4. METHODS AND MATERIALS

A. Experimental Approach

Determination of PAH concentrations in soil and ground water was necessary for site characterization and assessment of the grass phytoremediation system. Soil and ground water samples were obtained from the Norfolk Southern tie yard site, Oneida, Tennessee. Using a hand auger, soil boring samples were obtained. Composite and depth-specific soil samples were taken at various site locations. Grass plot soil samples were obtained with a "G" Series Oak Field tube-type soil sampler. The grass plot samples were obtained from a depth of six to eight-and-a-half inches below land surface (BLS). The two-and-a-half inch sample section was extracted to determine PAH concentrations at various locations within the grass plot test area. Ground water samples were collected from MLSs installed at the site by Virginia Tech. The MLS design allowed depth-dependent sampling after proper purging of the sample collection lines. Sample lines were color-coded with respect to depth. Ground water and soil samples were stored in coolers at the site. At Virginia Tech, samples were stored at 4 C.

USEPA extraction procedures for PAHs in soil and water were reviewed. The procedures were determined to require an exorbitant amount of lab time, solvent, and materials. It was desired to extract the ground water and soil samples within the USEPA holding time of 7 days and eliminate the need to outsource samples. The magnitude of samples collected on an average sampling trip would not have permitted satisfaction of both requirements. Soil and ground water samples were extracted using lab-validated

unconventional methods. See Figure 3 for MLS and soil boring locations. Poplar tree lines are shown in green on Figure 3.

B. Soil Boring Collection Dates

On July 21 and 22, 1997, auger holes SB-5, -9, -13, -14, and -19 were drilled and composite samples were collected in 500 mL amber jars with Teflon-lined caps. On July 31 and August 1, 1997, soil samples were collected at one foot intervals and many bedrock interfaces at ML-2, -3, -4, -14; SB-4, -6, -8, -10, -11, -12, -17 and P-6. On August 20, 1997, soil samples were collected at one-foot intervals and many bedrock interfaces in the following locations: SB-1, -2, -3, -7, -16 and SB-19. All of these soil samples were considered time zero (t=0) soil boring samples.

C. Grass Plot Maintenance and Soil Collection Dates

The grass plot area and each 4 foot by 4 foot cell was staked off on July 1, 1997. Cell layout was based on a statistically random design with emphasis on control cells (Figure 4). Cell 1 is located at the northwest end of the test area. All grass plots were initially seeded and fertilized on July 2, 1997. The surface soil was very hard and dry. One hundred thirteen and-a-half grams (0.25 pounds) of ladino clover and 227 grams (0.5 pounds) of perennial rye and KY 31 fescue seed was hand-dispersed to each of the appropriate cells. Six hundred ten grams (1.34 pounds) of 10-10-10 fertilizer was dispersed all cells. All seeds and fertilizer were purchased locally at Blacksburg Feed and Seed, Blacksburg, VA. Time zero (t=0) grass plot soil samples were collected on July 9 and 10,

Figure 3: Location of Soil Borings (SB), Multilevel
           Samplers (ML), Piezometers (P) and Grass
           Phytoremediation Study Area (figure3.gif, 141K)

Figure 4: Grass Plot Cell Layout (figure4.gif, 73K)

1997. One hundred forty-four samples were taken to permit adequate characterization of the test area.

As of July 21, 1997, growth was not experienced in planted plots due to dry weather conditions. On July 21, 1997, the top three inches of soil in plots 36, 35, 33, 32 thru 26, 22, and 19 was agitated with a shovel and a 32 pound bag of Scott's™ 3-in-1 organic mix was intermixed with the native soil. All planted plots were seeded again and none of the plots were fertilized. On July 31, 1997, all remaining plots were agitated and organic mix was added. Planted plots were reseeded. All plots were fertilized, covered with bale straw, and watered thoroughly. On July 21 and 31, 1997, the following weights of ladino clover, perennial rye, and KY 31 fescue were dispersed to the appropriate planted plots: 57 grams (0.12 pounds), 112.5 grams (0.23 pounds), and 113 grams (0.23 pounds).

Overall, an average amount of snow/rain was received at the site from November to March (Figure 5), but delivery of precipitation came from multiple, intense storms. Collection of time one samples was attempted on February 17, 1998, but highly saturated subsurface conditions made sample collection impossible. Fifty-nine (t=9 months) samples were successfully collected on March 12 and 13, 1998. At the time of collection, the subsurface was still very saturated in certain plots and soil samples could not be taken at specific plot positions. At t=9 months, subsurface conditions did restrict selection of some samples.

All soil samples were collected in clear plastic bags

Figure 5: Oneida Precipitation Data (figure5.gif, 76K)

and immediately transferred to a cooler to prevent PAH photodegradation. All t=0 samples were taken at cell positions A thru I (Figure 6). In addition to position A thru I samples, some t=9 month samples were taken directly above or below the sampling position by an indicated number of inches. Positions A and F are located at the northwest end of the cell. All t=0 and t=9 month samples were extracted and analyzed at Virginia Tech.

D. Ground Water Collection Dates

Time zero (t=0) ground water samples were collected at various MLSs on November 25, 1997. All t=0 samples were taken to EnviroTech Mid-Atlantic Laboratories for PAH and select BTEX analysis. The following MLSs were sampled at depth-specific points for PAH analysis: ML-1; ML-2; ML-3; ML-4; ML-5; ML-7; ML-8; ML-9 and ML-14. T=4 month ground water samples were taken on March 12 and 13, 1998. Select duplicate samples were analyzed at EnviroTech Mid-Atlantic laboratories for the 16 PAHs. All t=4 month samples were extracted and analyzed at Virginia Tech for 10 PAHs. Depth-specific and composite samples were obtained from the following MLSs: ML-1; ML-3; ML-4; ML-7 thru ML-12; ML-15 and ML-16. Duplicate samples of ground water were collected in 40 mL amber vials with no headspace.

Figure 6: Grass Plot Cell Sampling Positions
(figure6.gif, 36K)

E. Analytical Methods

i. Soil Auger Boring Extraction Procedure

The soil samples were air dried for four hours in the vent hood. Using a FisherBrand Scoopula™ spatula, the soil was chopped into fine grains. The Teflon septa of a 40 mL amber vial was pierced with a clean 0.22 gauge hypodermic needle. The pierce allowed the vials to self-vent without exploding on the rotating table. Five grams of soil was placed into a 40 mL amber vial and 15 mL of Fisher Optima grade methylene chloride (MeCl) was added with a volumetric pipette. At a speed of 1, each vial was agitated on the Fisher Vortex machine for ten seconds. A permanent line was marked around the circumference of the vial to indicate the MeCl level. The vials were placed on the rotating table and agitated for 34 to 38 hours. The vials were removed from the rack and carefully examined for loss of MeCl. Addition of MeCl may be required to attain the initial MeCl level. The vials were refrigerated at 4 C for 12 hours. One to one-and-a-half mL of the 40 mL vial supernatant was transferred to a 1.5 mL amber GC vial. A Pasteur pipette was used to transfer the supernatant extract to the GC vial.

ii. Grass Plot Soil Extraction Procedure

The grass plot soil extraction procedure is identical to the soil auger boring procedure, with a few exceptions. The exceptions follow:

a) Due to the very low moisture content of the t=0

samples, these samples were not air-dried. After removal from the 4 C refrigerator, extraction was immediate. The t=9 month soil samples were very saturated. T=9 month samples were air-dried in the vent hood for three to four to mimic t=0 soil moisture levels.

b) The Teflon septa of the t=0 40 mL vials were not pierced with a 0.22 gauge needle. T=0 samples were extracted prior to the soil auger boring samples and random vial explosions did not occur. In order to prevent potential explosions and sample loss, the septa of t=9 month vials were pierced.

c) The vials were not agitated on the Fisher Scientific Vortex Mixer.

iii. Ground Water Extraction Procedure

A 30:1 one-step sample concentration method was used to extract ground water samples. It is imperative that the ground water used for the extraction be free of any visible particles or colloidal material. 37.7 mL of sample was transferred into a 40 mL amber vial with Teflon cap. Vial caps were tightened. Using a 1 mL gas-tight syringe, 1.3 mL of Fisher Optima grade MeCl was dually injected through the septa. Each vial was vigorously hand-shaken for 1.5 minutes. Two minutes of static settling time were observed. Some MeCl will settle to the bottom of the vial, but much will remain as small bubbles throughout the solution. While shaking the vial in a downward motion, small MeCl bubbles are forced to the bottom of the vial. The vial should be tilted and rotated to promote agglomeration of the many smaller MeCl fractions into one or two larger bubbles of MeCl. The vial cap was removed and the MeCl bubbles were drawn into a 1 mL pipette. A

conscious effort was made to draw as much of the MeCl into the pipette as possible without drawing water into the pipette. Average recovery of MeCl was approximately 0.6 mL. The contents of the 1 mL pipette were directly transferred to a GC vial. Minimum GC vial refrigeration time prior to injection is 4 hours. GC vials should be analyzed within one week of extraction.

iv. Analytical Equipment

A Shimadzu GC-14A Gas Chromatograph (GC) equipped with a flame ionization detector (FID) was used for PAH analysis. A J&W Scientific DB5-MS fused silica capillary column with 0.25 micron film thickness, 0.25 millimeter internal diameter, and 30 meter length was used. The initial column oven temperature was 80 C for 1 minute. The rate of oven temperature increase was 10 C/min and the final temperature was 305 C for 9 minutes. At the end of the run, a cool-down program was employed to allow adequate time for detector equilibration. The cool-down program consisted of a 40 C/min oven temperature decrease to 80 C with a hold of 14 minutes. Total run time was 46.5 minutes. Injector and detector temperatures were maintained at 295 C. Auxiliary 2 temperature was 300 C.

Samples were analyzed in a splitless/split fashion. A Shimadzu AOC-20I autoinjector unit was used for sample injection. Injected sample size was 2 μL. Event 91, splitless mode, was programmed for one minute. Column helium flow rate was 1 mL/min. Air, hydrogen, helium (column), and helium (make-up) gauge pressures were maintained at 45, 55, 72, and 78 kPa, respectively. Purge

and split flow rates were 10 and 50 mL/min, respectively. Shimadzu CR501 integrator settings were: slope = 2000; width = 1; drift = 0; tdbl = 0; att = 3 or 4; stop time = 32.5 min.; chart speed = 10 mm/min; and min area = variable (range: 50-1500).

External standards were used to identify and quantify acenaphthene, acenaphthylene, anthracene, benzo(b)fluoranthene, chrysene, fluorene, fluoranthene, naphthalene, phenanthrene, and pyrene in respective ground water and soil samples. The standards were custom made mixes prepared by Supelco Inc., Bellefonte, PA. Six standard concentrations (0.5, 1, 5, 10, 25, 40 mg/L) were used to develop a standard curve for each constituent. In some cases, sample dilutions were required to avoid data extrapolation. All constituents produced linear standard curves up to 40 mg/L.

Carry over, a process in which constituents from a prior injection volatilize during the subsequent GC run, of HMW compounds was experienced in soil samples. Due to the presence of many HMW compounds in creosote, occurrence of carryover was expected with our soil samples. Therefore, it was standard procedure to place a clean vial of MeCl or methanol in between GC soil sample vials. Effects of carry over in ground water samples were also evaluated. Even the dirtiest ground water samples did not exhibit carry over effects. Since the aqueous solubility of PAHs decreases with molecular weight, few HMW PAHs were present in ground water samples. Thus, HMW carryover was not experienced with ground water injections and, accordingly, clean solvent vials were not required between sample vials.

The following equation was used to calculate constituent concentrations (mg/kg) in soil:

$$=(chromatogram\ area*15\ mL*1000mg/g)/(1000ml/L*5g*slope)$$

The following equation was used to calculate constituent concentrations (µg/L or parts per billion(ppb)) in water:

$$= (chromatogram\ area*1000)/(slope*30*avg.recovery(decimal))$$

The slope term in the above equations refers to the slope of the standard curve for each constituent.

v. Validation of Soil Extraction Procedure

The soil extraction procedures used in this study are modified versions of Brauner's procedure (Ph.D. thesis, unpublished). However, method validation and refinement occurred before analytical results were used to assess site contamination. The soil used for method refinement and validation was collected at the Oneida site in May 1997. The sample was very saturated and smelled of petroleum-related contamination. First, a 10:1 (MeCl volume:soil mass) ratio was evaluated with a solid clump and a fragmented mass of soil. A larger amount of soil-MeCl contact area resulted in sharper and more defined constituent chromatogram peaks for the fragmented sample in comparison to the clumped sample. Soil surface area available for contact with MeCl was a parameter that had to be maximized.

The next set of extractions involved identification of

the optimum MeCl volume:soil mass ratio. Three duplicate
extractions were performed at ratios of 3:1, 5:1 and 7:1.
Soil was cut into small squares and chopped as finely as
possible. However, soil moisture levels prevented the soil
from being chopped into the desired particle size. The
presence of clay in the soil further hampered the
situation. Nonetheless, the chromatograms for the 3:1 (15
mL MeCl: 5 grams soil) ratio provided the sharpest peaks
with ideal heights.

Soil moisture was addressed utilizing the 3:1 ratio.
The PAHs monitored in the soil were considered to be semi-
and non-volatile PAHs, so air-drying samples prior to
extraction should not result in loss of monitored PAHs.
This assumption was confirmed experimentally. At different
vent hood drying times, duplicate extractions were
performed. Selected drying times were 5 minutes, 1 and 4
hours. Chromatograms confirmed that drying times up to 4
hours did not have an adverse effect on the resulting
chromatogram areas of any monitored PAHs. Sample drying in
the vent hood was applied to 2 batches of samples, time
zero soil boring and time one grass plot samples.
Excessive soil moisture levels have been reported to
negatively affect extraction efficiencies (Schleussinger *et
al.*, 1996). During the extraction process, high soil
moisture levels also prevented adequate separation of the
soil matrix.

The 4 hour vent hood drying time was implemented in a
duplicate extraction at the 3:1 ratio to assess method
reproducibility. Taking into consideration site soil
heterogeneity, method reproducibility was acceptable
(Table 4). Area-based deviations ranged from 4 to 19%. A
duplicate extraction was performed on a time zero grass

plot sample, 11A (Table 5). Extraction deviation was
comparable to deviations experienced in the previous
duplicate extraction. Duplicate injections of several t=0
grass plot extractions were performed to assess GC sample
reproducibility (Table 6). Many peer-reviewed articles
stated error due to analytical equipment variations and
soil heterogeneity ranging from 15 to 25%. Comparison of
analytical results suggests concurrence with the peer-
reviewed literature.

Table 4: Chromatogram Area-Based Results from Duplicate Extraction of Site Soil

PAH Constituent	Peak Area-Trial 1	Peak Area-Trial 2	Percent Difference Between Trials
Naphthalene	32673	29278	11.6
Acenaphthene	22465	23461	4.4
Fluorene	20184	23237	15.1
Phenanthrene	59937	69152	15.4
Fluoranthene	34959	41117	17.6
Pyrene	29062	34622	19.1

Table 5: Results of Duplicate Soil Extraction: Grass Plot Cell 11, Position A

	Concentrations, mg/kg		
PAH Constituent	Trial 1	Trial 2	Percent Difference
Acenaphthene	9.0	9.8	9.0
Fluorene	8.7	10.5	19.8
Phenanthrene	28.8	35.7	24.0
Fluoranthene	80.1	86.6	8.1
Pyrene	80.9	86.1	6.4
Chrysene	45.8	50.1	9.4

Table 6: Results of Duplicate GC Injections: T=0 Grass Plot Cell Data

Plot ID	Chromatogram Areas					
	Acenaphthene	Fluorene	Phenanthrene	Fluoranthene	Pyrene	Chrysene
26 C	6403	6614	21845	38399	43765	22348
	6336	6467	21842	38323	43632	21984
% Difference	1.06	2.27	0.01	0.20	0.30	1.66
23 C	10023	10210	35719	45177	47936	23698
	9595	11087	35678	44555	48263	23728
% Difference	4.46	8.59	0.11	1.40	0.68	0.13

Several t=0 soil auger boring samples were taken to
EnviroTech Mid-Atlantic Laboratories for PAH analysis.
Until EPA methods were used to analyze the soil, soil
concentrations yielded from the unconventional extraction
method were only relative to one another. USEPA Method
8100 analytical results were expected to be lower than
unconventional extraction method results for a given soil.
Intensive destruction of the bulk soil sample into fine
particles is not part of the USEPA extraction procedure.
To reduce excessive moisture levels, the USEPA method
allows addition of sodium sulfate. The addition of sodium
sulfate and vent hood drying may provide different
reductions in soil moisture.

For all samples, EnviroTech soil concentrations were
lower, or in some cases below detection, than
concentrations obtained with the unconventional method.
(Tables 7 and 8). Average unconventional method
concentrations were 2 times to 2 orders of magnitude
greater than USEPA method concentrations. Therefore,
unconventional method-derived soil concentrations can be
directly compared to one another and will likely be higher
than concentrations derived from the EPA extraction
procedure.

Table 7: Virginia Tech Analytical Results for T=0 Soil Auger Boring Extractions

		Soil Conc., mg/kg					
SB ID #	Depth	Acenaphthene	Fluorene	Phenanthrene	Fluoranthene	Pyrene	Chrysene
SB-13	9-9.5'	0.61	1.07	3.93	29.74	29.40	15.83
SB-3	5'	55.9	71.3	281.7	271.6	259.2	128.3
	7'	2.8	2.7	7.6	20.3	18.8	7.3
	8'	207.4	258.1	909.5	665.9	583.4	347.4
ML-4	6'	0.34	1.07	0.71	8.08	11.82	9.18
	8' 2 "	0.18	1.46	3.56	2.94	1.70	0.23
	10' 8 "	3.00	3.63	10.55	10.80	9.44	3.71

Table 8: EnviroTech Analytical Results for T=0 Soil Auger Boring Extractions

		Soil Conc., mg/kg					
SB ID #	Depth	Acenaphthene	Fluorene	Phenanthrene	Fluoranthene	Pyrene	Chrysene
SB-13	9-9.5'	<0.008	<0.008	<0.008	17.00	16.00	7.00
SB-3	5'	<1.4	4.80	12.00	<1.4	<1.4	<1.4
	7'	<0.14	<0.14	<0.14	<0.14	<0.14	0.79
	8'	<1.4	<1.4	<1.4	3.30	2.60	<1.4
ML-4	6'	<0.14	<0.14	<0.14	0.90	1.40	1.20
	8' 2 "	<0.14	0.22	0.88	0.76	0.41	<0.14
	10' 8 "	1.50	1.60	5.60	6.60	5.50	1.70

vi. Validation of Ground Water Extraction Procedure

The reproducibility of this unconventional method and comparability to USEPA extraction method results were assessed. USEPA Method 625/8100 concentrates the ground water 1000:1 in a multi-step extraction process (USEPA, 1982). After sample concentration, several dilutions may occur providing more opportunity for error. Development of a one-step extraction procedure that required less lab time was essential for this project. A 30:1 one-step sample concentration method was used to extract ground water samples. The method is similar to that of Priddle and MacQuarrie (1994).

All t=0 ground water samples were taken to EnviroTech Mid-Atlantic Laboratories for PAH analysis. EnviroTech followed USEPA Method 8100 to extract, analyze, and report concentrations of the 16 PAHs. Duplicate t=0 sample collection occurred to allow testing and refinement of the unconventional extraction method. Once the method was refined, the samples provided a means of testing method reproducibility. Four extraction concentration ratios were evaluated, 20:1, 25:1, 30:1, and 40:1. The best combination of constituent peak heights and chromatogram areas was achieved with a 30:1 concentration ratio for 2 MLS samples with varying degrees of contamination.

Reproducibility of the method was assessed using a field sample, ML2-OR. Using the 30:1 concentration ratio, ML2-OR was extracted in triplicate. Aqueous concentrations for 6 PAHs were calculated for the triplicate extractions and constituent concentration deviations were low. In addition, EnviroTech analytical results for ML2-OR were comparable (Table 9).

Extraction efficiency was examined by spiking vials containing 37.7 mL of clean distilled water with three Supelco standards. 1.3 mL of each 0.25, 0.5, and 1 mg/L standard was injected into a vial (Table 10). A linear fit (R^2=0.988 to 0.998) between initial spike concentration and chromatogram area was observed. Ground water spikes were performed again prior to extraction of t=4 month ground water samples. The results are also in Table 10. In both trials, average percent recoveries of common PAHs were consistent and standard deviations were relatively low.

Table 9: Comparison of Analytical Results: Triplicate
Unconventional Extraction and EnviroTech
Extraction of ML2-OR Ground Water Sample

Table 10: Percent Recoveries of Standard Spikes into Clean
Distilled Water (table9and10.gif, 79K)

All percent recoveries were higher than 100%. Initially, this caused concern. A more thorough understanding of the procedure is required to explain the high recoveries. 1.3 mL of MeCl is injected into a 40 mL vial and only approximately 0.6 mL of MeCl is recoverable. Due to the fact that headspace is present in the extraction vial, a reasonable percentage of MeCl resides in the vapor phase after the vial has been shaken for 1.5 minutes. Methylene chloride is an extremely volatile chemical with a Henry's constant of 3×10^{-3} atm $\times$ m^3/mol (Bedient *et al.*, 1994). MeCl also has a relatively high liquid to gas exchange rate (Bedient *et al.*, 1994). Thus, under atmospheric conditions MeCl will migrate from liquid to gas phase. A decrease in MeCl volume with the same PAH mass present will produce extraction recoveries in excess of 100 percent. As long as the recoverable volume is consistent, the percent recoveries will be approximately the same. Recoverable MeCl volumes were consistent for all time one samples extracted.

All t=4 month ground water samples were analyzed at Virginia Tech and select duplicates were taken to EnviroTech Mid-Atlantic Laboratories for comparison of analytical results (Table 11). In the field, samples collected for Virginia Tech analysis were taken prior to samples collected for EnviroTech analysis. Virginia Tech 40 mL vials had zero headspace and EnviroTech 1 Liter sample jars normally had headspace. A combination of these factors can be attributed to typically higher concentrations of naphthalene, acenaphthylene, acenaphthene, fluorene, and phenanthrene in the Virginia Tech samples. Aqueous concentrations of anthracene,

Table 11: Comparison of Analytical Results for Select
        Time=4 Mos. Ground Water Samples
        (table11.gif, 56K)

fluoranthene, and pyrene were very consistent with EnviroTech results. This unconventional ground water extraction method has proven to be reliable and produced results comparable to USEPA method results.

One concern regarding this and other water extraction procedures is the presence of solids in the sample. While sampling ground water at PAH-contaminated sites, Backhus *et al*.(1993) found that increased ground water sample turbidity correlated to increased ground water concentrations of many hydrophobic PAH's, such as pyrene, chrysene, and phenanthrene. Ground water concentrations of naphthalene, a PAH with higher solubility, did not covary with turbidity. Backhus *et al*. (1993) stated, "The significant excess of measured chrysene/benz(a)anthracene may suggest that some of this low-solubility contaminant is associated with the colloidal material observed in the ground water." Therefore, care should be taken to not include solids and/or colloidal materials in the ground water extraction vial.

F. Determination of Soil Characteristics

i. Moisture Content

A known amount of soil was placed on a tin pan of known weight. The pan was placed in the 105 C oven for one week. The tin was weighed and the mass of water lost was calculated for each sample. The mass of water lost divided by the initial soil weight was used to quantify a mass-based soil moisture content.

ii. Total Organic Carbon (TOC)

The amount of TOC in select grass plot soil samples was determined using an unconventional method. Burgos (1995) used a similar method to measure soil TOC. Soil samples were placed in ceramic crucibles of known weight and dried in the 105 C oven for 15 minutes. The crucible was weighed and then immediately placed in the muffle furnace for 45 minutes at 430 C. The crucible was removed from the muffle furnace and weighed. The weight difference divided by the initial soil weight was used to quantify soil TOC.

iii. Soil Characterization

A set of 6 sieves was used to obtain a grain size distribution for the grass plot soils. The set consisted of the following sieves (#, sieve opening (mm)): #10, 2 mm; #18, 1 mm; #30, 0.6 mm; #35, 0.5 mm; #60, 0.25 mm; #200, 0.074 mm and #325, 0.045 mm. Samples were air dried for 48-72 hours and ground with a mortar and pestle. Each sample was agitated on a mechanical sieve shaker for 15 minutes. Soil retained on each sieve was weighed. Grain sizes greater than 0.074 mm and less than 0.074 mm comprised the sand and clay/silt fractions, respectively.