plants in various soils at week 0 (empty bar), 4 (grey bar), and 8 (black bar). $N = 4$, except for *L. cardinalis* x Soil C (week 4, $N = 3$), *L. cardinalis* x soil F (week 8, $N = 1$), *A. gerardii* x soil C (week 8, $N = 3$), *L. cardinalis* x soil C (week 8, $N = 1$), *L. cardinalis* x Soil F (week 8, $N = 1$). In these instances sample sizes were reduced due to mortality.

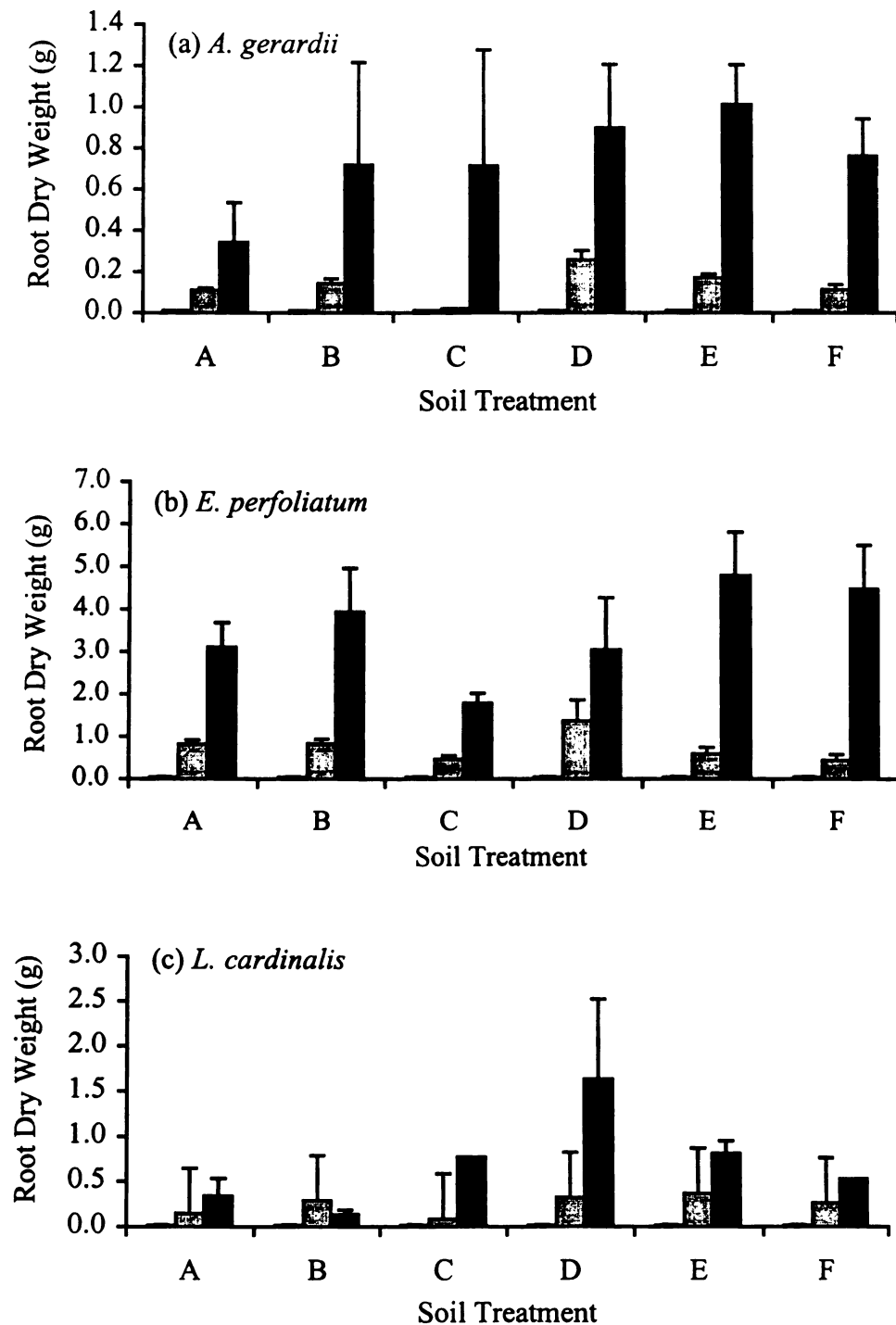


Figure A5.2 (a-c). Plant root dry weight (mean + SE) for plants in various soils at week 0 (empty bar), 4 (grey bar), and 8 (black bar). $N = 4$, except for *L. cardinalis* x Soil C (week 4, $N = 3$), *L. cardinalis* x soil F (week 8, $N = 1$), *A. gerardii* x soil C (week 8, $N = 3$), *L. cardinalis* x soil C (week 8, $N = 1$), *L. cardinalis* x Soil F (week 8, $N = 1$). In these instances sample sizes were reduced due to mortality.

DISCUSSION

Soil Properties & Nutrients

Soil characteristics were varied among the different treatment soil mixtures.. The contaminated soil had higher nutrient concentration, which may partially reflect its higher cation exchange capacity (Table A5.2). The pH increased from week 0 to week 8, which is likely because the tap water in the MSU greenhouse has a high pH ~8 (Dave Freville, personal communication), though the starting pH of the Rouge soil was observed to be ~ 8.0 – 8.5 (Table A5.2). In this greenhouse study, soil compost amendments, specifically the yard compost, decreased soil pH and increased soil organic matter, thereby improving soil conditions for plant growth.

Soil [tPAH]

The soil [tPAH] values were highly variable and this variability masked any effects of plant species and soil treatments. The soil from the Rouge Manufacturing Complex Coke Oven area was highly variable in soil [tPAH], ranging from 500 – 900 mg/kg prior to mixing with uncontaminated soil. The experimental results may have been improved with better homogenization of the contaminated soils prior to distribution among the plant treatments. To reduce variability of soil [tPAH] for the samples in a given treatment, more soil could be used for a single extraction, or soil samples could be pulverized or finely sieved (Dr. G. Phil Robertson, personal communication). In addition, the sample variation was very high and potentially not enough samples were taken to accurately describe the variation, in particularly at time 0. Future studies may also include more subsamples. Alternatively, the failure to observe reduction of soil [tPAH]

may indicate that 8 weeks was insufficient to demonstrate phytoremediation of such highly PAH-contaminated soils.

The results in this study are not in accordance with the majority of previous studies. There are several potential reasons that soil [tPAH] concentrations were not observed to decrease in this study as has been shown in other studies (Aprill and Sims, 1990; Pradhan *et al.*, 1998; Yateem *et al.*, 2000) including the APGEN study and Chapter 2 of this thesis. The starting soil [tPAH] concentration for this experiment is higher and more heterogeneous than in the Phyto Demo field study. It is possible that phytoremediation may not be as effective at high concentrations. This explanation does not hold for the comparison of the data in this study with the APGEN data, however, since the APGEN soil [tPAH] data were just as high and in some cases higher (Appendix 1 vs. Table A5.5). The APGEN study was conducted differently using only Perlite as a soil amendment and analysis of the entire pot contents, rather than only for rhizosphere soil exclusive of roots as performed in this experiment. Concentration of PAHs have been found to be 4- 5 times higher around plant roots as a result of increased mobility of PAHs (Liste and Martin, 2000). A hypothesis explaining higher PAH concentration near roots is that roots increase PAH mobility and exude organic compounds, resulting in sorption of PAHs to these exudates and to root surfaces (Liste and Martin, 2000). Sampling differences, therefore, may account for some discrepancy between this study and the APGEN study. By week 8, plants roots filled the entire pot for most treatments and separation of roots from soil was difficult. Small plant roots may have been included in the extraction sample and this too may have contributed to the variability. Multiple

factors, alone or synergistically, may have led to discrepancies between this and previous studies.

Plant [tPAH]

The exceedingly high concentrations of PAHs detected in plants in this study cannot be explained by previous literature. Previous literature reports various results for PAH concentration in plant tissue. Some previous studies have shown biomagnification of PAHs occurs in plant tissues, though others indicate background levels at parts per billion or lower. Data presented by (Sims and Overcash, 1983) indicate natural background levels of PAHs such as anthracene, fluoranthene, benz(a)anthracene, pyrene and benz(a)pyrene in plants at concentrations from 10-90 µg/kg dry weight for each PAH compound. Thus, the plant PAH concentration values in this thesis are substantially higher than values previously reported in the literature and the reasons for these high concentrations in unknown at this time.

Plant Dry Biomass

There were differences in plant growth among plant species for the various treatments. Transplanting stress and nutrient deficiency stress may have led to slow growth rates of plants during the first weeks of the experiment. *E. perfoliatum* had greater shoot dry weight than *A. gerardii* or *L. cardinalis*. Greater plant growth was observed in uncontaminated soils than the contaminated soils. The higher plant biomass observed in uncontaminated soils may indicate that plants were stressed in the contaminated soil perhaps by the lack of nutrient availability or by the PAH contaminants.

The poultry manure amendment may have caused some toxic effects to the plants in this study. The poultry manure had not been fully composted (Andy Fogiel, personal

communication) and the odor of ammonia was evident. At week 4, plants in the yard compost + poultry manure-amended soil had lower shoot and root dry weights and higher plant mortality than other treatments (data not shown). By contrast, at week 8, *A. gerardii* and *E. perfoliatum* showed greater growth in uncontaminated soil amended with yard compost + poultry manure than all other treatments. As plants grew bigger, they may have become less susceptible to ammonium or salt toxicity and as a result slow growth rates were not observed between weeks 4 and 8. It is therefore important to consider the levels and quality of soil amendments prior to large-scale application in remediation efforts.

SUMMARY & CONCLUSION

This study raised some issues and considerations for future greenhouse studies. The experimental results in these trials were confounded by high variability in soil contaminant concentrations and high plant contaminant concentrations. The source of the elevated plant tissue [tPAH] levels is unknown and remains to be resolved by re-analysis of the apparent leaf PAH compounds by additional analytical procedures, such as GC-MS. In this 8-week greenhouse study, no effect of plant treatment or soil amendments on soil [tPAH] could be seen because high soil [tPAH] variation may have masked these effects. Future phytoremediation greenhouse experiments could be improved by more thorough homogenization of soils before treatment and upon sample analysis. Additionally, larger sample volumes could be used to buffer the influence of heterogeneous soil “hot spots” on soil [tPAH] determination. This study has raised questions about high [tPAH] concentrations in plant tissues, provided important lessons

in sampling and analyses of PAH-contaminated soil samples, and has shed light on how analytical protocols may be improved for future phytoremediation experiments.

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