Phytoremediation: Investigating a Means of Removing PAHs from the Floodplain of the Chattanooga Creek

by
Eric Matthew Cohen

Departmental Honors Thesis
The University of Tennessee at Chattanooga
Biology Department

Project Director: Dr. Sean Richards
Examination Date: March 29, 2007

Dr. Doug Kutz
Dr. Henry Spratt
Dr. Hill Craddock

Examining Committee Signatures:

____
Project Director

____
Department Examiner

____
Department Examiner

____
Liaison, Departmental Honors Committee

____
Chairperson, University Departmental Honors Committee

Table of Contents

Abstract……………………….............……………........……………………….……3
Introduction……………………..........……………...…………………………...4
Materials and Methods……………….....................................……………………13
Results………………………...............……………………………………..…24
Discussion..28
Literature Cited..37
Acknowledgements...46

List of Figures

Figure 1: UTC Greenhouse Growth Experiment..15
Figure 2: Phytoremediation Experimental Set-up..17
Figure 3: Pyrene Standard Curve...21
Figure 4: HPLC Pyrene Peak Detection...22
Figure 5: Growth Experiment: *F. arundinacea* graph..24
Figure 6: Growth Experiment: *C. pepo* graph...25
Figure 7: Phytoremediation Experiment: *F. arundinacea* graph................................26
Figure 8: Phytoremediation Experiment: *C. pepo* graph..27

List of Tables

Table 1: Sixteen Priority Pollutant PAHs 7
Table 2: HPLC Mobile Phase...20

Abstract

As a result of uncontrolled dumping of coal-tar for much of the past century, polycyclic aromatic hydrocarbons (PAHs) are abundant in the floodplain of Chattanooga Creek. The area is deemed a Superfund site and a danger to public health. Phytoremediation has shown to be a cost-effective, ecologically friendly method to remove toxicants from the environment. The present study concerns two candidate plants for phytoremediation, *Festuca arundinacea* (tall fescue) and *Cucurbita pepo ssp. pepo* (zucchini). Each plant was grown for 28 days in pyrene (a representative PAH) amended soil. Shoot heights were recorded every 3 to 4 days to test plants for sensitivity to PAHs. Results indicate that both species were not affected by the low concentrations of pyrene in the soil. Each plant was also grown in window boxes with pyrene amended (treated) soil and untreated soil to test for the plants ability to reduce pyrene in the soil. The right side of each box was the unplanted control, to test for degradation of pyrene due to soil-borne factors. Multiple soil samples were taken from each window box before planting and then after 24 days for *F. arundinacea* and 56 days for *C. pepo*. Pyrene was then extracted from the soil samples and analyzed via reverse-phase HPLC. Results indicated that *F. arundinacea* and *C. pep*o did not significantly contribute to a reduction of pyrene in the soil. Pre-existing microorganisms are thought to be responsible for the degradation of pyrene. Longer studies should be conducted to allow for *F. arundinacea* and *C. pep*o to influence remediation and to conclusively determine the feasibility of phytoremediating the floodplains of the Chattanooga Creek.

Introduction

Background of Tennessee Products Superfund Site

The Chattanooga Creek originates on Lookout Mountain, Georgia and flows approximately 26 miles into the Tennessee River, passing many south Chattanooga neighborhoods, such as Alton Park and Piney Woods (Vulava 2002). For much of the 20th century there was uncontrolled dumping of coal-tar wastes into the Chattanooga Creek and along the bank. Various companies from 1918 to 1987 operated a coal carbonization facility in Chattanooga, Tennessee (EPA 1999). Notably, the Tennessee Product Corporation (TPC) operated a facility for the longest period, from 1926 to 1964 (Searfoss 2003).

In the coal carbonization process, gases are removed from coal through heating. The typical process produces 80% coke, 12% off-gases which are used as energy for residential heating and lighting, 3% coal-tar (composed of various polycyclic aromatic hydrocarbons) and 1% light oils (Vulava 2002). Water waste, containing coal-tar, was regularly discharged into the Chattanooga Creek from a direct pipe (EPA 2002). Coal-tar was also dumped by the truck load; with some locations having 6 to 8 feet of coal-tar deposits in the stream bed (EPA 1992).

These events caused the area to be hazardous. In 1993, the Agency for Toxic Substances and Disease Registry (ATSDR) issued a health advisory for approximately 2.5 miles of sediment in the Chattanooga Creek, deeming the creek itself a Superfund Site (EPA ID: TND071516959, Vulava 2002). The Tennessee Products Superfund (TPS) site begins approximately at Hamill Rd. and ends at Dobbs

Branch (EPA 1999). The ATSDR has stated that the area contains carcinogenic polycyclic aromatic hydrocarbons (PAHs) at levels higher than the acceptable minimum set by the EPA for potential cancer risk (Searfoss 2003).

The soil in the floodplains of the Chattanooga Creek poses potential health risks. In 1993, the EPA placed a fence between Alton Park Middle School and the Chattanooga Creek to protect children from possible exposure (ATSDR 2006). Despite the potential for contaminant exposure, the Trust for Public Land has proposed building a greenway in South Chattanooga with part of this greenway being a public hiking trail along the Chattanooga Creek (Chattanooga Greenways Program 2003). For these reasons there is an intense interest at the federal, state and local level for clean up of this contaminated site. The initial clean-up of the Chattanooga Creek was completed in 1998, excavating 25,350 cubic yards of waste from the site, and costing 12 million dollars (EPA 2002). This large-scale, expensive clean-up however, may not have completely diminished the human health risk and ecological threat of the contaminated site because it did not clean-up the floodplains. The TPS is still highly contaminated with PAH concentrations ranging from 18.8 to 506 mg/kg of soil in the floodplains of Chattanooga Creek (ATSDR 2006). Flooding of the Chattanooga Creek has the potential to spread PAHs to all parts of the floodplains, making expensive excavation techniques unfeasible.

Physiochemical Properties of PAHs

PAHs are composed of two or more fused benzene rings and vary widely in molecular structure. In general, PAHs are hydrophobic, with aqueous solubility decreasing almost linearly as molecular mass increases (Zhang et al. 2006). High-molecular-weight PAHs (PAHs with 4 or more rings) have high resonance energies due to their dense clouds of π electrons surrounding the aromatic rings; making them persistent in the environment and recalcitrant to microbial degradation (Johnsen et al. 2005, Klevens 1949). Low aqueous solubility and high soil sorption also contribute to their persistence and recalcitrance (Parrish et al. 2004, Potter et al. 1999). The EPA designated 16 PAHs as priority pollutants (Table 1) to emphasize the importance of removing these PAHs from the environment. These 16 PAHs are designated priority pollutants because of their recalcitrance in the environment and their potentially toxic, carcinogenic or mutagenic effects to organisms (EPA 1984).

PAHs	Number of Rings	Carcinogenic Classification
Napthalene	2	NC
Acenapthene	3	3
Acenapthylene	3	NC
Fluorene	3	3
Anthracene	3	3
Phenanthrene	3	3
Fluoranthrene	4	3
Chrysene	4	2B
Pyrene	4	3
Benz[a]anthracene	4	2B
Benzo[b]fluoranthene	5	2B
Benzo[k]fluoranthene	5	2B
Benzo[a]pyrene	5	1
Dibenz[a,h]anthracene	5	2A
Benzo[g,h,i]perylene	6	3
Indeno[1,2,3-c,d]pyrene	6	2B

Table 1: Sixteen PAHs designated as priority pollutants by the EPA, the number of aromatic rings that each chemical possess, and their carcinogenic classification given by the International Agency for Research on Cancer (IARC). Carcinogenic classification is based on the risk to humans. Group 1: Carcinogenic, Group 2A: Probably Carcinogenic, Group 2B: Possibly Carcinogenic, Group 3: Carcinogenicity Not Classifiable, and NC: Not Classified by the IARC (IARC 2006).

<u>*Health Effects of PAHs*</u>

All sixteen of the PAH priority pollutants are present at the TPS site, and many of these are probably carcinogenic (Table 1; ATSDR 2006). PAHs can cause cancer by covalently binding to DNA and interfering with accurate replication, eventually leading to mutation and tumor initiation (IARC 2006, EPA 2002). Particular PAHs present in the TPS (benz[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, chrysene, dibenz[a,h]anthracene, and indeno [1,2,3-c,d]pyrene) have been shown to cause tumors in mice when the mice

were exposed to these PAHs through inhalation, ingestion, or dermal contact for extended periods of time (ATSDR 1995). Benzo[a]pyrene has also been shown in mice studies to reduce fecundity and increase the incidence of birth defects (ATSDR 1995). Occupational studies of coal carbonization facility workers who were exposed to high levels of PAHs have also shown increased risk of cancer (IARC 2006). These studies suggest that exposure to PAHs through inhalation, ingestion or dermal contact over extended periods of time may cause significant human health problems.

For the next clean-up, the EPA (2005) explored many costly options for clean-up of the toxic waste, however phytoremediation was not mentioned. Phytoremediation is an *in* situ, ecologically-friendly method to remove toxicants from soil. In phytoremediation, plants are employed to remove toxicants from the soil, promote their degradation, make the toxicant unavailable to the air or groundwater, and reduce human exposure to carcinogenic dust (Pivetz 2001, Ahn et al. 2005).

Phytoremediation has the potential to be a cost effective option for removing organic toxicants from the floodplain of the Chattanooga Creek and elsewhere. However, the phytoremediation of PAHs is complicated by the tight binding of PAHs to soil particles. This tight binding renders the PAHs unavailable to some plants and (or) results in poor uptake (Ahn et al. 2005). Two plants that can stimulate the removal of PAHs from the soil are *Festuca arundinacea* (tall fescue, Chen et al. 2003) and *Cucurbita pepo ssp. pepo* (zucchini, Parrish et al. 2006). These two species differ in their mechanism of removal of PAHs. *F. arundinacea* removes PAHs from

the soil via rhizodegradation whereas; *C. pepo* mechanism of removal is phytoextraction (details below).

F. arundinacea: Rhizodegradation of PAHs

The rhizosphere is the area 1mm from the root in any direction (Pivetz 2001). This area provides a positive growing environment for microorganisms by releasing root exudates, a significant source of energy such as organic acids, amino acids, sugars, and growth factors (Banks et al. 1999, Pivetz 2001). *F. arundinacea* has a deep, extensive fibrous root network maximizing the root-soil contact and the influence of the rhizosphere (Hannaway et al. 1999). *F. arundinacea* uses the influence of plant roots in the rhizosphere to increase the number and activity of rhizosphere microorganisms. The increased number of rhizosphere microorganisms leads to an increase in the degradation of PAHs in the rhizosphere by the microorganisms (Chen and Banks 2004, Pivetz 2001). Microbial activity is also increased by plant roots actively pumping oxygen into the rhizosphere (Hutchinson et al. 2001). The release of energy sources and oxygen into the rhizosphere by the plant is commonly termed the rhizosphere effect. For example, in a 190 day study, Chen et al. (2003) found that soil planted with *F. arundinacea* degraded 37.7% of the pyrene, whereas there was only 4.3% degradation for the unplanted control. Different rhizosphere microorganisms use various pathways to aerobically degrade PAHs; however, most possess the key enzymes dioxygenase or monoxygenase (Zhang et al. 2006). These enzymes catalyze the degradation reaction by incorporating oxygen

molecules into the PAH, which is then further oxidized through a series of complex reactions until the PAH is fully biodegraded (Samanta et al. 2002, Launen et al. 2000). The specific mechanisms for the biodegradation of PAHs is dependent on the particular PAH, microorganism, available nutrients and co-factors (Zhang et al. 2006). *F. arundinacea* has great potential to phytoremediate the floodplains of the Chattanooga Creek because it can promote rhizodegradation of PAHs and can grow well under stressful conditions (Huang et al. 2004*b*).

C. pepo: Phytoextraction of PAHs

Whereas *F. arundinacea* has a fibrous root network, *C. pepo* has a deep tap root system, allowing *C. pepo*'s roots to extend to greater depths in the soil (2-3 feet; Mills 2001). *C. pepo* utilizes phytoextraction to directly uptake PAHs from the soil into its roots and then translocates the PAHs into aerial tissue (Parrish et. al 2006). It would be thought that highly hydrophobic compounds (low aqueous solubility) such as high-molecular-weight PAHs would be sequestered in the soil and unable to cross the plant plasma membrane, however, *C. pepo* has the distinct ability to extract these compounds (White et al. 2005)

C. pepo can phytoextract PAHs that are weathered. The term weathered refers to hydrophobic compounds that are sequestered and sorbed strongly to soil particles over an extended period of time, greatly limiting their bioavailability (Chen and Banks 2004). Parrish et al. (2006) found that *C. pepo* only phytoextracted approximately 0.7% of the total weathered PAHs in the soil; however, a significant

amount of the weathered PAHs accumulated were highly persistent 5 and 6-ring PAHs. Potter et al. (1999) describes that the high hydrophobicity and recalcitrance of 5 and 6-ring PAHs greatly limits the success of remediative technologies because these PAHs bind so tightly to soil particles. The accumulation of 5 and 6-ring PAHs by *C. pepo* is a remarkable and distinct ability for plants. Bioremediation of 5 and 6-ring PAHs are greatly limited by the PAHs' high resonance energies (Klevens et al. 1950). Wang et al. (2004) reported that *C. pepo* was able to phytoextract 1.3% of weathered p,p'-DDE with 98% in the above ground tissue. Despite the low removal percentages, these findings are important because highly weathered, hydrophobic compounds like PAHs and p,p'-DDE are often unavailable to other forms of phyotremediation (Potter et al. 1999). Interestingly enough, Parrish et al. (2006) explained that the uptake and translocation of hydrophobic compounds in *C. pepo* is a newly discovered phenomenon for which the exact mechanisms still remains unknown. Although there is little literature on *C. pepo's* ability to phytoextract, the preliminary research shows much promise for the removal of PAHs from the Chattanooga Creek floodplains.

Study Rationale and Thesis Statement

The effectiveness of different phytoremediation techniques are influenced by biological factors such as soil composition and microbial population and composition and by PAHs' physiochemical properties, which ultimately determine its bioavailability (Chekol et al. 2002, Ahn et al. 2005). *F. arundinacea* and *C. pepo*

represent two contrasting methods of phytoremediation of PAHs. The aim of this study is to determine which plant would be most effective in removing PAHs from the floodplains of the Chattanooga Creek. Based on the information presented above, the working thesis is: *phytoremediation by F. arundinacea and (or) C. pepo will significantly decrease soil concentrations of a model PAH (pyrene) as compared to unplanted soil.*

Materials and Methods

Soil Collection

All of the soil for the present experiment was obtained from the floodplain of Chattanooga Creek, 18.28 m from the nearest bank, close to the border of Georgia and Tennessee, and near the intersection of the Chattanooga Creek and Wilson Road. This location was chosen because it is upstream from the TPS site and therefore the soil was assumed to be relatively free from pyrene. The soil was used from this site due to its similar texture and composition of the soil from the TPS site. At the dig site, 45 plastic pots (27.94 cm in diameter, with a volume of 11.36 L) were filled three-quarters full with soil. The soil was mixed by hand and any waste or debris was removed. The 45 pots were then transported back to the UTC greenhouse.

Experimental Design: Growth Experiment

A large-scale growth experiment was conducted for 28 days using 45 plastic pots so that the conditions were realistic as possible. The pots were housed in the UTC greenhouse and lined with an impermeable tarp in order to contain soil leachate (Fig. 1). The experimental design consisted of 15 pots with 0 mg pyrene/kg of soil, 15 pots with 1 mg of pyrene/kg of soil, and 15 pots with 10 mg pyrene/kg of soil. Each group of 15 pots were divided into 5 pots planted with *C. pepo* (Each pot contained 5 seeds), 5 pots planted with *F. arundinacea* (Each pot contained 23.62 g of seeds), and 5 pots planted with neither species (control group). *C. pepo* and *F. arundinacea* seeds were acquired from Burpee (Warminster, PA). In order to ensure that each pot

received its respective amount of pyrene, the top 3 inches of 5 pots from the 1 mg of pyrene/ kg of soil group were put in a large metal basin. The top three inches of the pots were defined as the topsoil. The total mass of the soil in the metal basin was estimated to be approximately 9 kg. The proper amount of pyrene was mixed with acetone so it could be dissolved. The organic solution was then added to the soil in the metal basin, and mixed thoroughly. Pyrene and all other chemicals were obtained from Sigma (St. Louis, MO.). The soil was then put back in the respective pots. This method was repeated 2 more times to get a total of 15 pots with 1 mg of pyrene/kg of soil in their top soil. This same procedure was repeated to make 15 pots with 10 mg of pyrene/kg of soil. The pots were placed on a greenhouse bench in 3 rows of 15 pots; treatments were arranged in a random block design. Measurement of the shoot heights for each pot planted with *C. pepo* and *F. arundinacea* were conducted bi-weekly and recorded. The shoot heights were measured from where the plant penetrates the soil to the apical meristem of each plant. Measurements of the height of *F. arundinacea* were taken in 5 separate locations (the middle, north, south, east and west sides of the box) and the average taken to determine the height of *F. arundinacea* in each particular box.

Figure 1: Picture of the Growth Experiment in the UTC greenhouse.

Experimental Design: Phytoremediation Experiment

Twelve plastic window boxes (19 x 58 x 19 cm) were each filled with approximately 3 kg of soil. These boxes were housed in the UTC greenhouse and lined with an impermeable tarp in order to contain soil leachate. The control group consisted of six boxes of untreated soil from the floodplains of the Chattanooga Creek, three of which were planted with *C. pepo* and three were planted with *F. arundinacea*. Six boxes were treated with 20 mg/kg of pyrene; 3 of which were planted with *C. pepo* and 3 were planted with *F. arundinacea* to test for the phytoremediation of pyrene. In order to ensure that each box received its respective amount of pyrene, the mass of the soil in each box was estimated to be approximately 3 kg. The pyrene was then weighed, and dissolved in acetone. This organic solution was then distributed evenly to the soil of the respective box. This was repeated five times to get a total of six boxes with 20 mg/kg of pyrene in the topsoil. The seeds (6 *C. pepo* seeds or 23.62 g of *F. arundinacea* seeds per box) of each plant were planted in the left and middle of their respective boxes. The right side of each box was the unplanted control, which tested for degradation of pyrene due to soil-borne factors. There was no physical barrier between the right side and the rest of the boxes. Soil samples were taken at the beginning and end of the experiment from three locations in each box. In order to determine the phytoremediative capabilities of *C. pepo* and *F. arundinacea*, the samples at the end of the experiment were taken from the rhizosphere (1mm from the root) of each plant. This was accomplished for *F. arundinacea* by lifting the vegetation out of the boxes and collecting the soil located

in-between *F. arundinacea's* extensive root system. For *C. pepo* the tap roots were first located in the soil and then soil samples were taken approximately 1mm from the roots. Two samples were taken from the right (unplanted control), one sample from the middle (heavily planted) and two samples from the left side (heavily planted) of the box (Fig. 2). The experiment was conducted for three weeks for *F. arundinacea* and two months for *C. pepo*.

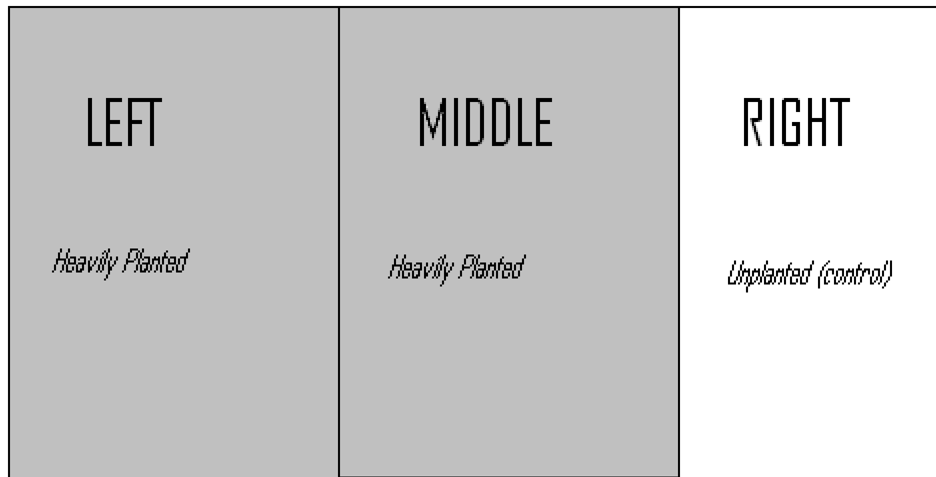

Figure 2: Diagram of experimental set-up of the window boxes for the phytoremediation experiment. The left and middle parts of the window box were planted with either (6 *C. pepo* seeds or 23.62 g of *F. arundinacea* seeds). The right side of the window box was the unplanted control to test for the degradation of pyrene due to natural factors.

Pyrene Extraction

Pyrene was extracted from the soil by sonication using a modified method of EPA 3550B (EPA 1995). Soil samples were individually placed in a dish and then dried in an oven at 20°C. After the sample was dry, it was crushed by mortar and pestle, and then weighed. Individual soil samples were transferred to an Erlenmeyer flask and 50 ml of dichloromethane was added. The sample was then sonicated for ten minutes in an ultrasonic bath. The sample was filtered by vacuum filtration using 5.5 cm Quantitative Filter Paper (Fisher Scientific, Hampton, NH). The extract was then run through a 0.45 μm, laminated Teflon filter (Fisher Scientific, Hampton, NH) to rid the sample of all soil particles. The extract was allowed to air dry for twenty-four hours. The extract was then re-suspended with 3 ml of dichloromethane, and thoroughly mixed. Then 1 ml was pipetted out, added to a HPLC vial and then capped. This procedure was repeated for all soil samples. This extraction procedure was tested for efficiency by adding known amounts of pyrene to pyrene-free soil similar in composition to the floodplains of the Chattanooga Creek and then using the extraction method presented above. The extraction procedure was found to be 99% efficient.

HPLC Analysis

Soil pyrene concentrations were quantified by reverse-phase high-pressure liquid chromatography (HPLC) system (Waters, Milford, MA) with a slightly modified EPA Method 8310 (EPA 1986). Samples were injected into a Waters

2690 Alliance HPLC system equipped with a Waters 996 photodiode array detector and a Alltima (C18, 250 × 4.6 mm, 5 µm, 114Å) column (Alltech, Deerfield, IL). The stationary phase was a column composed of octadecyl bonded silica gel. The flow rate was 1.20 ml/min and the total run time was 50 minutes. The injection volume was 10 µL. A gradient mobile phase was used in order to separate the chemicals in the analyte, based on hydrophobicity (Table 2). The elution gradient started out at 60% HPLC-grade water and 40% HPLC-grade acetonitrile. Over the course of 15 minutes, the elution gradient linearly changed to 100% HPLC-grade acetonitrile. The gradient remained at 100% HPLC-grade acetonitrile from 15-24 minutes. The column re-equilibrated for the final 10 minutes; returning linearly to 60% HPLC-grade water and 40% HPLC-grade acetonitrile. The final 10 minutes also cleared the column of any chemical deposits before the next run. Reverse-phase HPLC separates compounds using a non-polar, hydrophobic stationary phase and polar mobile phase (Hamilton and Sewell 1982). The analyte is forced through the column by a pressurized mobile phase. The hydrophilic chemicals, in the analyte, will remain in the mobile phase or elute from the stationary phase earlier, thus exiting the column first. The more hydrophobic chemicals have a higher affinity for the stationary phase and therefore exit the column at later times. As the elution gradient of the mobile phase becomes more organic, hydrophobic compounds elute from the stationary phase and exit the compound. The time which a chemical exits the column is called the retention time (Smith 1988). As the eluents exit the column they are passed

through a photodiode array detector which recorded the UV-Vis absorption spectra from 210-400 nm.

Time (min)	**Flow (ml/min)**	**HPLC-grade Water (%)**	**HPLC-grade Acetonitrile (%)**
---	1.20	60	40
1.00	1.20	60	40
15.00	1.20	0	100
25.00	1.20	0	100
40.00	1.20	60	40
50.00	1.20	60	40

Table 2: HPLC gradient mobile phase used to separate chemicals by hydrophobicity, allowing for proper elution, separation and identification of pyrene.

Identification and Quantification of Peaks

Quantitative measurements were taken using Empower software (Waters, Milford, MA). Quantitative measurements were facilitated by using a known pyrene standard (>99% Purity, Sigma, St. Louis, MO.) to first determine the retention time and then create a standard curve. The standard curve was created by making single injections of multiple different concentrations of pyrene dissolved in dichloromethane (Fig. 3). It was determined that the unknown eluents should be scanned at 254 nm because of pyrene's efficient absorbance at that wavelength. Peaks from extractions of experimental soil were analyzed by comparing retention times of the unknown to that of our standard solutions. The retention time for pyrene was determined to be 5.6 min (Fig. 4). The peak within this retention time was then integrated to calculate the area under the curve. To determine the area under the curve (peak), the Empower

software monitored each new data point. When data points showed an upward trend from the baseline that was named the beginning of the peak. After the maximum height of the peak, the data points began to decline until it reaches the baseline level again, this point is the end of the peak (a.k.a., curve; Fig. 4). The area under the curve was found by calculating a definite integral between the determined beginning and end point of the peak (Smith 1988). To determine the unknown sample's pyrene concentration, the standard curve's linear trend line was determined ($y = 6177.1x - 1.4817$) and the unknown sample's area was inserted in for y (area) and the equation was solved for x (concentration).

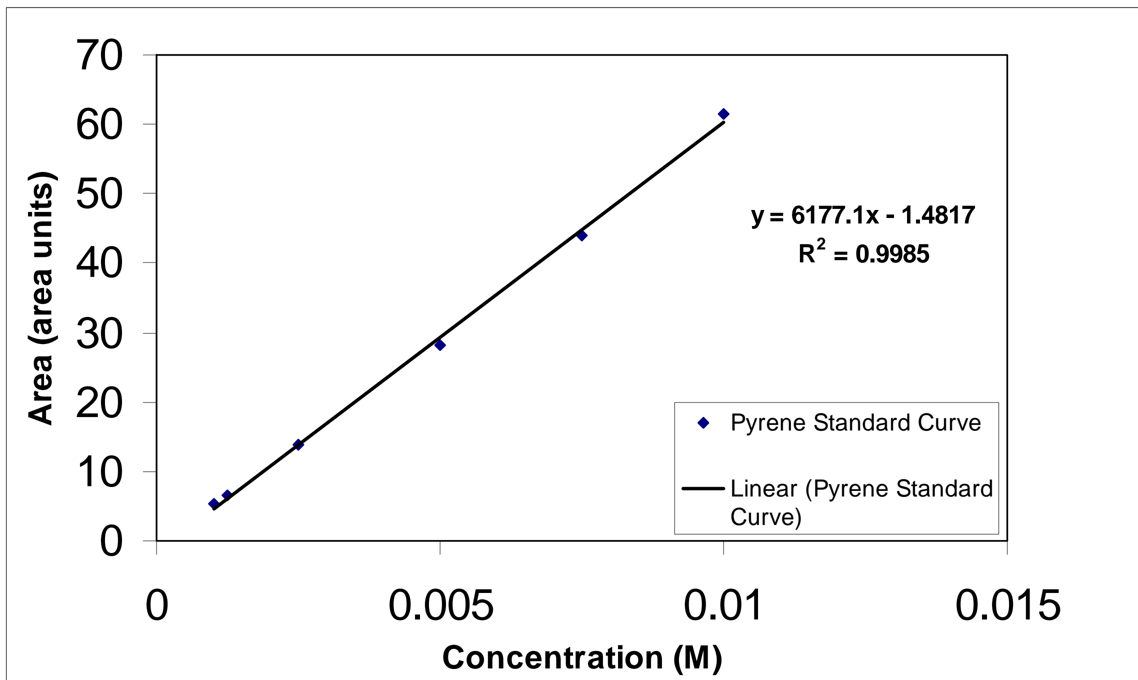

Figure 3: Standard Curve for pyrene detection (n=6).

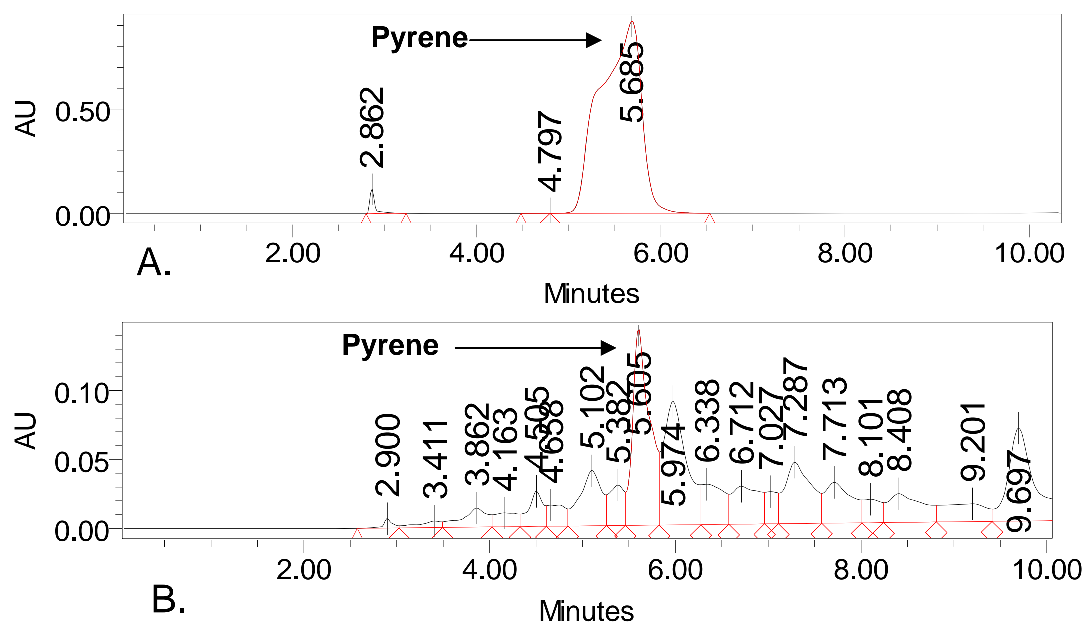

Figure 4: A: Reverse-phase HPLC analysis at 254 nm of a standard solution of pyrene (0.001 M) with the identified pyrene peak having a retention time of 5.685 minutes. B: Reverse-phase HPLC analysis at 254 nm of a final sample of *C. pepo* with the identified pyrene peak having a retention time of 5.605 minutes.

Statistical Analysis

Using Microsoft Office Excel spreadsheets (Microsoft, Redmond, WA), phytoremediation (pyrene removal) data for both plants were arranged into eight groups: initial and final planted treated soil, initial and final planted untreated soil, initial and final unplanted treated soil, and initial and final unplanted untreated soil. Growth data for both plants were arranged by day and height. Data from each group were analyzed for their mean, standard deviation, and standard error. To determine statistical significance, a non-parametric Kruskal-Wallis One-Way Analysis of Variance (ANOVA) by ranks ($P \leq 0.001$) was conducted followed by post-hoc

comparisons with Dunn’s method ($P < 0.050$). Dunn’s method was conducted to determine which specific groups were statistically significant. Kruskal-Wallis ANOVA and Dunn’s Method were conducted via SigmaStat (Systat Software Inc., San Jose, CA).

Results

F. arundinacea Growth Data

The growth of *F. arundinacea* was not affected by pyrene concentrations of 1 mg/kg and 10mg/kg in the Chattanooga Creek floodplain soil (Fig. 5). The median growth of *F. arundinacea* was significantly affected by time (days after planting); however; pyrene concentrations in the soil did not statistically affect differences in height.

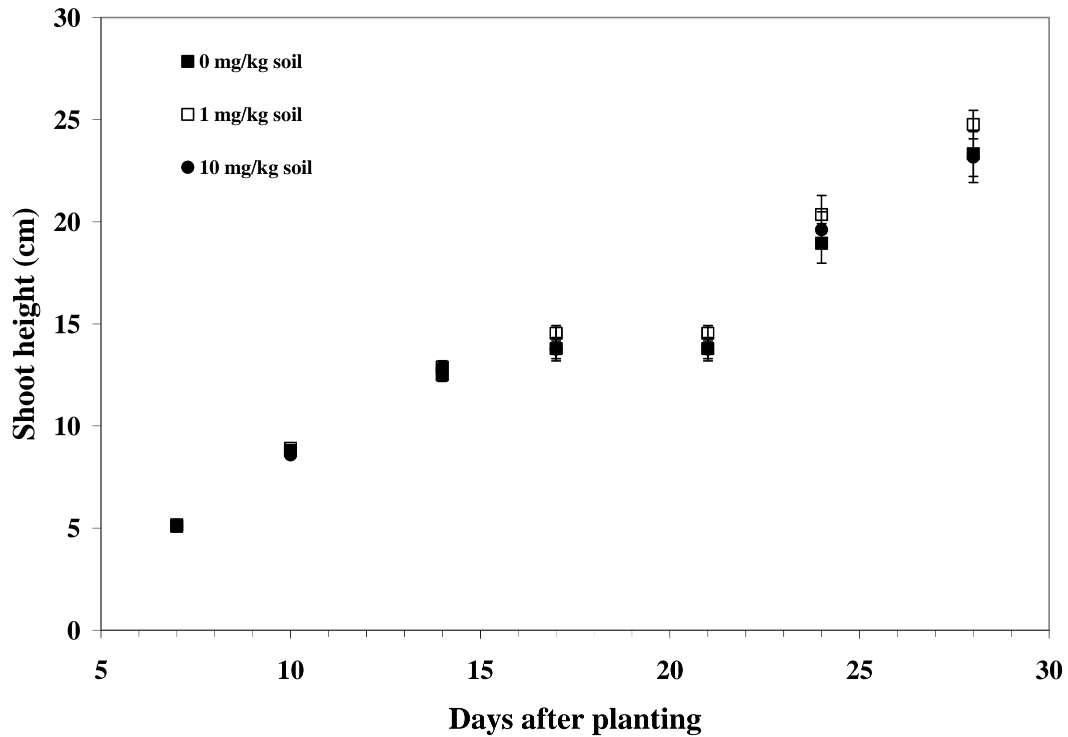

Figure 5: Growth of *F. arundinacea* in soil with pyrene concentrations of 0 mg/kg, 1 mg/kg, and 10 mg/kg from the floodplain of Chattanooga Creek (n = 5). Error bars represent one standard error of mean (σ_M).

C. pepo Growth Data

The growth of *C. pepo* was not affected by pyrene concentrations of 1 mg/kg and 10 mg/kg in the soil of the floodplain of the Chattanooga Creek (Fig. 6). The median growth of *C. pepo* was significantly affected by time (days after planting) however; pyrene concentrations in the soil did not statistically affect differences in height. An interesting, albeit anecdotal, observation was that the leaves of *C. pepo* exposed to pyrene concentrations of 1 mg/kg and 10 mg/kg in the soil were affected. Some leaves of the plants growing in pyrene laden soil, were smaller in size and encrusted with a brown hardened material. None of the control plants exhibited these effects.

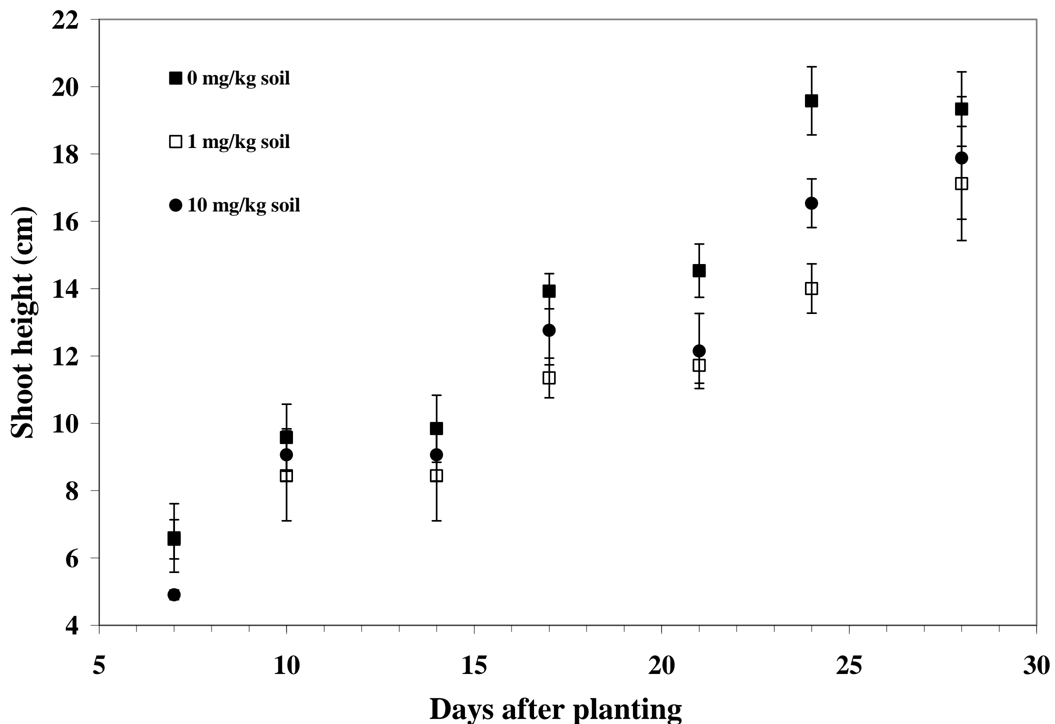

Figure 6: Growth of *C. pepo* in soil with pyrene concentrations of 0 mg/kg, 1 mg/kg, and 10 mg/kg from the floodplain of Chattanooga Creek (n=5 for 0 mg/kg soil, n=4 for 1 mg/kg and 10 mg/kg soil). Error bars represent one standard error of mean (σ_M).

F. arundinacea Phytoremediation Data

Over 24 days, the pyrene concentration in the treated soil was reduced by 45.87% in the planted boxes and by 41.06% in the unplanted boxes (Fig. 7). The differences in pyrene removal between planted and unplanted boxes were not significant. Therefore, *F. arundinacea* was not the cause of the reductions of pyrene in the soil. There were significant differences in pyrene concentrations between treated and untreated boxes but this was expected because the treated soil was amended with 20 mg/kg of pyrene. In summary, both the planted and unplanted control had similar reductions in pyrene; therefore, *F. arundinacea* cannot be attributed with causing the reduction of pyrene in the soil.

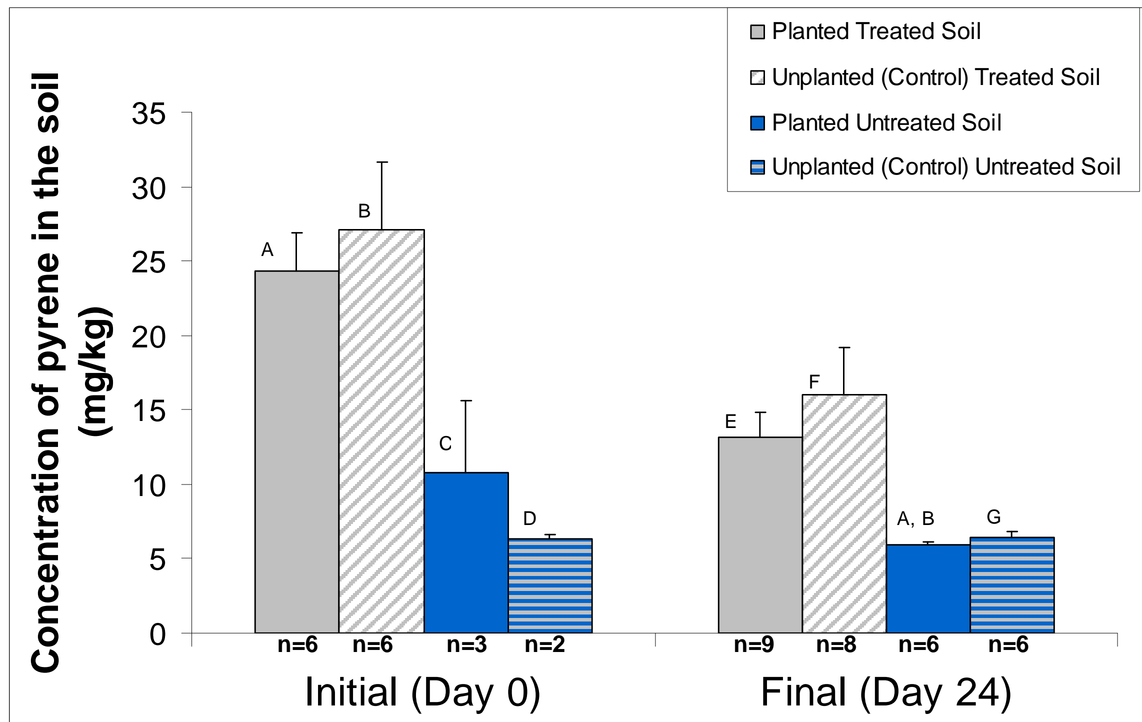

Figure 7: Concentration of pyrene before and after growth of *F. arundinacea*. Columns with the same letter are significantly different ($P<0.05$; i.e., column A is significantly different from column A, B). Sample sizes (n) are located under their respective bars. Error bars represent one standard error of mean (σ_M).

C. pepo Phytoremediation Data

Over 56 days, the pyrene concentration in the treated soil was reduced by 60.38% in the planted boxes and by 54.77% in the unplanted boxes (Fig. 8). The differences in pyrene removal between planted and unplanted boxes were not significant. Therefore, *C. pepo* was not the cause of the reductions of pyrene in the soil. There were significant differences in pyrene concentrations between treated and untreated boxes but this was expected because the treated soil was amended with 20 mg/kg of pyrene. In summary, both the planted and unplanted control had similar reductions in pyrene; therefore, *C. pepo* cannot be attributed with causing the reduction of pyrene in the soil.

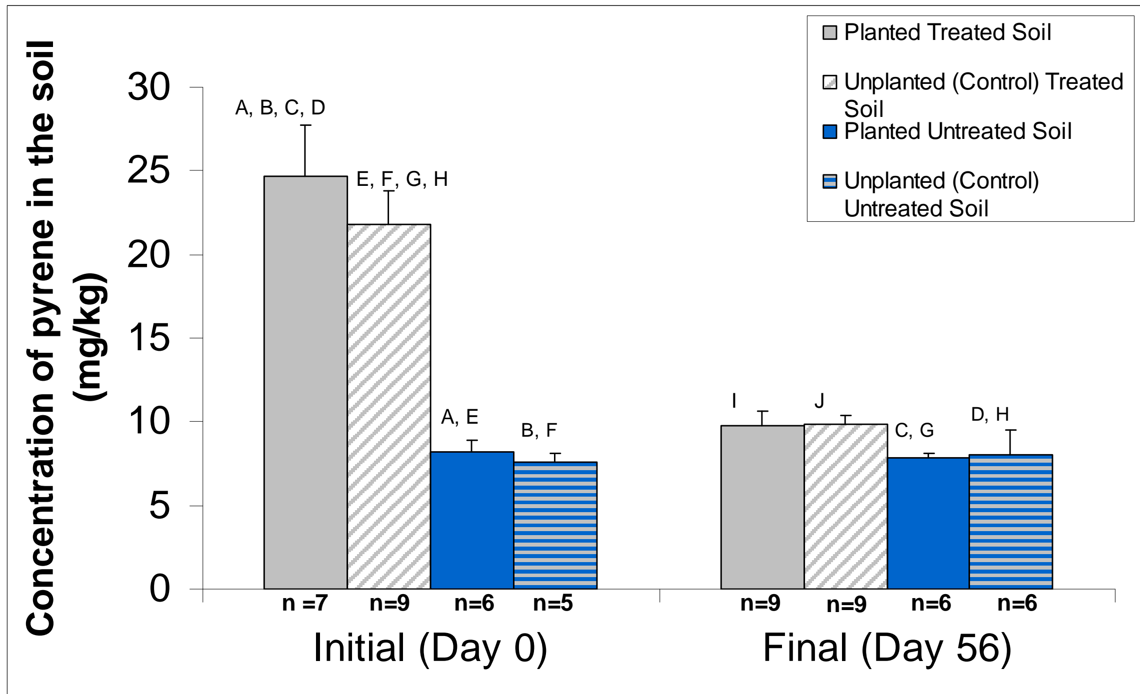

Figure 8: Concentration of pyrene before and after growth of *C. pepo*. Columns with the same letter are significantly different ($P<0.05$; i.e. column A, B, C, D is significantly different from column A, E). Sample sizes (n) are located under their respective bars. Error bars represent one standard error of mean (σ_M).

<u>**Discussion**</u>

<u>*F. arundinacea* Phytoremediation Summary</u>

F. arundinacea did not cause a significant reduction of pyrene in the soil. Although there was a 45.87% reduction of pyrene in the treated soil planted with *F. arundinacea*, there was also a 41.06% reduction of pyrene in the treated soil that was unplanted. Therefore, the loss of pyrene cannot be attributed to *F. arundinacea's* promotion of rhizodegradation. There are three possible explanations for the loss of pyrene: pre-existing microbial populations, lag time, and weathering (details below).

Park et al. (1990) determined that photodegradation and volatilization were not significant removal pathways of pyrene and found the half-life of pyrene to be 199-260 days which is far greater than the length of this study. Banks et al. (1999) established that *F. arundinacea's* uptake of benzo[a]pyrene was less than 0.12% and was not a significant removal pathway. Therefore, the loss of pyrene is likely due to biodegradation. The soil used for this experiment was from the vegetated floodplains of the Chattanooga Creek and contained pyrene concentrations ranging from 5 to 10 mg/kg prior to subsequent amendment. Therefore, the soil likely contained pre-existing microorganisms before the experiment started, some of which may have been capable of PAH degradation. Both the planted and unplanted control experimental soil would have contained these pre-existing microorganisms. This hypothesis is supported by Gunther (1996) and Chen and Banks (2004). In Gunther's 12 week study, planted pots reduced the PAHs from 85 mg/kg to 15 mg/kg and unplanted control pots reduced the PAHs from 85 mg/kg to 14 mg/kg. Gunther explained that

the soil used was agricultural top soil with no known toxicants and the significant reductions in the PAHs was due to the pre-existing microbial community's extraordinary ability to degrade 3 and 4-ring PAHs. Similarly, Chen and Banks (2004) reported rapid pyrene degradation by pre-existing microorganisms in both the planted and unplanted pots after 21 days. Our experiment had comparable results to both researchers (Gunther 1996, Chen and Banks 2004), with both planted and unplanted boxes rapidly degrading pyrene.

The time frame of the present experiment may not have been long enough for *F. arundinacea's* rhizosphere to have a significant effect or influence on the pre-existing microbial population, explaining the similar reductions of pyrene between planted and unplanted groups. Chen and Banks (2004) reported that it took 35 days for *F. arundinacea* to have an effect on the pre-existing microbial community and significantly degrade more pyrene than the unplanted. Chen et al. (2003) also found that *F. arundinacea's* rhizosphere had a distinctive lag period of 20 days on pyrene degradation. They concluded that this lag was probably due to the limited development and influence of the rhizosphere. Their study was conducted for over 190 days, and found that pots planted with *F. arundinacea* degraded 37.7% of the pyrene in the soil, whereas there was only 4.3% degradation for the unplanted control (Chen et al. 2003). Similarly, Banks et al. (1999) reported a 30 day lag period before the rhizosphere influenced bacterial growth. After 6 months, the researchers found that soil planted with *F. arundinacea* had an order of magnitude more bacteria than the unplanted control (Banks et al. 1999). The reported lag period of the *F.*

arundinacea's rhizosphere effect on pre-existing microbial populations fluctuates from 20 to 35 days. Our study was conducted for only 24 days and it is likely that *F. arundinacea's* rhizosphere was still in its lag period. Therefore, longer studies should be conducted to allow for the development of roots and influence of the rhizosphere.

In the present study, pre-existing microorganisms may have caused significant reductions in pyrene in the treated planted and unplanted boxes; however, there was limited reduction in the pyrene in the untreated planted and unplanted boxes. This implies that the pre-existing microorganisms did not degrade the pyrene in the untreated planted and unplanted boxes. This may have been due to the limited bioavailability of weathered pyrene to microorganisms in the untreated boxes (Zhang et al. 2006, Huang et al. 2004*a*). The soil collected from Chattanooga Creek, contained 5 to 10 mg/ kg of pyrene prior to subsequent amendment in the laboratory. Treated boxes contained pyrene from the contaminated site and were additionally amended with pyrene from the laboratory, whereas, untreated boxes contained only pyrene from the contaminated site. Ahn et al. (2005) found that experiments where PAHs are amended may significantly overestimate biodegradation because these PAHs tend to sorb to the outer surfaces of soil particles, allowing them to still be available to biodegradation. Whereas PAHs present at coal carbonization by-product sites (i.e. the floodplains of the Chattanooga Creek) may be trapped in the interior of soil aggregates, limiting their availability to biodegradation (Ahn et al. 2005). The present study had similar results. The treated (pyrene amended) planted and unplanted

boxes had great reductions in pyrene, whereas, the untreated (weathered pyrene) planted and unplanted boxes did not.

Weathered PAHs are difficult to remediate; however, Ho and Banks et al. (2006) reported over a 10 month period that *F. arundinacea* degraded approximately 300 mg/kg of weathered pyrene, 36% more than the unplanted control. They explained that *F. arundinacea* facilitated rhizodegradation by creating a favorable environment in the rhizosphere for the bacterial population, which in turn, increased degradation. Thus, weathered compounds are not completely inaccessible to microrganisms. Furthermore, the rhizosphere has been reported by White et al. (2005) to increase the availability of weathered PAHs in the soil matrix by releasing root exudates which have been shown to compete for binding sites in the soil. Root penetration can further enhance degradation by introducing oxygen into the soil, since degradation of pyrene is an aerobic process (Chen and Banks 2004). Root penetration can also break up the soil aggregates which may contain sorbed PAHs in the interior, making them available for biodegradation (Pivetz 2001). Therefore, it would be expected in our experiment that untreated planted boxes would be able to degrade some weathered pyrene due to the unique influences of the rhizosphere; however the untreated planted boxes did not reduce the weathered pyrene. This was most likely due to the short time frame of the study which limited the rhizosphere and roots' influence on the weathered pyrene and not the unavailability of weathered pyrene in the floodplains.

C. pepo Phytoremediation Summary

C. pepo did not cause a significant reduction of pyrene in the soil. Although over 56 days, there was a 60.38% reduction of pyrene in the treated soil planted with *C. pepo*. There was also a 54.77% reduction of pyrene in the treated soil that was unplanted. The difference was not statistically significant. Therefore, the remediation of pyrene cannot be attributed to *C. pepo's* phytoextraction. Plant uptake is the major pathway of pyrene removal for *C. pepo*, however, the aerial tissue and roots were not analyzed for pyrene uptake in this experiment. Demonstrating that plant uptake is the major pathway, Wang et al. (2004) reported that *C. pepo* was able to phytoextract 1.3% of weathered p,p'-DDE with 98% in the above ground tissue. Similarly, Parrish et al. (2006) found that *C. pepo* phytoextracted approximately 0.7% of the total weathered PAHs in the soil. This is a very small percent reduction by *C. pepo* and would have made up an insignificant portion of the 60.38% reduction of pyrene seen in this experiment. Therefore, the majority of pyrene loss is likely attributed to biodegradation, not phytoextraction. Pre-existing microorganisms in the soil, some of which may have been PAH degraders, explain the considerable reduction of pyrene in the treated soil of both planted and unplanted boxes. The same lack of reduction of pyrene that occurred in *F. arundinacea's* untreated planted and unplanted boxes occurred in the *C. pepo's* untreated planted and unplanted boxes. This was likely due to the limited availability of weathered pyrene to the pre-existing microbial population. Furthermore, *C. pepo* has been reported (Wang et al. 2004, Parrish et al. 2006) to have success in phytoextracting weathered compounds, but in the present

study there was no difference in the reduction of pyrene in the untreated planted and unplanted boxes. This implies that *C. pepo* did not phytoextract the weathered pyrene thought to be present. The inability of C. pepo to phytoextract pyrene in both treated and untreated boxes is most likely due to the short time frame of the study, the slow growth of *C. pepo* in the greenhouse and limited root influence. Longer studies should be conducted with *C. pepo* and aerial and root tissues analyzed for pyrene to conclusively determine the phytoextraction ability of *C. pepo*.

<u>*Limitations of Phytoremediation*</u>

A potential limitation of phytoremediation is that many plants are sensitive to contaminants such as PAHs in the soil. During chemical stress, plants produce ethylene which inhibits plant growth, especially in the roots (Huang et al. 2004*b*). Inhibition of root growth would greatly affect the capabilities of *F. arundinacea* and *C. pepo* to effectively carry out rhizodegradation and phytoextraction, respectively. Furthermore, PAHs can affect photosynthesis by blocking electron transport (Huang et al. 1997, Mallakin et al. 2002). Plants use photosynthesis to convert light energy into chemical energy which is then used to fix carbon dioxide into carbohydrates and other organic compounds. The inhibition of photosynthesis would prevent the production of useable energy and adversely affect the growth of the plant. Any inhibition of growth may affect the phytoremediative capabilities of the plants. During our growth experiment it was observed that the shoot heights of *F. arundinacea* and *C. pepo* were not affected by low concentrations of pyrene in the

soil, however, *C. pepo* was observed to have some smaller leaves with abnormal morphological characteristics that may have affected *C. pepo's* photosynthesis (details below).

When PAHs block electron transport, an increase in the Chl a/b ratio results (Huang et al. 1997, Marwood et al. 2001). Therefore, Chl a/b ratios can be an indicator of chemical stress. Huang et al. (2004*b*) found that the biochemistry and physiology of the *F. arundinacea* made it tolerant to soil with creosote concentrations of 0.5 g/kg to 3 g/kg. In highly contaminated creosote soil, *F. arundinacea* was able to maintain chlorophyll content and a fairly constant chlorophyll (Chl) a/b ratio. They hypothesize that *F. arundinacea* maintained a fairly constant Chl a/b ratio by increasing root growth, increasing water content in shoots and slowing the growth of shoots (Huang et al. 2004*b*). Increased root growth allows for more nutrients and water uptake to overcome the stress of creosote. Increased water content and slowed growth of shoots, dilutes and diminishes the effects of creosote in the photosynthetic parts of the plant (Huang et al. 2004*b*). Our growth experiment showed that pyrene concentrations did not affect the shoot growth of *F. arundinacea*, contrasting with Huang et al.'s (2004*b*) observations. The differences in results is most likely due to the considerably lower PAH concentration (1, 10 mg/kg vs. 0.5, 1, 2, 3 g/kg) in our growth experiment and shorter time frame (28 days vs. 120 days).

There is limited information on the tolerance of *C. pepo* to the effects of PAHs on growth and photosynthesis. Anecdotal and qualitative observations indicate that during our growth experiment *C. pepo* grown in 1 mg/kg and 10 mg/kg pyrene

soil had some smaller leaves with an abnormal morphological characteristic. They were encrusted with a hardened brown material. This may have decreased the photosynthetic capabilities of those plants and if a longer study was conducted the difference shoot heights among those grown in soil with pyrene and those without may have become apparent. This abnormal morphological characteristic was not observed in subsequent experiments, although it is possible that photosynthetic capabilities of those leaves could have been affected to a lesser degree than visible to the eye. In future experiments, chlorophyll assays and photosynthetic measurements would give a more definitive conclusion to the possible photosynthetic inhibition of *C. pepo* by pyrene.

Further greenhouse studies should be conducted in which *F. arundinacea* and *C. pepo* are grown in soil with PAH concentrations of 506 mg/kg (the highest PAH soil concentration found in the floodplains) to accurately replicate the most stressful and contaminated soil conditions of the floodplains of the Chattanooga Creek. In addition, the measurement of shoot height, photosynthesis, chlorophyll content and the fresh and dry weight of root and shoots should be measured to conclusively determine if PAHs have adverse effects on the growth of *F. arundinacea* and *C. pepo*.

Data Gaps and Uncertainties

Defining the rhizosphere and taking samples from the rhizosphere (1mm from the roots) proved a daunting task and may have contributed to the standard error. Dealing with large soil samples (approximately 30g), small sample sizes and an

extensive extraction procedure left room for error and variability. Uncertainties exist about how much pyrene covalently bonded to soil particles (Eschenbach 1998). This could affect the extractability of pyrene, however, with an extraction procedure that is 99% efficient and under such a short time frame it is unlikely that extraction efficiency was affected. Uncertainties also exist about the amount of pyrene lost due to photodegradation and volatilization, however, the amount is likely negligible because these are not major degradation pathways of pyrene due to its high resonance stability (Park et al. 1990).

Conclusion

Phytoremediation is a potential solution to the PAHs in the floodplains of the Chattanooga Creek. However, Chekol et al. (2002) explains that the capabilities of phytoremediation to remove toxicants varies greatly due to the differences in interactions between the plant, soil, microbial populations, and contaminant. Therefore, more research on *F. arundinacea* and *C. pepo* is needed. Especially their interactions between soil, microbial populations and PAHs in order to fully understand the specific interactions of the floodplains of the Chattanooga Creek and recognize whether phytoremediation is the best solution for the floodplains.

Literature Cited

Ahn S, Werner D, Luthy RG. 2005. Physicochemical Characterization of Coke-plant Soil for the Assessment of Polycyclic Aromatic Hydrocarbon Availability and the Feasibility of Phytoremediation. *Environmental Toxicology and Chemistry* 24: 2185-2195.

[ATSDR] Agency for Toxic Substances and Disease Registry. 1995 [accessed 2006 Dec 4]. Toxicological Profile for Polycyclic Aromatic Hydrocarbons (PAHs). *ATSDR*. Available at: http://www.atsdr.cdc.gov/toxprofiles/tp69.html.

[ATSDR] Agency for Toxic Substances and Disease Registry. 2006. Health Consultation: Glover Site, Chattanooga, Hamilton County, TN. The Department of Health and Human Services (US).

Banks MK, Lee E, Schwab AP. 1999. Evaluation of Dissipation mechanisms for Benzo[a]pyrene in the Rhizosphere of Tall Fescue, *Journal of Environmental Quality* 28: 294-8.

Chattanooga Greenways Program. 2003 [accessed 2006 Nov 13]. *The Trust for Public Land*. Available at: http://www.tpl.org/tier3_cd.cfm?content_item_id=1179&folder_id=670.

Chekol T, Vough LR, Chaney RL. 2002. Plant-Soil-Contaminant Specificity Affects Phytoremediation of Organic Contaminants. *International Journal of Phytoremediation* 4(1): 17-26.

Chen YC, Banks MK, Schwab AP. 2003. Pyrene Degradation in the Rhizosphere of Tall Fescue (*Festuca arundinacea*) and Switch Grass (*Panicum virgatum*). *Environmental Science and Technology* 37: 5778-5782.

Chen YC, Banks MK. 2004. Bacterial Community Evaluation During Establishment of Tall Fescue (*Festuca arundinacea)* in soil Contaminated with Pyrene. *International Journal of Phytoremediation* 6(3): 227-238.

Eschenbach A, Weinberg R, Mahro B. 1998. Fate and Stability of Nonextractable Residues of [^{14}C] PAH in Contaminated Soils under Environmental Stress Conditions. *Environmental Science and Technology* 32: 2585-2590.

[EPA] Environmental Protection Agency (US). 1984 [accessed 2006 Dec 15]. Priority Pollutant Ranking System. *EPA*. Available at: http://www.epa.gov/waterscience/library/wqcriteria/ranking.pdf.

[EPA] Environment Protection Agency (US). 1986. Method 8610: Test Methods for Evaluating Solid Waste, SW-846, 3rd Ed. Washington (D.C.): U.S. Government Printing Office.

[EPA] Environmental Protection Agency (US). 1992. Chattanooga Creek Sediment Profile Study. Chattanooga (TN): EPA Environmental Services Divisions.

[EPA] Environmental Protection Agency (US). 1995. Method 3550B: Test Method for Extracting Nonvolatile and Semivolatile Organic Compounds from Solid Samples, SW-846, 3rd Edition. Washington (D.C.): U.S. Government Printing Office.

[EPA] Environmental Protection Agency (US). 1999. Revised Focused Feasibility Study: Tennessee Products Superfund Site, Chattanooga Creek. Chattanooga (TN): US Army Corps of Engineers.

[EPA] Environmental Protection Agency (US). 2002. EPA Superfund Update: Proposed Plan Fact Sheet. Cleanup of Chattanooga Creek. *EPA*.

[EPA] Environmental Protection Agency (US). 2005 [accessed 2006 Nov 13]. Tennessee NPL/NPL Caliber Cleanup Site Summaries. *EPA*. Available at: http://www.epa.gov/region4/waste/npl/npltn/tennprtn.htm.

Gunther T, Dornberger U, Fritsche W. 1996. Effects of Ryegrass on Biodegradation of Hydrocarbons in Soil. *Chemosphere* 33(2): 203-215.

Hamilton RJ, Sewell PA. 1982. Introduction to High Performance Liquid Chromatography, 2nd ed. New York (NY): Chapman and Hall. pp. 1-12.

Hannaway D, Fransen S, Cropper J, Teel M, Chaney M, Griggs T, Halse R, Hart J, Cheeke P, Hansen D, Klinger R, Lane W. 1999 [accessed 2006 Nov 13]. Tall Fescue. PNW 504 (OR): *Pacific Northwest Extension Publications*. Available at : http://extension.oregonstate.edu/catalog/html/pnw/pnw504/.

Ho CH, Banks MK. 2006. Degradation of Polycyclic Aromatic Hydrocarbons in the Rhizosphere of *Fesuca arundinacea* and Associated Microbial Community Changes. *Bioremediation Journal* 10(3): 93-104.

Huang XD, El-Alawi Y, Penrose DM, Glick BR, Greenberg BM. 2004*a*. A Multi-process Phytoremediation System for Removal of Polycyclic Aromatic Hydrocarbons from Contaminated Soils. *Environmental Pollution* 130: 465-476.

Huang XD, El-Alawi Y, Penrose DM, Glick BR, Greenberg BM. 2004*b*. Responses of Three Grass Species to Creosote During Phytoremediation. *Environmental Pollution* 130: 453-463.

Huang XD, McConkey BJ, Babu ST, Dixon DG, Greenberg BM. 1997. Mechanisms of Photoinduced Toxicity of Photomodified Anthracene to Plants: Inhibition of Photosynthesis in the Aquatic Higher Plants *Lemna gibba* (duck weed). *Environmental Toxicology and Chemistry* 16: 1707-1715.

Hutchinson SL, Banks MK, Schwab AP. 2001. Phytoremediation of Aged Petroleum Sludge. *Journal of Environmental Quality* 30: 395-403.

[IARC] International Agency for Research on Cancer. 2006 [accessed 2007 Jan 10]. Polycyclic Aromatic Hydrocarbons. *IARC*. Available at: http://monographs.iarc.fr/ENG/Meetings/92-pahs.pdf.

Johnsen AR, Wick LY, Harms H. 2005. Principles of Microbial PAH-degradation in Soil. *Environmental Pollution* 133: 71-84.

Klevens HB. 1949. Solubilization of Polycyclic Hydrocarbons. Presented at the 23rd National Colloidal Symposium. American Chemical Society: Division of Colloid Chemistry. Minneapolis, MN.

Launen LA, Pinto LJ, Percival PW, Lam SFS, Moore MM. 2000. Pyrene is Metabolized to Bound Residues by *Penicillium janthinellum* SFU403. *Biodegradation* 11(5): 305-312.

Mallakin A, Babu TS, Dizon DG, Greenberg BM. 2002. Sites of Toxicity of Specific Photoxidation Products of Anthracene to Higher Plants: Inhibition of Photosynthetic Activity and Electron Transport in *Lemna gibba* L. G-3 (duck weed). *Environmental Toxicology* 17(5): 462-471.

Marwood CA, Solomon KR Greenberg BW. 2001. Chlorophyll Fluorescence as a Bioindicator of Effects on Growth in Aquatic Macrophytes from Mixtures of PAHs. *Environmental Toxicology and Chemistry* 20: 890-898.

Mills J. 2001 [accessed 2006 Nov14]. University of Georgia Cucumber page. Available at: http://www.uga.edu/vegetable/cucumber.html

Park KS, Sims RC, Dupont R, Doucette WJ, Matthews JE. 1990. Fate of PAH Compounds in Two Soil Types: Influence of Volatilization, Abiotic Loss and Biological Activity. *Environmental Toxicology and Chemistry* 9(2): 187-195.

Parrish ZD, Banks MK, Schwab AP. 2004. Effectiveness of Phytoremediation as a Secondary Treatment for Polycyclic Aromatic Hydrocarbons (PAHs) in Composted Soil. *International Journal of Phytoremediation* 6(2): 119-137.

Parrish ZD, White JC, Isleyen M, Gent MPN, Iannucci-Berger W, Eitzer BD, Kelsey JW, Mattina MI. 2006. Accumulation of Weathered Polycyclic Aromatic Hydrocarbons by Plant and Earthworm species. *Chemosphere* 64: 609-618.

Pivetz BE. 2001. Phytoremediation of Contaminated Soil and Ground Water at Hazardous Waste Sites. *Ground Water Issue* EPA publication number EPA/540/S-01/500.

Potter CL, Glaser JA, Chang LW, Meier JR, Dosani MA, Herrmann RF. 1999. Degradation of Polynuclear Aromatic Hydrocarbons under Bench-scale Compost Conditions. *Environmental Science and Technology* 33:1717-1725.

Samanta SK, Singh OV, Jain RK. 2002. Polycyclic Aromatic Hydrocarbons: Environmental Pollution and Bioremediation. *TRENDS in Biotechnology* 20(6): 243-248.

Searfoss R. 2003 [accessed 2006 Feb 21]. Activities in Tennessee. *Agency for Toxic Substances and Disease Registry.*. Available at: http://www.atsdr.cdc.gov/ statefactsheets/sfs-tn.html.

Smith RM. 1988. Gas and Liquid Chromatography in Analytical Chemistry. Suffolk (UK): St. Edmundsbury Press. pp. 273-284.

Vulava VM. 2002 [accessed 2006 Nov 14]. Summary of the "Remedial Investigation Report for the Tennessee Products Site, Chattanooga, Tennessee" for the UT-Chattanooga Creek Initiative. Available at: http://web.utk.edu/~hydro/ChatCreek/TPSiteReportSummary.pdf

Wang X, White JC, Gent MPN, Ianucci-Berger W, Eitzer BD, Mattina MI. 2004. Phytoextraction of Weathered p,p'-DDE by Zucchini (*Cucurbita pepo*) and Cucumber (*Cucumis sativus*) Under Different Cultivation Conditions. *International Journal of Phytoremediation* 6(4): 363-385.

White JC, Parrish ZD, Iseleyen M, Gent MPN, Berger WI, Eitzer BD, Kelsey JW, Mattina MI. 2005. Influence of Citric Acid Amendments on the Availability if Weathered PCBs to Plant and Earthworm Species. *International Journal of Phytoremediation* 8: 63-79.

Zhang X, Cheng S, Zhu C, Sun S. 2006. Microbial PAH-Degradation in soil: Degradation Pathways and Contributing Factors. *Pedosphere* 16(5): 5655-5665.

Acknowledgments

I would like to thank: the late Dr. Stephen Halperin for his wonderful visions of phytoremediating the floodplains of the Chattanooga Creek and his guidance in the laboratory. Dr. Sean Richards for all the time he dedicated as project director, his insightful input on the project and, of course his numerous revisions. Dr. Doug Kutz, Dr. Henry Spratt, and Dr. Hill Craddock for being on my Departmental Honors Committee, their time and contributions to the project. Dr. Steven Symes for his assistance and knowledge of the HPLC. David Percy and Joe Simpson for their invaluable assistance in the laboratory and greenhouse. Nicholas Fiacco for his assistance in preparing the literature cited. The UHON faculty for their advice and guidance. The UTC Biology Department for the use of their laboratories, greenhouse and for funding this project.