Relationships Between Hybrid Poplar Tree Extractives and Ground Water Contamination at a Phytoremediation Site

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ABSTRACT

In 1997, a phytoremediation program began at a creosote-contaminated former railroad tie yard in Oneida, Tennessee with the planting of over 1000 hybrid poplar trees onsite. Creosote, a mixture of hazardous chemicals composed of 85% polycyclic aromatic hydrocarbons (PAH) had entered the site soil and ground water. After planting, a seasonal ground water testing program began that monitored the progress of remediation by measuring the concentration of the 10 predominant PAHs in the contaminant plume: naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, and benzo(b)fluoranthene. The concentrations of these compounds steadily decreased over time, but the role the trees played in the remediation was unclear.

In order to gain a clearer understanding of the role the trees played in contaminant remediation, chemical analysis of tree tissue began. It was not known whether the trees were taking up PAH contaminants or their metabolites or if the rhizosphere zone created by the trees simply enhanced the ability of the site microflora to degrade the PAH. The objectives of this research were to (1) develop a suitable method for the chemical analysis of tree tissue collected from a field site, (2) determine if there were any chemicals not usually found in poplar trees that occurred in the trees growing over contamination, (3) determine if bud, bark, and twig tissue differed in their ability to predict ground water contamination, and (4) determine if a spatial correlation existed between the aromatic compounds in the tree tissue and the ground water total PAH plume.

Two types of tree tissue/ground water comparisons were performed: spatial distribution of isoeugenol concentration in tree tissue with spatial distribution of total PAH in ground water over the area of interest; and the spatial distribution of the quantity of aromatic compounds in tree tissue with the spatial distribution of total PAH concentration in ground water. Due to unit discrepancies between the quantities of interest, all comparisons were made on a percentile basis.

Initial tree sampling revealed that several compounds not usually present in poplar trees occurred only in those trees growing over contamination. In the first part of this study, the concentration of one of these chemicals, the substituted phenol isoeugenol, was compared with the concentration of total PAH in ground water from samples collected from February-March 2002. The bark tissue percentiles fell within 20 percentiles of ground water total PAH concentrations in 60% of the study area. The twig tissue showed slightly better agreement, with 67% of the study area differing from ground water by twenty percentiles or less.

The second comparison took place over three sampling events: March 2001, July 2001, and February-March 2002. The number of unique aromatic compounds in bark, bud, and twig tissue was compared with the total PAH concentration in ground water. Twig tissue aromatic compound content was the most accurate predictor of ground water contamination among the tissue types. After excluding those chemicals likely to be interferences from consideration, twig tissue aromatic content agreed with ground water total PAH concentration to within 20 percentiles over 2/3 or more of the study area during each sampling event, suggesting the potential uptake of PAHs or their microbial metabolites as a mechanism of phytoremediation at the site.

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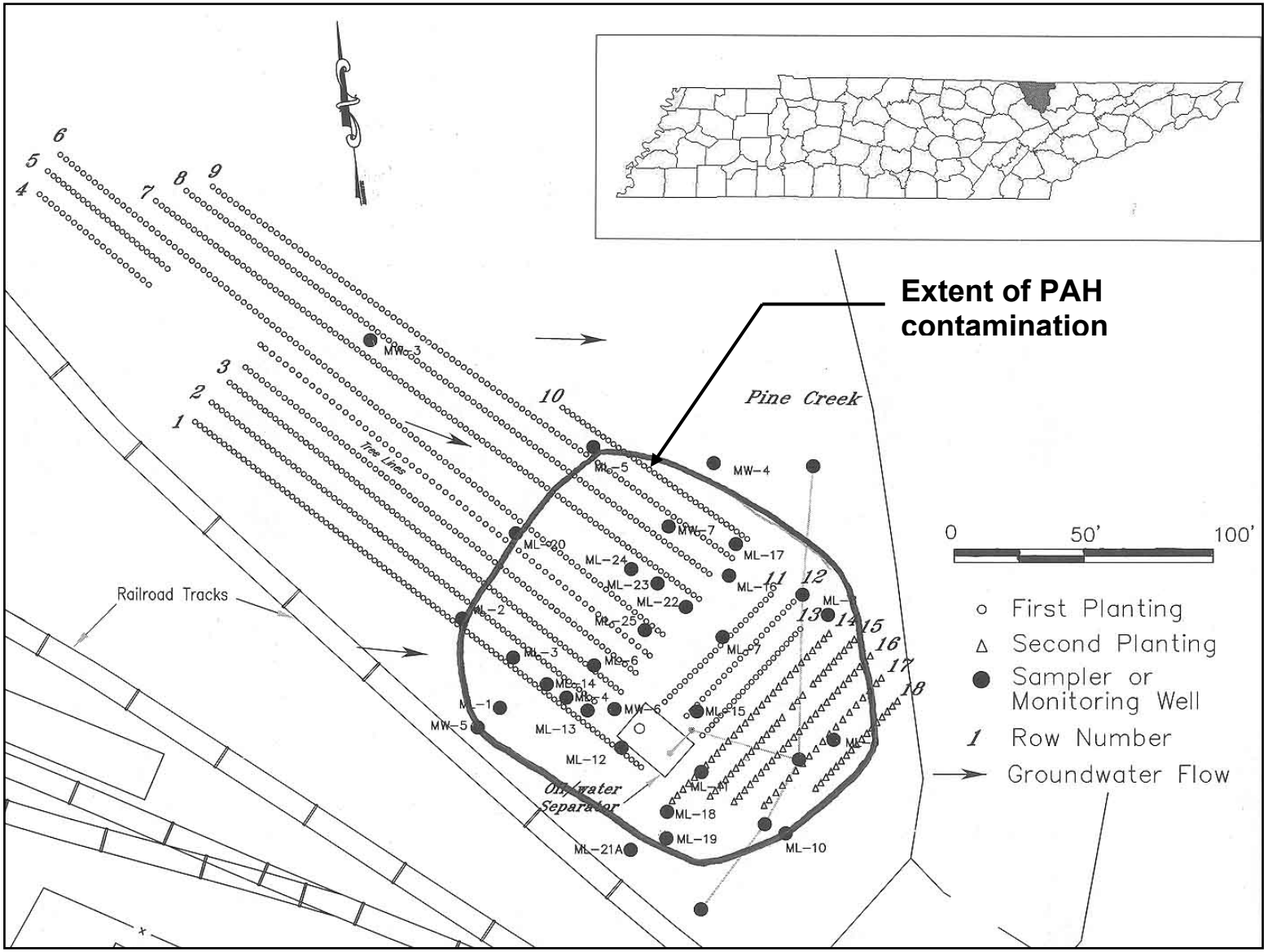
Chapter 1: Introduction

In 1990, the Army Corps of Engineers, while repairing a drainage channel at Pine Creek in Oneida, Tennessee, discovered creosote pollution in the waterway. The contamination resulted from years of wood-preserving activity at a railroad tie yard upgradient of the stream. Under orders from the Tennessee Department of Environment and Health to prevent further migration of creosote to the creek, the property owner hired an environmental consulting firm, ARCADIS Geraghty and Miller, to begin site investigations, determine the extent of the contamination, and propose remedial alternatives. After hiring a subcontractor to repair a trench designed to intercept contaminated ground water and prevent it from entering the creek, ARCADIS Geraghty and Miller recommended that their client use phytoremediation at the site, and so in 1997 over 1000 hybrid poplar trees were planted onsite, as shown in Figure 1-1.

After initiation of the phytoremediation system to treat the waste site, Virginia Tech proceeded to monitor the site soil and ground water for evidence of remediation. After planting, a seasonal ground water testing program began that monitored the progress of remediation by measuring the concentration of the 10 predominant PAHs in the contaminant plume: naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, and benzo(b)fluoranthene. Over time, the trees grew without inhibition, and creosote levels decreased in the site soil and ground water (Robinson, 2001). The mechanism through which remediation was taking place, however, remained unelucidated. Soil bacteria from the site, when grown in culture, had been able to utilize various polycyclic aromatic hydrocarbons as a sole carbon source, but the interactions between the trees and the soil bacteria were not well understood. In order to gain a clearer understanding of the role the trees played in contaminant transformation, chemical analysis of tree bark, bud, and twig tissue began in March of 2001. Subsequent sampling events took place in July 2001 and February-March 2002.

Figure 1-1: Oneida, Tennessee Phytoremediation Site



The objectives of this research were to (1) develop a suitable method for the chemical analysis of tree tissue collected from a field site, (2) determine if there were any chemicals not usually found in poplar trees that occurred in the trees growing over contamination, (3) determine if bud, bark, and twig tissue differed in their ability to predict ground water contamination, and (4) determine if a spatial correlation existed between the aromatic compounds in the tree tissue and the ground water total PAH plume.

Chapter 2: Literature Review

Phytoremediation Mechanisms

The Environmental Protection Agency defines phytoremediation as using plants to decontaminate any medium from environmental pollution, in whole or in part (Pivetz, 2001). Like other remediation methods, phytoremediation may lead to several different acceptable outcomes and has several distinct modes of action. These are phytostabilization, immobilization, accumulation, volatilization, phytodegradation, and rhizodegradation.

In phytostabilization, the contaminant is not destroyed, but simply prevented from migrating offsite or doing further damage to the ecosystem. A disadvantage of this technique is that ensuring that contaminant migration does not occur may involve monitoring a site for the foreseeable future.

In immobilization or stabilization, the pollutant sorbs to the plant roots and is prevented from migrating, or, in reacting with root exudates, forms an insoluble precipitate which is no longer bioavailable and does not enter ground water. Immobilization prevents the contaminant from having a deleterious effect on the ecosystem, but, since it does not destroy the contaminant, care must be taken to ensure that the pollutant does not mobilize again at a later date due to environmental changes or the death of the plant (Schnoor *et al.*, 1995; Schnoor, 2000).

In accumulation, also known as phytoextraction, the chemical of concern is taken up into the plant, but its form does not change significantly. The contaminant is thus removed from the original matrix, and the plant material is harvested and usually landfilled. The main advantage of this technique is the ability to concentrate the pollutant into a small volume and preventing the pollutant from extended interaction with the ecosystem. Disadvantages are that the contaminant is not destroyed, and a suitable disposal method and location must be found for the contaminated plant tissue.

In volatilization, the plant takes up the contaminant of concern from soil, water, or a mixed soil and water matrix, converts it to a volatile form, and releases it to the atmosphere, usually through the leaf stomata (Aitchison *et al.*, 2000; Burken and Schnoor, 1998). This technique is only suitable for contaminants that do not pose a significant air pollution hazard.

Phytodegradation is the uptake and metabolism of a contaminant by the plant where it is converted into less toxic forms. It is a permanent solution for organic chemical contamination, and has the advantage that the site no longer needs monitoring after target concentrations are reached.

Rhizodegradation, or enhanced rhizosphere degradation, takes place at the intersection of bioremediation and phytoremediation. In this process, the presence of the plant and its roots creates a rhizosphere zone more amenable to the microbes that degrade the contaminant. Root exudates such as organic acids and ketones may promote microbial growth, as may the increase in soil organic matter caused by the roots.

Investigators at various institutions have performed many studies to verify the ability of plants to remediate both inorganic and organic contaminants. Plants have demonstrated ability to treat both inorganic and organic contaminants. Inorganic contaminants may be remediated through phytostabilization, immobilization, and volatilization, while organic pollutants may be treated through any method.

Phytoremediation Case Studies

In the phytoremediation of metals and metalloids, phytoextraction through hyperaccumulation is becoming the remediation method of choice. The contaminant is not destroyed, but simply removed from the more sensitive matrix. Researchers at the University of Florida have discovered the ability of the Chinese brake fern, *Pteris vittata,* to hyperaccumulate arsenic. In a field test, the ferns were planted at a wood-preserving site containing soil contaminated with from 18.8 to 1,603 parts per million arsenic, and accumulated from 3,280 to 4,980 parts per million arsenic in its tissues (Ma *et al.*, 2001).

Sunflowers, *Helianthus annus* have proven effective in the remediation of radionuclides and certain other heavy metals. The flowers were planted as a demonstration of phytoremediation in a pond contaminated with radioactive cesium-137 and strontium-90 as a result of the Chernobyl nuclear disaster in the Ukraine. The concentration of radionuclides in the water decreased by 90% in a two-week period. According to the demonstration, the radionuclide concentration in the roots was 8000 times that in the water. In a demonstration study performed by Phytotech for the Department of Energy, *Helianthus annus* reduced the uranium concentration at the site from 350 parts per billion to 5 parts per billion, achieving a 95% reduction in 24 hours (Schnoor, 1997).

Brassica juncea, Indian mustard, has shown itself to be an effective accumulator of lead. In studies carried out at Rutgers University, researchers found that cultivar 426308 of Brassica juncea possessed the greatest ability to translocate lead to the shoots, accumulating nearly 3500 ppm lead in its above-ground biomass. Though this cultivar had the greatest ability to translocate lead to the shoots from the roots, it still maintained a higher root concentration of lead, approximately 100,000 ppm, or 10%, than shoot concentration. (Kumar, P. B. A. N. *et al.*, 1995).

Several researchers have attempted to find a way to increase the root-to-shoot translocation of lead accumulators, since the above-ground biomass is easier to harvest and dispose of and phytoextraction, as opposed to phytoimmobilization, would reduce the need for long term monitoring of the site to ensure that re-release of sequestered contaminants did not occur. Usually, the limiting factor in the phytoremediation of lead is its low water solubility and resulting low bioavailability for the plants, resulting in limited uptake and unfavorable rates of root-to-shoot translocation (Blaylock *et al.*, 1997; Huang *et al.*, 1997).

The method of choice to increase root-to-shoot translocation rates of lead in soil has been to add synthetic chelates to increase the solubility, and thus, bioavailability, of the heavy metal (Blaylock *et al.*, 1997; Huang *et al.*, 1997). Blaylock *et al.* performed three short-term studies: one in which they added lead as lead carbonate to a clean soil, one "using a hydroponic system in a growth chamber" in which lead was added as lead nitrate, and one using bioreactors containing contaminated soil from a cable-manufacturing site. In the first study, *Brassica juncea* seeds were planted in pots containing 350 grams of soil amended with fertilizers and heavy metals. After three weeks of growth under controlled conditions, the chelating agents *trans*-1, 2-cyclohexylenedinitrilotetraacetic acid (CDTA), diethylenetrinitrilopentaacetic acid (DTPA), ethylenedinitrilotetraacetic acid (EDTA), ethylenebis[oxyethylenetrinitrilo]tetraacetic acid (EGTA), citric acid, or malic acid were surface applied to the soil as a potassium salt solution. One week after the chelator addition, the researchers excised the stem one centimeter above the soil surface and measured lead concentrations in plant tissues and the soil.

EDTA proved the chelating agent most effective in promoting root-to-shoot translocation of lead. In a pot with soil lead concentration of 600 mg/kg and amended with 10 mmol/kg EDTA, a lead shoot concentration of 15,000 ppm by mass was obtained, and a maximum shoot concentration of 5000 mg/kg was obtained from soil containing with the addition of 0.5 mmol EDTA/kg as opposed to 20 mg Pb/kg in the control. (Blaylock *et al.*, 1997).

Creosote Composition and Characteristics

Though often referred to as a wood-preserving chemical, creosote is actually a complex mixture in which approximately 300 chemicals have been identified, although up to 10,000 may be present. The overwhelming majority of chemicals in creosote are polycyclic aromatic hydrocarbons (PAHs) (Dementi and Wasti, 1981; Mueller, Chapman, and Pritchard, 1989). The six-membered benzene ring forms the basic structural unit of most PAHs, although some PAHs do exist that contain seven-membered rings (Deleuze, 2002). As a class, PAHs tend to have relatively high molar masses and low water solubilities (Mueller *et al.*, 1989).

PAHs typically are difficult and expensive to remediate by conventional means, such as pump-and-treat or incineration (Glass, 2000). Due to their low water solubility and tendency to form a DNAPL layer, bioremediation usually acts relatively slowly to remediate these compounds (Breedveld and Karlsen, 2000).

Creosote has long been used as a wood preservative, and, in the United States, has been used for this purpose more than any other chemical (ATSDR, 1990). In 1985, an estimated 13,000 tons of the substance were manufactured in the United States (Wright *et al.,* 1985). Creosote is produced by distillation of the tar produced during the coking of coal (Worsham, 1993). The substance, though not fully characterized, has been found to contain about 85% polycyclic aromatic hydrocarbons, and 3 % phenols (Ellis, 1994). Approximately 300 pure compounds have been identified in the mixture, though as many as 10,000 may be present. (Dementi and Wasti, 1981).

Wright *et al.* (1985) separated commercial creosote into four fractions: aliphatic hydrocarbons, polycyclic aromatic hydrocarbons, nitrogen-containing polycyclic aromatic hydrocarbons (NPAHs), and hydroxy-functional polycyclic aromatic hydrocarbons (HPAHs). They found that aliphatics formed 7% of the mixture, PAHs 69%, nitrogen PAHs 11%, and hydroxy- PAHs 11%, making the product 91% aromatic (Wright *et al.,* 1985). The two percent that these authors could not account for could be the phenols identified by Ellis (1994).

Of the commercial creosote PAH fraction, the authors could positively identify approximately 545.3 mg/g. The most significant components they found were phenanthrene (169 mg/g), acenaphthene (82.5 mg/g) naphthalene (75.9 mg/g), fluoranthene (74.6 mg/g), pyrene (52.6 mg/g), fluorene (51.9 mg/g) and dibenzofuran (38.8 mg/g), together making up 75.7% of the PAH fraction.

Many of the chemicals identified in the PAH and NPAH fractions of creosote by Wright *et al.* (1985) have been found in the contaminated ground water at the Oneida site, specifically naphthalene, benzo(b)thiophene, 2-methylnaphthalene, biphenyl, acenaphthene, dibenzofuran, fluorene, and phenanthrene.

These compounds tend to be semivolatile, relatively insoluble in water, and difficult to bioremediate (Meulenberg *et al.*, 1997). Most polycyclic aromatic hydrocarbons (PAHs) will sorb strongly to the soil matrix. Their low solubility, high molecular weight, and high density, cause the compounds to customarily occur as a dense non-aqueous phase liquid at the bottom of the saturated zone.

Poplar Trees in Phytoremediation

Poplar trees have been used in the remediation of both inorganic and organic contaminants. In one study, the trees successfully stabilized a Superfund site contaminated with arsenic and cadmium, where ground water had been polluted as a result of mining activity. Using one acre of the site, researchers at the University of Iowa planted 3100 hybrid poplar trees along Whitewood Creek. The trees, planted to a depth of 1.6 m, were able to confine the metals to the upper 1.8 m of the site and prevent them from entering the creek (Schnoor, 2000).

Poplar trees have also been shown to be effective in the remediation of recalcitrant organic contaminants. Several studies have demonstrated the ability of the trees to take up trichloroethylene or bind the chemical to its roots (Orchard *et al.*, 2000a; Burken and Schnoor, 1998). Aitchison *et al.*, in a laboratory study, showed that hybrid poplar trees could volatilize 1,4-dioxane, a persistent contaminant. For concentrations of 5 mg/L or less, in aqueous solution, the researchers determined that inhalation of the airborne concentrations of 1,4-dioxane produced by phytovolatilization did not pose a significant health risk.

Characteristics of Poplar Trees

Several characteristics of hybrid poplar trees make them suitable for the performance of phytoremediation. The hardwood trees are phreatophytes, requiring large volumes of water and able to pull water from the ground water table. This property alone enables them to perform hydraulic control of ground water and prevent contaminant migration through that pathway. Typically, they aid in the remediation of contamination in one of four ways: stabilization and erosion control, hydraulic control, rhizosphere effects, and direct uptake (Schnoor, 1997).

The trees are hardy, flood tolerant, fast-growing, and easy to propagate (Schnoor, 2000; Schnoor *et al.*, 1995). They are native to the eastern to midwestern portions of the United States, and grow well in climates as diverse as those found from Georgia to Iowa. Poplars can grow from cuttings or "whips" due to pre-formed root initials that they possess in their stems. Another important facet of poplars is their ability to tolerate many types of contamination, both by organic and inorganic chemicals. Part of the poplar resistance to organic contamination, especially that of PAHs and other aromatics, may lie in the chemistry of the trees.

Poplars are members of the family *Salicaceae*, which includes willows and aspens, and is known for the aromatic compound salicylic acid, which occurs in the barks of several trees in the family. Many trees in this family, including poplars, contain various naturally-occurring aromatic compounds such as salicylic acid, salicylic alcohol, aromatic ketones, terpenoids, fatty and organic acids, 2-benzoylsalicin, benzyl alcohol, β-phenylethanol, p-ethylphenol, methyl benzoate, phenol, pyrocatechol (1,2-benzenediol), salicortin, salicin, ω-salicyloylsalicin, and other compounds (Pearl and Darling, 1977; Abramovitch and Koleoso, 1966; Hossfeld and Kaufert, 1957; Pearl and Darling, 1971a; Pearl and Darling, 1971b; Browning, 1967; Sentsov *et al.*, 1997; Steele and Ronald, 1973) . The presence of so many naturally-occurring aromatic compounds in poplar wood means that investigators must take great care in analyzing the wood for aromatic compounds as evidence of phytodegradation.

Poplar Extractives

Hybrid poplar trees, as members of the family *Salicaceae,* typically possess aromatic compounds among their extractives. Salicylic acid is perhaps the best-known naturally-occurring aromatic compound found in this family. Upon analysis, however, several aromatic compounds eluted from the poplar tissue of certain trees which do not usually occur in this tree species. Because the trees at the Oneida site are genetic clones of one another, were planted within three years of each other, and were sampled during the same seasons, taxonomy, age, and season do not satisfactorily explain the presence of these unusual compounds (Steele, Ronald, and Bolan, 1973; Steele and Ronald, 1973; Browning, 1967). Environmental stress, while known to change the composition of the extractives in poplars, typically does so by changing the proportion of one extractive component relative to the others. It has not been found to promote the production of new extractive chemicals (Pelah *et al.*, 1997; Picard, Chenault, and Augustin, 1994; Tholakalabavi, Zwiazek, and Thorpe, 1994; Constabel *et al.*, 2000; Gebre, Brandle, and Kuhns, 1997).

While environmental stress seemed unlikely to promote the appearance of new extractive chemicals in poplar trees, exposure to environmental pollution emerged as a plausible cause of this chemical change. Several studies have documented the ability of hybrid poplar trees to take up organic contaminants and the resultant presence of contaminant or contaminant metabolites in the tree tissue (Aitchison *et al.*, 2000; Orchard *et al.*, 2000a; Newman *et al.*, 1997). Exposure to environmental contamination has been found to add new chemicals to the extractive complement of hybrid poplar trees.

Isoeugenol

Isoeugenol, a substituted phenol found in the tissue of trees at the Oneida site, does not typically occur in the *Salicaceae* family in general or hybrid poplar trees in particular. Used in the manufacture of vanillin, isoeugenol occurs naturally in cinnamon and cloves. This compound appears both in cigarette smoke and as a fragrance component in perfumes (Smith et al., 2002; Rastogi et al., 2001). It is a skin irritant and has been identified as a tumorigen and a mutagen (SIRI, 2000). Its material safety data sheet identifies isoeugenol as a moderate health hazard with skin and respiratory tract irritation as the primary effects of exposure (Fisher, 2000).

Wood Chemistry and Associated Methods

Wood substances may be divided into two major categories: the cell-wall components and the extraneous components (Browning, 1967). The cell-wall components give wood its strength and structure. They consist of lignin and polysaccharides, including cellulose. The balance of the material is the extraneous matter, which includes proteins, inorganic materials and other components. The extraneous components that can be removed from the wood by the use of neutral solvents are known as the extractives.

The chemical analysis of wood and wood products, especially pulp and paper, has been practiced for some decades both by professionals in forestry and the pulp and paper industry. Wood science and wood chemistry are developed fields, and several protocols exist for the analysis of woods and the isolation of extractives. Browning (1967), in his text on wood chemistry, observes that no one solvent is capable of removing all the

extractives present in wood, and presents several solvent systems for the use of the investigator. The solvents in these systems gradually go from one polarity extreme to the other, while the author notes that it falls to the investigator to determine the most appropriate series of solvents for the wood under analysis (Browning, 1967).

The American Society for Testing and Materials developed the Standard Test Method for Moisture and Creosote-type Preservative specifically for creosote compounds in wood, with the aim of measuring the efficacy of the wood treatment process, specifically for railroad ties and other large timbers. The method uses a reflux technique to calculate the water content and measure the quantity of creosote and volatile oils in the timber sample. If dry solvent will not be used for the extraction or the reflux apparatus has not been dried, the first step of the method is to prepare the reflux apparatus, which consists of an extraction flask, condenser, water trap, and an extraction cup.

In the preparation, 200 mL of solvent and one or two mL of water are added to the flask in the assembled apparatus and boiled under reflux for 30 minutes. Any residual water on the condenser or the walls of the water trap is transferred to the storage portion of the trap by the use of a hydrophobic rod and the volume of water in the trap recorded to the nearest 0.01 mL.

Wood samples in the method are collected by the use of a boring tool, which must be calibrated before each use in creosote determination. In order to calibrate the borer, 20 boring samples are collected with each sample being measured longitudinally and transverse to the grain to the nearest 0.025 mm. For each boring, the square of the mean is taken of the transverse and longitudinal measurements. The square root of the mean of the calculated mean-squares then is determined as the calibrated diameter of the borer to the nearest 0.025 mm.

Masses of the extraction cup and stoppered weighing bottle are then determined to the nearest 0.01 g and the extraction cup is inserted into the weighing bottle. Twenty carefully measured borings are then collected, placed into the weighing bottle, and weighed, while taking care to minimize contact of the samples and the air inside the bottle with the ambient air. The entire contents of the weighing bottle are then massed to the nearest 0.01 g. The extraction cup containing the samples is then placed into the extraction apparatus, and the emptied weighing bottle is again massed. The difference

between this mass and the original mass of the stoppered weighing bottle represents water that has condensed from the samples onto the bottle and is the first portion of water in the sample.

At this point, the reflux of the approximately 200 mL of toluene, xylene, or toluene-xylene solution in the flask is begun and continues for at least two hours at a minimum rate of one drop from the condenser per second. The reflux continues for two hours for freshly creosoted timber, and five hours for timber to which a creosote-coal tar solution has just been applied. No time parameters are given for aged creosote-treated piles, which likely would be more difficult to extract due to irreversible sorption (Hatzinger and Alexander, 1995).

At the end of the reflux period, the trap is allowed to cool to room temperature and any water in the condenser or on the trap walls is transferred into the graduated portion of the water trap. The volume of water in the trap is read to the nearest 0.01 mL. The difference between this volume and the volume in the trap at the first reading is the second portion of water from the wood sample.

After measuring the second portion of water, the experimenter removes the extraction cup with the sample from the extraction apparatus and dries it in a fume hood for 15 minutes and then in an oven at 125 ˚ C for two hours. After drying, the investigator transfers the extraction cup and sample to an acetone-rinsed, oven-dried weighing bottle that has been pre-weighed and stored in a dessicator. The researcher then places the extraction cup with boring samples in the weighing bottle and let them come to room temperature in a dessicator and mass them. From this measurement he or she finds the mass of the dry extracted wood.

From this method it is possible to calculate the water content of the wood, the mass of creosote in the wood, and the concentration of creosote in the wood, on a mass per volume basis (ASTM, 2000). The ASTM did not provide precision information for the method. An advantage of the method is that it could be used to consider volatile and semi-volatile components of creosote. Disadvantages of the method are that it includes no way to quantify the different components of the creosote, and that it is doubtful if it can be used in a situation where samples are taken in the field and transported to a laboratory for analysis. It also requires significant preparatory steps in the calibration of

the borer and pre-refluxing of the apparatus. The method, like other methods, also requires the boiling of toxic, flammable solvents.

The TAPPI presents a solvent extraction method that seeks to quantify the total amount of non-volatile extractive material in wood or pulp. The solvents the association recommends for this procedure are dichloromethane, 1/3 ethanol with 2/3 benzene, or 1/3 ethanol with 2/3 toluene (TAPPI, 1994). The TAPPI, in issuing its protocol, did so for wood that had already been pulped, and so most of the polar compounds had already been removed. Thus, the TAPPI procedure only makes use of the less polar solvents.

In the procedure, approximately 2 to 4 g of wood are dried and in a Wiley mill ground to sawdust that passes a 40-mesh (0.40-mm) screen and extracted using a Soxhlet apparatus. The mass of sawdust is extracted with 150 mL of boiling solvent over a four to five hour period. After extraction, the spent solvent is concentrated to 20-25 mL, and this volume is then placed into a tared weighing dish. The dish and solvent are then placed in an oven at 105°C, and brought to room temperature in a dessicator. The mass of the residue remaining is then measured to the nearest tenth of a milligram. An aliquot of pure solvent will also undergo the same procedure to correct for solvent residue.

The major advantage of the TAPPI method is its high degree of precision. Highly qualified operators can expect results to agree within 21%. (TAPPI, 1994). A significant disadvantage of the method is that it requires at least 4g of wood tissue per solvent used, and usually at least two solvents are required in order to analyze the full range of extractive components in a sample, thus necessitating 8g of wood tissue per sample run. For non-sacrificial samples, which seek to measure changes in the composition of extractives in different tree parts over time, such a large quantity of sample is not feasible. The harvesting of 8 grams each of bud, twig, and bark tissue necessary for the method would likely kill or severely stress a living tree. Another drawback of the TAPPI method is the requirement to boil a volatile solvent continuously for four or five hours.

The boiling presents a safety concern, in that four of the six solvent choices recommended for use in the method involve benzene or toluene, both of which are significant health and flammability hazards. Boiling the solvents increases the risk of inhalation of the vapors as well as the risk of injury due to accidental splash. Chemical resistant gloves may or may not be heat resistant as well.

The literature provides estimates of the precision of wood chemistry methods. The Technical Association of the Pulp and Paper Industry, in their published method for the preparation of extractive-free wood, a related procedure, state that a measure of precision is not applicable to the procedure. (TAPPI, 2000). In the TAPPI method for the determination of total non-volatile extractives in wood, the association provides values for two precision-related parameters: repeatability and reproducibility.

Repeatability refers to the range within which agreement is obtained 95% of the time between results performed on samples with the same characteristics by a single operator. Reproducibility indicates the range within which agreement is obtained 95% of the time between results performed on similar samples by different, equally well-qualified operators. The association provides its precision values by solvent system, so that for ethanol-benzene, the expected repeatability is 11% with a reproducibility of 20%; for dichloromethane the expected repeatability is 4% and expected reproducibility is 21%. For ethanol-toluene, the other recommended solvent in this published method, the association has not yet determined measurements of precision (TAPPI, 2000).

Wood Chemistry Methods Associated with Phytoremediation

Most of the researchers who are involved in wood chemistry in order to monitor phytoremediation use radio labeling and liquid scintillation counters to quantify the amount of contaminant taken up by the tree (Orchard *et al.*, 2000; Schnabel *et al.*, 1997; Nair *et al.*, 1993). All of the radiolabelled experiments appear to have been on a laboratory scale using bioreactors (Aitchison *et al.*, 2000; Orchard *et al.*, 2000; Nair *et al.*, 1993). A known quantity of mixed radiolabelled and non-radiolabelled organic contaminant is introduced into the tree system, which is closed to the atmosphere and contains carbon dioxide and volatile organic compound traps.

Orchard *et al.* (2000) examined *Populus deltoides* tissue for evidence of phytoremediation by the performance of tissue analysis on the microscale. The authors set up a hydroponic system to monitor the fate of trichloroethylene (TCE) in a planted system. These authors used radiolabelling to verify the uptake of TCE by the poplars and devised a procedure to analyze the tree tissue so that they could identify and quantify the TCE metabolites dichloroacetic acid, trichloroacetic acid, and trichloroethanol.

In the procedure, five grams of fresh-ground tree tissue were placed into a 50-mL teflon centrifuge tube to which 15 mL of 0.25N sodium hydroxide were added. The tubes was then shaken for ten minutes on a reciprocating shaker and subsequently centrifuged for ten minutes at a speed of 9,750 rpm. The supernatant was transferred to a 50-mL polyethylene centrifuge tube. The material remaining in the teflon tube was subsequently extracted two more times by the same process, and all the supernatants combined in the same polyethylene tube. The combined supernatants were then acidified to a pH less than 0.5 through the addition of 50% sulfuric acid, saturated with sodium chloride, and extracted for five minutes three more times with 3 mL of methyl *tert* butyl ether (MTBE). The ether solution was then centrifuged for five minutes at 5000 rpm, and the combined supernatant from the three MTBE extractions combined, dried over anhydrous sodium sulfate, and brought to a 10-mL volume. A one-milliliter aliquot of the extract was then injected into a Shimadzu gas chromatograph with an electron capture detector (Orchard *et al*., 2000).

There are several advantages to the analytical method employed by Orchard *et al*. (2000). The method functions efficiently on a reasonably small scale and allows a single investigator to analyze a quantity of samples at one time. The analyst also is able to quantify the metabolites produced by the poplar trees. One concern about the method relates to the degree of hazard posed to the investigator. MTBE, as an ether, has the potential to form peroxides when dry and become extremely explosive. Wisely, the investigators chose not to heat the solvent solution. The use of strong base, strong acid, and a moderately reactive solvent (MTBE) all serve to increase the intrinsic hazard of the procedure.

Another safety concern of the procedure is the addition of MTBE to a solution having a pH of less than 0.5. According to its MSDS, MTBE is incompatible with acids and is unstable in the presence of acid solutions (Fisher, 2000). Other caveats to the method have to do with the reagents chosen: 0.25N sodium hydroxide and MTBE. Strong base has the potential to react with organic materials, so that peaks appearing in the chromatograms could be a result of reaction with the solvent and may not have been originally present in the trees. The use of derivatizations and the possibility of chemical reactions occurring in solution due to the presence of strong acid and base makes the procedure usable only in laboratory settings where the metabolites produced are already known.

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Chapter 3: Chemical Relationships Between Ground Water Contamination and Hybrid Poplar Tree Extractives at a Phytoremediation Site

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Abstract

In 1990, significant creosote contamination of soil and ground water was discovered at an abandoned railroad tie yard in Oneida, Tennessee. In 1997, over 1000 hybrid poplar trees were planted onsite, followed by a program for monitoring polycyclic aromatic hydrocarbons (PAHs) in soil and ground water. In March of 2001, tree tissue samples were collected and analyzed to aid in determining the mechanism of remediation and to attempt to quantify PAHs or their metabolites in the tree tissue. Sampling and analysis protocols were developed for the tree tissue samples so that the plants could be sampled for an indefinite period in a non-sacrificial manner. Drawing upon the available literature in wood chemistry, the expertise of a wood chemist, and methods previously developed for the analysis and sampling of soil at the site, a new method was developed that attempted to identify and quantify metabolites of PAHs in the tissue samples. Isoeugenol, a chemical not usually found in trees, emerged as a potential PAH metabolite and was quantified in tree bark and twig tissues.

The research evaluated the ability of isoeugenol concentration in tree tissue to predict ground water total PAH concentration by using geographic information systems (GIS) to compare the spatial distribution of isoeugenol concentration in tree tissue with the spatial distribution of total PAH concentration in ground water. A strong trend emerged, linking isoeugenol concentrations in the tree tissue and PAH concentration in the ground water. The bark tissue percentiles fell within 20 percentiles of ground water total PAH concentrations in 60% of the study area. The twig tissue showed slightly better agreement, with 67% of the study area differing from ground water by twenty percentiles or less.

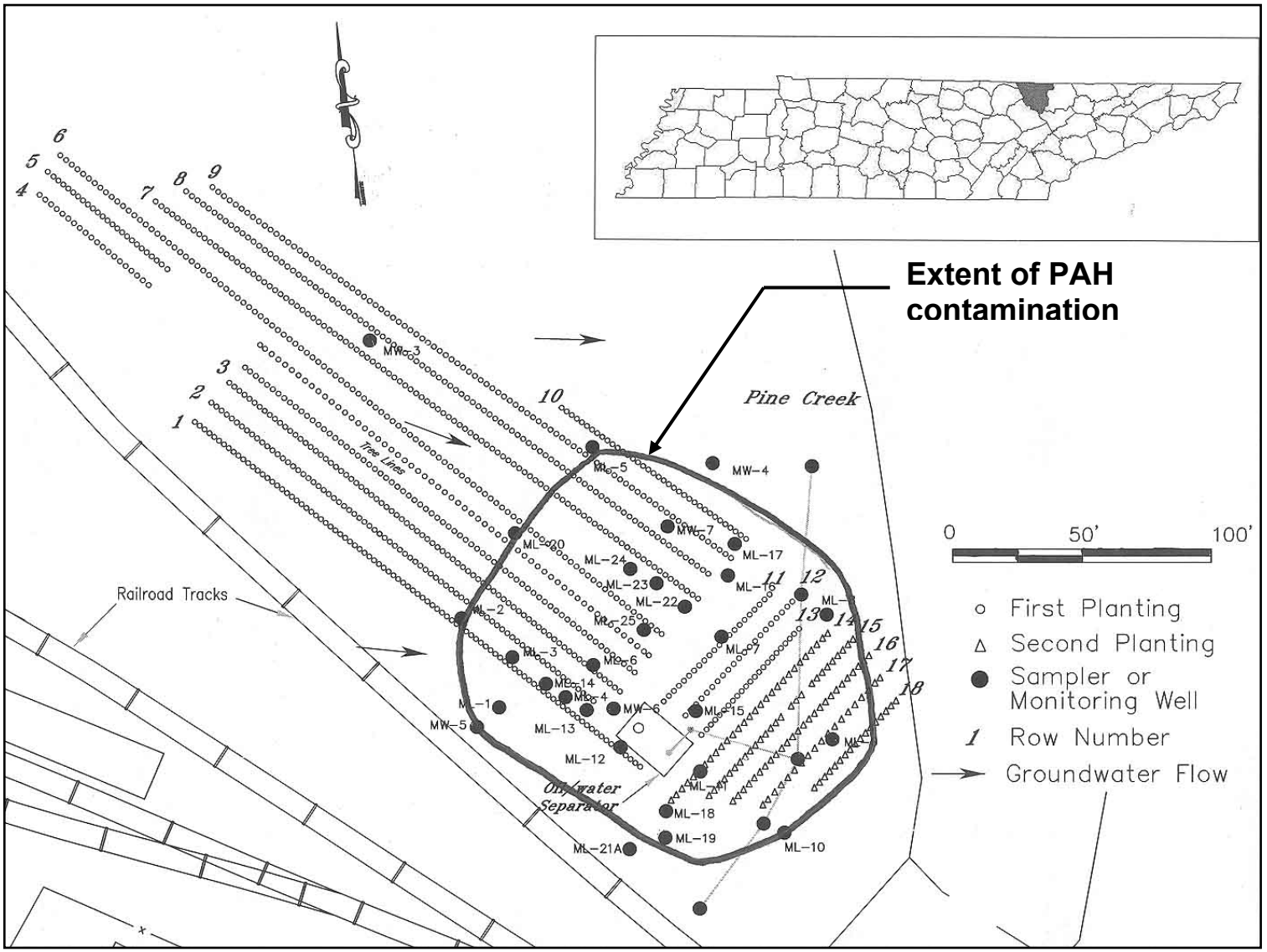
Introduction

In 1997, remediation of a creosote-polluted railroad tie yard in Oneida, Tennessee was initiated. Creosote, a wood preservative and waterproofing agent, contaminated the site's soil and ground water. Though sometimes referred to as a wood-preserving chemical, creosote is actually a mixture of over 300 compounds (Dementi and Wasti, 1981; Mueller *et al.*, 1989). Creosote consists primarily of polycyclic aromatic hydrocarbons (PAH). Due to the aromatic nature of the majority of creosote's components, this mixture tends to be difficult or expensive to treat by conventional methods, such as pump-and-treat or incineration (Glass, 2000).

PAH contamination exists at the site in three phases: a dense, non-aqueous phase liquid (DNAPL) overlying bedrock, in aqueous solution in ground water, and as an adsorbate attached to soil. In order to remediate the site in the most economical way possible, ARCADIS Geraghty & Miller, an environmental consulting firm, recommended the use of phytoremediation. Over 1000 hybrid poplar trees were planted at the site, some directly over the contamination in order to promote degradation of the contaminant. Others were planted beyond the periphery of the plume to take up contaminated ground water and prevent its migration offsite. The site is shown in Figure 3-1.

Several characteristics of poplar trees make them particularly suitable for the remediation of ground water. Their phreatophytic nature, requiring them to take in large volumes of water, enables them to draw water from below the water table and provide hydraulic control. Hybrid poplars also grow quickly and have a reputation for hardiness. Typically, they aid in the remediation of contamination in one or more of four ways: stabilization and erosion control, hydraulic control, rhizosphere effects, and direct uptake (Schnoor, 1997). Native to the southeastern United States, poplar trees are hardy, flood tolerant, fast-growing, and easy to propagate. Known to tolerate organic contamination, the trees have been documented to take up organic contaminants such as trichloroethylene and 1,4-dioxane (Orchard *et al.*, 2000a; Burken and Schnoor, 1998).

Figure 3-1: Site Layout



After the tree planting in 1997, monitoring of the site for evidence of soil and ground water remediation began. Over time, the presence of the trees seemed to enhance contaminant reduction, but the role of various mechanisms through which this enhancement has taken place remained unelucidated (Robinson, 2001). Though it had been shown that PAH degradation was promoted via rhizosphere effects, the impact of direct uptake by the trees was unknown (Robinson, 2001). Soil bacteria from the site, when grown in culture, have been able to utilize various polycyclic aromatic hydrocarbons as a sole carbon source, but the interactions between the trees and the soil bacteria were not well understood.

In March of 2001, four years after the tree planting, the first bud, bark, and twig tissue samples were collected and analyzed to aid in determining the mechanism of remediation and to attempt to quantify PAHs or their metabolites in the tree tissue. Subsequent tree tissue sampling events occurred in July 2001 and March 2002. Solvent extractions were performed on the tree tissue to determine if ground water contaminants or similar compounds were present in the trees and to quantify these contaminants in the tree tissue. The objectives of this research were (1) to develop a method of tree tissue chemical analysis suitable for an ongoing field study (2) to determine if there were any chemicals not usually found in poplar trees that occurred solely in the trees known to be growing over contamination at the site (3) to determine if a spatial correlation existed between aromatic compounds in tree tissue and the ground water TPAH plume.

Sampling Plan

Based on the standard deviation of total PAH concentrations from site ground water samples, the number of trees needed to provide valid results for a significance level, α, of 0.10, within a range of 1000 ppb was selected. The trees were divided into two strata: one for those growing over areas thought contaminated based on previous ground water data, the other for trees growing over areas thought to be uncontaminated based on previous ground water data. It was assumed that no spatial correlation existed between samples independent of ground water contamination level. Minimum necessary sample size, n, was calculated after Gilbert (1987):

$$n = \frac{z^2_{1-\alpha/2} \sum_{h=1}^{L} W_h s_h^2 / d^2}{1 + z^2_{1-\alpha/2} \sum_{h=1}^{L} W_h s_h^2 / d^2 N} \qquad (1)$$

where

z = cumulative probability density function for standard normal distribution

s_h = standard deviation of a given sample stratum h

$\alpha = 0.10$, significance level

d= 1000 ppb, minimum detectable difference

N = total number of population units (maximum number of samples that could be taken)

L = number of strata

N_i = number of population units in stratum i, $N = \sum_{i=1}^{L} N_i$

$W_i = N_i/N =$ stratum weight

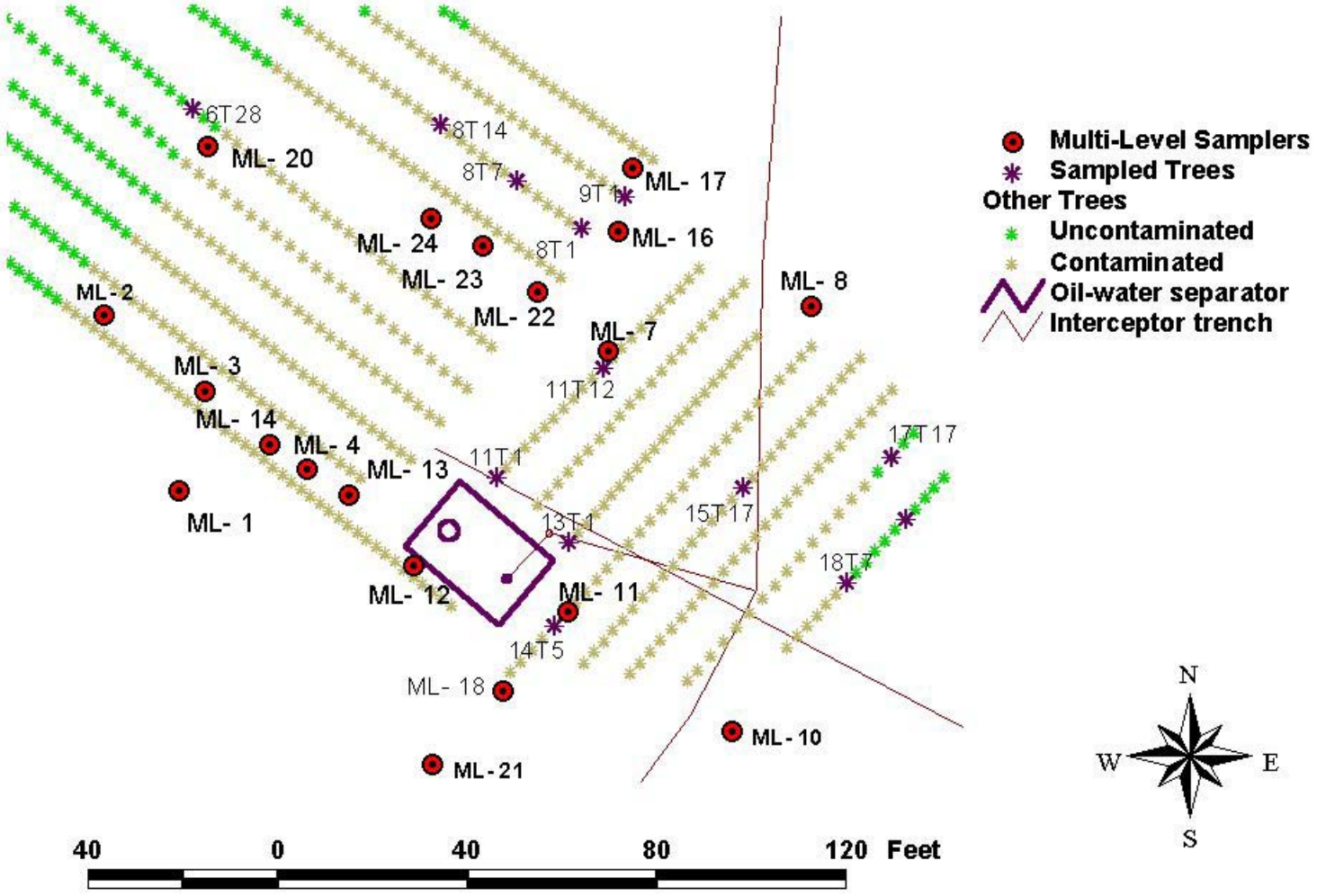
n = total number of samples taken

n_i = number of samples taken from stratum i

Based on the results from Equation (1), the minimum number of trees that needed to be sampled in March of 2001 was 25, 19 in areas identified as contaminated, and 6 in uncontaminated areas. A random number generator was employed to help ensure complete coverage of the site. Figure 3-2 shows sampling locations of both trees and ground water.

After the initial tree sampling trip in March 2001, the number of trees needed to be sampled was recalculated based on the new standard deviation of the number of aromatic compounds found in the tissues. According to the new calculation, only 8 trees needed to be sampled to provide statistically significant results for an alpha of 0.10, 6 in growing over contamination and 2 growing in uncontaminated areas.

Figure 3-2: March 2001 Sampling Locations



Bud, bark, and twig samples were collected during the winter and summer seasons in order to control for seasonal and anatomical variations in phytochemistry. The bark was collected from the lower bole on the south side of the trees, while buds and twigs were gathered from the low-hanging branches. In order to gather twig and bud samples, the twig and attached buds were removed from the tree by snapping off the twig at a joint or by cutting off the twig at the joint with a knife. Bark samples were collected by making a shallow cut with a knife and removing one or several strips of tissue. After collection in the field, the samples were placed into plastic bags and sealed, and transported to the laboratory on ice. The samples were stored at 4° C or frozen until analysis. Only one control tree was able to be processed from each of the March 2002 and July 2001 sampling events, though an excess of contaminated trees was processed.

Analytical Goals

Several goals were established for the sample analysis protocol of the tree tissue. Since samples were to be collected from the same trees over time, the method had to function on a much smaller scale than usual for wood chemistry experiments, the microscale, so that the removal of tissue for analysis would not unduly stress or kill the tree. The method should have the potential to provide positive identification and quantification of the chemicals under analysis. Like all processing protocols, the method should yield relatively precise results, which are replicable within a reasonable margin of error by both a given operator and by other operators performing the same procedure.

In addition, the method required the minimization of chemical changes in the tree tissue. This requirement excluded from consideration procedures that subjected the samples to elevated temperatures or strongly reactive conditions. For this reason high-temperature drying, boiling solvents, and solvents containing strong oxidizing agents, such as acids, bases, or peroxides were rejected.

Maintaining the safety of the method was another analytical goal. Steps taken to meet this goal included avoiding Soxhlet extractions, avoiding potentially reactive combinations of reagents, and selecting solvents with minimal toxicity.

While Soxhlet extractions are commonly used for the elution of aromatic compounds from solid media, often yielding a high level of precision, they were not used in this instance due to safety concerns. The Soxhlet extraction procedures for wood often require the boiling of wood samples in an organic solvent (ASTM, 2000). Since the extractable components in the wood were uncharacterized and flashpoints and autoignition temperatures unknown, Soxhlet extraction was deemed inappropriate for this research.

The goals of solvent selection were to maximize solvent effectiveness while minimizing solvent toxicity. Benzene, while commonly used and a very effective solvent, was rejected for use in the procedure due to its extreme toxicity. Dichloromethane posed a lesser health hazard and facilitated the comparison of results from the trees with results from the ground water, as the ground water extraction procedure utilized dichloromethane as a solvent. Several of its properties led to the selection of acetone as the second solvent. Acetone's hydrophilic nature complemented the hydrophobicity of dichloromethane and allowed a wider variety of chemicals to be extracted from the tree tissue. In addition, acetone acts effectively as an organic solvent and poses a relatively low toxicity risk.

Wood Chemistry Methods

Several published methods exist for performing solvent extractions of woody tissue. Two classes of methods were considered: standard methods developed by wood chemists and methods in the published literature developed to verify or monitor phytoremediation.

Methods for the examination of the extractives, the class of wood materials that are extractable in pH neutral solvents, have been published by Browning (1967), the Technical Association for the Pulp and Paper Industry (TAPPI), and the American Society for Testing and Materials (ASTM). Typically, the methods use more than one solvent and involve performing a Soxhlet extraction in which the solvents are heated to boiling temperatures and the vapors recondensed and the condensate collected. The methods often seek to measure the total quantity of extractable material present in wood.

Wood chemistry methods did not to provide a way to quantify individual chemicals in wood, did not function on the microscale, involved the use of undesirable solvents, and could have potentially heated unknown contaminants or their metabolites beyond their flashpoints (Browning, 1967; TAPPI, 1994; ASTM, 2000). Therefore, none of the standard methods developed by wood chemistry professionals for the chemical analysis of wood met enough of the stated analytical goals to be considered for this research.

Most of the researchers involved in monitoring phytoremediation use radio labeling and liquid scintillation counters to quantify the amount of contaminant taken up by the tree (Orchard *et al.*, 2000a; Schnabel *et al.*, 1997; Nair *et al.*, 1993). All of the radiolabelled experiments appear to have been on a laboratory scale using bioreactors. (Aitchison *et al.*, 2000; Orchard *et al.*, 2000a; Nair *et al.*, 1993). A known quantity of mixed radiolabelled and non-radiolabelled organic contaminant is introduced into the tree system, which is closed to the atmosphere and contains carbon dioxide and volatile organic compound traps. Usually sacrificial in nature, methods cited in the phytoremediation literature required that metabolites be fully characterized. Radiolabelling, the usual method for quantification of contaminant uptake, was not feasible for the current field study. Due to the infeasibility of using the lab-scale wood chemistry methods to analyze field data, none of the methods published in the phytoremediation literature emerged as a suitable alternative. Since neither the wood chemistry nor the phytochemistry literature provided a method suited to the current research, a new method was developed.

Materials and Methods

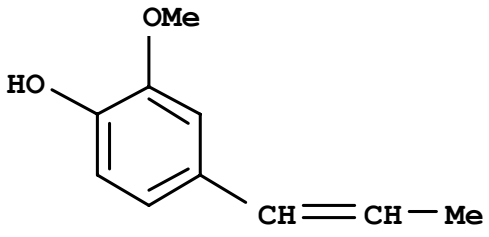
The tree tissue was analyzed using a modified form of the soil extraction procedure developed by Glendon Fetterolf as an alternative to the Soxhlet extraction (Fetterolf, 1998). The tree tissue was dried for 24 hours at 30 degrees centigrade and ground in a 10 mesh Wiley mill. The ground samples were then placed in glass centrifuge tubes and extracted in methylene chloride for 36 hours on a shaker table. After shaking, the samples were chilled below 4 °C for at least 24 hours to encourage the flocculation and settling of fine particulates so that these particles would not interfere with the equipment used in the analysis. Subsequent to chilling, the samples were

centrifuged for two hours at a refrigeration temperature of 0° C at 2600 to 2800 rpm to encourage the further settling of fines. An aliquot of the supernatant was then drawn up into a 1-mL gas-tight syringe and injected into 2-mL GC target vials. The vials were then placed in an autosampler and injected into a GC/MS for analysis.

The instrumentation used consisted of an Agilent 5973 Network Mass Selective Detector and an Agilent 6890 Series GC system. Column flow was maintained at 1.0 mL per minute with helium carrier gas at 24.1 mL per minute. Initial oven temperature was 80˚ C for one minute then increasing at 10˚ C per minute to a maximum of 305˚ C which was maintained for nine minutes. The capillary column used had dimensions of 30 m by 0.25 mm with a 0.25μm film thickness. The column was composed of fused silica and was 5% crosslinked.

After being extracted in methylene chloride, the solvent remaining in the centrifuge tubes was filtered off using pre-extracted Whatman filter paper to prevent loss of particulate matter. The samples were then placed in a chemical fume hood to allow any remaining solvent to be removed by evaporation. Five milliliters of acetone were then added to each of the centrifuge tubes, which were then placed on a shaker table for 36 hours.

The compounds of interest included 2,3-dihydrobenzofuran and 2-methoxy-4-(1-propenyl)phenol, also known as isoeugenol, whose structure is shown below. Like their counterparts eluting in dichloromethane, they only appeared in trees known to be growing over contamination and are not naturally-occurring in poplar trees. These chemicals were quantified due to the availability of pure forms for making standards. Only compounds identified with 85% confidence or greater were reported. Concentrations were reported on an air-dry basis of tree tissue.



Isoeugenol **Me = Methyl group**

Poplar Extractives

Hybrid poplar trees, as members of the family *Salicaceae,* typically possess aromatic compounds among their extractives. Salicylic acid is perhaps the best-known naturally-occurring aromatic compound found in this family. Upon analysis, however, several aromatic compounds eluted from the poplar tissue of certain trees which do not usually occur in this tree species. Since the trees at the Oneida site are genetic clones of one another, were planted within three years of each other, and were sampled during the same seasons, taxonomy, age, and season do not satisfactorily explain the presence of these unusual compounds (Steele, Ronald, and Bolan, 1973; Steele and Ronald, 1973; Browning, 1967). Environmental stress, while known to change the composition of the extractives in poplars, typically does so by changing the proportion of one extractive component relative to the others. It has not been found to promote the production of new extractive chemicals (Pelah *et al.*, 1997; Picard, Chenault, and Augustin, 1994; Tholakalabavi, Zwiazek, and Thorpe, 1994; Constabel *et al.*, 2000; Gebre, Brandle, and Kuhns, 1997).

While environmental stress seemed unlikely to promote the appearance of new extractive chemicals in poplar trees, exposure to environmental pollution emerged as a plausible cause of this chemical change. Several studies have documented the ability of hybrid poplar trees to take up organic contaminants and the resultant presence of contaminant or contaminant metabolites in the tree tissue (Aitchison *et al.*, 2000; Orchard *et al.*, 2000; Newman *et al.*, 1997).

Exposure to environmental contamination has been found to add new chemicals to the extractive complement of hybrid poplar trees. Isoeugenol, a substituted phenol, does not typically occur in the *Salicaceae* family in general or hybrid poplar trees in particular. Used in the manufacture of vanillin, isoeugenol occurs naturally in cinnamon and cloves. This compound appears both in cigarette smoke and as a fragrance component in perfumes (Smith *et al.*, 2002; Rastogi *et al.*, 2001). It is a skin irritant and

has been identified as a tumorigen and a mutagen (SIRI, 2000). Its material safety data sheet identifies isoeugenol as a moderate health hazard with skin and respiratory tract irritation as the primary effects of exposure (Fisher, 2000).

Since isoeugenol does not occur naturally in poplar trees and did not appear in the tissues of any trees not growing over contamination, the ability of isoeugenol concentration in tissues to predict ground water contamination was investigated. Isoeugenol only appeared in trees known to be growing over contamination, and as such could be a metabolite of the PAHs present at the site or a potential indicator of contamination. If PAH contamination in ground water causes the presence of isoeugenol in tree tissue, then a correlation between depth-averaged ground water PAH concentration and isoeugenol concentration in tree tissue would be expected. Isoeugenol did not appear in the trees during the July 2001 sampling trip, and standards could not be ordered until after the analysis of samples collected during the March of 2001.

Methods and Computations

In order to gain more insight into the mechanism whereby the contaminant compounds were transformed, the tree tissue samples were extracted with methylene chloride and acetone and the extract analyzed by GC/MS, allowing the quantification and positive identification of tree compounds. Isoeugenol, an aromatic compound not usually found in poplar tree tissue, eluted with acetone and was quantified through the use of authentic standards (Pearl and Darling, 1970; Pearl, 1969; Pearl and Darling, 1971a). Several other compounds only eluted from trees growing over contamination. Previous research identified β-selinene, a sesquiterpene with a naphthalene-based structure as a compound unique to the trees growing over contamination at the site (Robinson, 2001). Isoeugenol was the only one of these chemicals which was commercially available and so was used in the research comparisons.

Because for most of the site trees and samplers were not in close enough proximity for direct comparisons of concentration to be made, the researchers sought a way to compare the spatial distribution of isoeugenol levels in the trees with the spatial distribution of PAH concentrations in the ground water at the site. Percentiles emerged

as the statistic most suitable for this comparison for several reasons. As a dimensionless quantity, percentiles removed the unit discrepancy in a direct comparison between ground water TPAH, measured in µg/L and tree tissue isoeugenol, measured in µg/g. Since a data point's rank within the dataset determines its percentile, the parameter allows the comparison of the relative rank of the ground water PAH concentration of a given location with the relative rank of the isoeugenol concentration in the tree tissue for that same location.

ArcView GIS Spatial Analyst™ was used to compare the tree and ground water percentiles. All of the computed percentiles were associated with a specific ground water multi-level sampler or tree location. First two point themes were created: one containing the calculated percentiles for the sampled trees, one the percentiles for the multi-level samplers. Using Spatial Analyst™, percentile grid themes were interpolated for both the tree tissue and the ground water for the area of the site enclosed by the respective point themes. Using the Map Calculator™, these extents of these two themes were then modified to contain only the area fully enclosed by both themes. The new themes created by this latest operation now covered precisely the same area. Next, the absolute value of the percentile differences between the themes, or percentile absolute difference (PAD) was computed such that:

$$\mathbf{PAD} = |\mathbf{P}_{\mathbf{Tree\text{-}Iso}} - \mathbf{P}_{\mathbf{GW\text{-}TPAH}}| \qquad (2)$$

Next, the value of the difference, or percentile difference (PD) between the two congruent percentiles themes was computed and added as a new grid theme to the view, using the following formula:

$$\mathbf{PD} = |\mathbf{P}_{\mathbf{Tree\text{-}Iso}} - \mathbf{P}_{\mathbf{GW\text{-}TPAH}}| \qquad (3)$$

where,

$\mathbf{P}_{\mathbf{Tree\text{-}Iso}}$ = Grid theme representing the percentiles of isoeugenol concentration (µg/g) in tree tissue

$\mathbf{P_{GW\text{-}TPAH}}$ = Grid theme representing the percentile values of TPAH concentration in ground water (μg/L)

From the sequences above, two sets of maps were created. One compared bark isoeugenol concentration to depth-averaged ground water total PAH concentration, while the other compared twig isoeugenol concentration with depth-averaged ground water total PAH concentration.

Results

As a means to investigate the role of direct uptake by the trees in the remediation of creosote at the Oneida site, isoeugenol concentrations in bark and twig tissue were compared with depth-averaged total PAH concentrations in ground water collected within the study area in March of 2002. Separate comparisons were made for each tissue type. The comparisons used GIS to compare the spatial distribution of depth-averaged ground water total PAH concentration with the spatial distribution of tree tissue isoeugenol concentration over the area of interest, as shown in Figures 3-3 and 3-4.

Depth-averaged ground water concentrations allowed a single measurement to represent PAH contamination at each multi-level sampler location, similar to the way that one measurement represented tree contamination in a given tissue type. Because the tree roots draw water from every depth that they penetrate, and depth-averaged concentrations provide a reasonable estimate of PAH concentration experienced by the trees, especially since the roots had likely reached bedrock by March 2001 (Robinson, 2001). Table 3-1 shows isoeugenol concentrations by tissue type for each of the sampled trees, while Table 3-2 shows TPAH concentrations for each of the multi-level samplers.

Table 3-1: February-March 2002 Tree Isoeugenol Concentrations

Tree Name	Bark Isoeugenol (ppm)	Twig Isoeugenol (ppm)
R11T8	9.2	non-detect
R13T10	13.71	7.66
R3T9	10.54	13.1
R5T9	7.52	non-detect
R6T28	non-detect	non-detect
R8T14	11.57	9.19
R8T7	13.35	9.45
R9T1	8.16	11.82

Table 3-2: February-March 2002 Multilevel Sampler Total PAH Concentrations

Sampler I.D.	Depth-Avg PAH µg/L
ML-1	26.22
ML-2	94.73
ML-3	72.17
ML-4	198.67
ML-5	0.32
ML-7	5925.77
ML-8	225.50
ML-9	9.72
ML-10	9.90
ML-11	281.25
ML-12	11.82
ML-13	190.92
ML-16	696.63
ML-17	42.55
ML-19	2.15
ML-20	5.98
ML-21	2.31
ML-22	640.30
ML-23	3.36
ML-24	70.40
ML-25	2615.60
ML-26	357.63

Figure 3-3: Percentile Absolute Difference Comparison of Bark Isoeugenol Concentration with Depth Averaged Ground Water TPAH

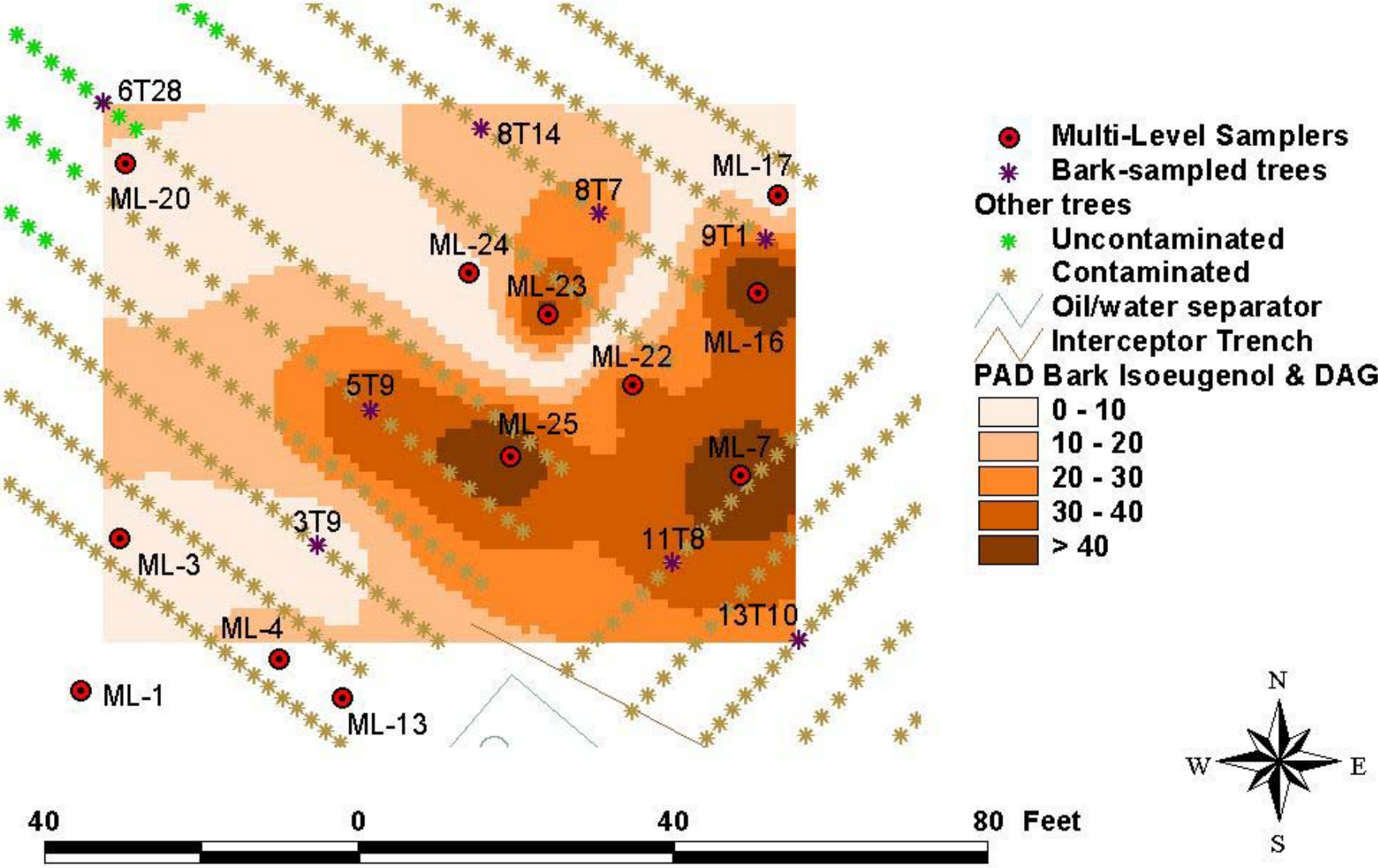
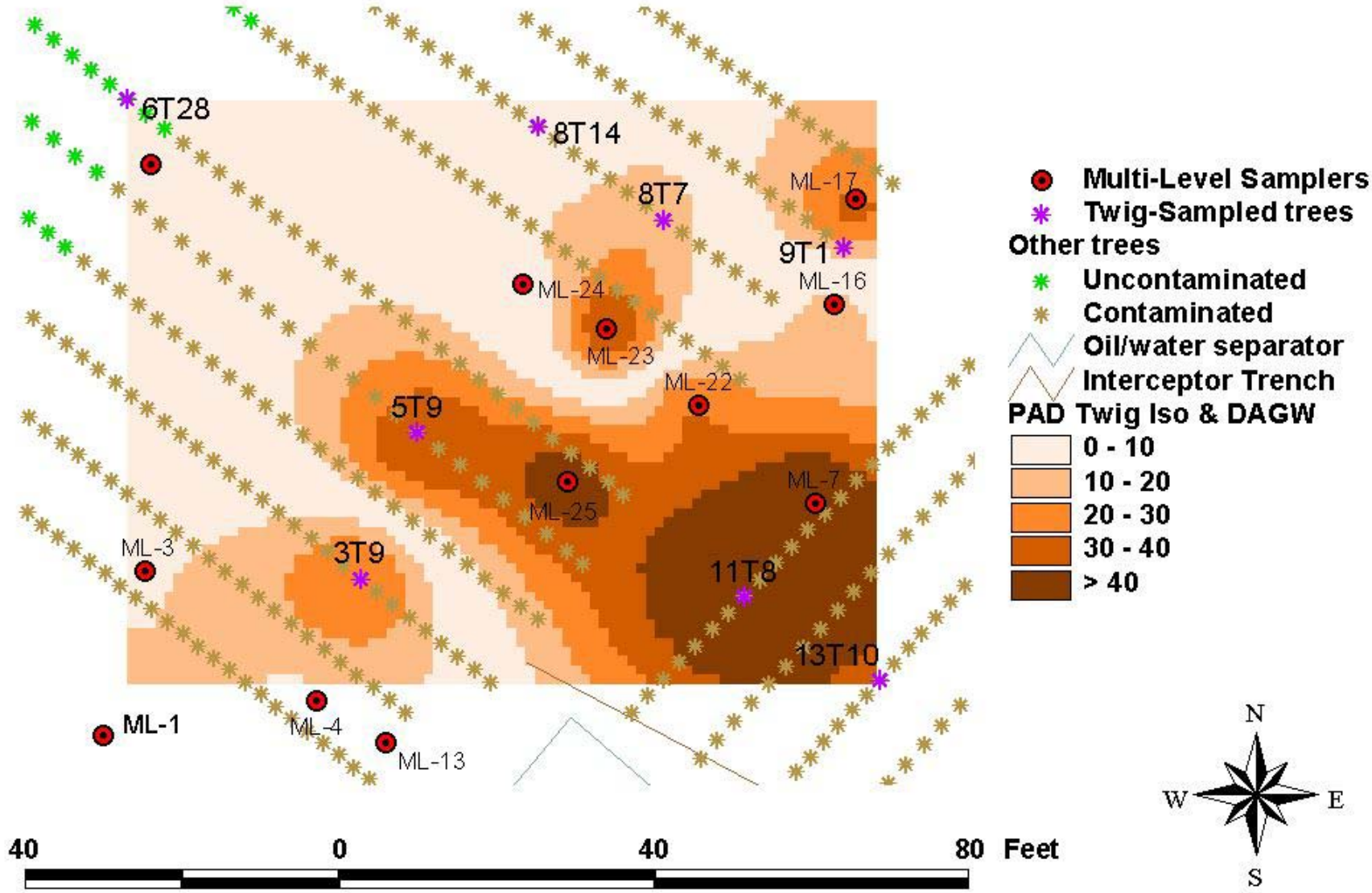


Figure 3-4: Percentile Absolute Difference Comparison of Twig Isoeugenol Concentration with Depth-Averaged Ground Water Total PAH



Discussion

Based on the spatial percentile comparisons shown in Figures 4-3 and 4-4 and summarized by Tables 3-3 and 3-4, both bark and twig tissue isoeugenol percentiles show good agreement with depth-averaged ground water total PAH percentiles. The bark tissue percentiles fall within 20 percentiles of ground water total PAH concentrations in 60% of the study area. The twig tissue shows slightly better agreement, with 67% of the study area differing from ground water by twenty percentiles or less.

As shown in Table 3-3, the bark tissue tends to predict moderate levels of contamination most accurately, with 88% of the ground water that falls between the 40^{th} and 60^{th} percentiles differing from tree isoeugenol percentiles by 20 percentiles or less. Areas of low ground water contamination had the highest proportion of agreement within 10 percentiles between tree isoeugenol and depth-averaged ground water total PAH. Bark predicted high levels of ground water contamination at a much lower rate than either low or moderate levels of ground water contamination. Only 21% of the area containing highly contaminated ground water was predicted within 20 percentiles by the bark tissue, with 7.4 % of these areas of highly contaminated ground water predicted within 10 percentiles.

Twig tissue predictive ability followed the same pattern as that of bark tissue, as shown in Table 3-4, predicting moderate ground water contamination most accurately at the 20-percentile agreement level and low ground water contamination most accurately at the 10-percentile agreement level. For areas of moderately contaminated ground water, twig isoeugenol measurements agreed with 20 percentiles 90% of the time and within 10 percentiles 63% of the time, as shown in Table 3-4. For areas of low ground water contamination, twig isoeugenol measurements fell within 20 percentiles of ground water measurements 82% of the time and within 10 percentiles 76% of the time. Like bark, twig isoeugenol percentiles predicted areas of highly contaminated ground water much less accurately than all other levels. Only 35% of the most contaminated ground water percentiles agreed fell within 20 percentiles of the twig isoeugenol measurements. Similarly, only 15% of the areas containing the most contaminated ground water also contained trees with the highest concentration of isoeugenol. The nature of the trend between tree isoeugenol concentration and ground water TPAH concentration suggests one of three alternatives: the chemical stress to the trees of growing in contamination causes them to

produce isoeugenol, the trees themselves take up creosote compounds and convert them into isoeugenol, or the trees take up isoeugenol as a microbial metabolite of creosote degradation. In either of these cases, isoeugenol could serve as a contaminant marker in tree tissue.

Table 3-3: February-March 2002 Bark Isoeugenol vs. Depth-Averaged Ground Water TPAH Areal Statistics

		February-March 2002 DAGW vs Bark Isoeugenol			
Percentile Range	Range Area, sq. ft.	Area within 20 Percentiles, sq. ft.	Zone % within 20 Percentiles	Zone area within 10 Percentiles	Zone % within 10 Percentiles
0 - 40	598.9	481.6	80.4	409.8	68.4
40 - 60	3549.2	3126.9	88.1	1720.8	48.5
60 - 100	2893.7	607.2	21.0	215.3	7.4
total	7041.7	4215.7	59.9	2345.9	33.3

Table 3-4: February-March 2002 Twig Isoeugenol vs. DAGW TPAH Areal Statistics

		February-March 2002 DAGW vs Twig Isoeugenol			
Percentile Range	Range Area, sq. ft.	Area within 20 Percentiles, sq. ft.	Zone % within 20 Percentiles	Zone area within 10 Percentiles	Zone % within 10 Percentiles
0 - 40	598.9	488.5	81.6	456.8	76.3
40 - 60	3549.2	3195.9	90.0	2242.4	63.2
60 - 100	2893.7	1004.6	34.7	437.4	15.1
total	7041.7	4689.0	66.6	3136.6	44.5

In studying contaminant uptake by poplar trees, Schnoor has noted that a contaminant must first sorb to the roots and then be transported into the tree tissue (Schnoor *et al.*, 1995). As with soil, an equilibrium would likely be established between the concentration of contaminant sorbed to the roots and the concentration dissolved in the ground water (Burken and Schnoor, 1998). The concentration of contaminant in the tree tissue would be limited by the maximum quantity of contaminant that can sorb to the root surfaces.

Evaluation of the Method

Upon obtaining the results, it was necessary to evaluate the method based on the stated method goals. The first goal of the method, that of functioning on the microscale, was verifiably attained, since the trees were sampled over three seasons and no impairment of the growth of the sampled trees was apparent when compared to other trees at the site.

The use of a GC/MS provided a method for reliable identification of compounds, thus fulfilling the second analytical goal. Several of the same chemicals were identified in the samples from all three time periods with greater than 85% confidence. A significant number of these chemicals are usually present in poplar trees (Pearl and Darling, 1977; Abramovitch and Koleoso, 1966; Hossfeld and Kaufert, 1957; Pearl and Darling, 1971a; Pearl and Darling, 1971b; Browning, 1967; Sentsov *et al.*, 1997). Though spectral identification is a good method of identification, it is not infallible. Isoeugenol was consistently identified with greater than 90% confidence and the compound identified in the tree tissue coeluted with authentic standards. However, 2,3-dihydrobenzofuran, for which a standard was ordered, eluted two minutes after the standard. Though identified in tree tissue with greater than 85% confidence, apparently the GC/MS had misidentified an unknown chemical whose spectral signature was more similar to 2,3-dihydrobenzofuran than any other chemical in its library. Rarely, the MS would misidentify the spectral signature of authentic standards. For identification of any compound identified in tree tissue to be certain, its spectral signature and elution time must match those of genuine standards.

The use of authentic standards allowed the quantification of the potential degree of contamination in the ground water by measuring the concentration of isoeugenol in tree tissue. Though most of the trees sampled in the winter of 2002 contained concentrations of isoeugenol that could be measured by the use of standards, the samples had a tendency to regain water during the evaporation of methylene chloride such that the mass of dry sample in the tube after evaporation was more than the dry mass present before extraction in methylene chloride. Consequently, the isoeugenol concentration in tree tissue was calculated based on the dry sample mass present before extraction in dichloromethane, rather than the corresponding mass present

before extraction in acetone, the solvent in which isoeugenol elutes, potentially introducing error into the quantification.

How well the chemical method met the next goal, precision, remains uncertain. Extraction efficiencies, a common metric for this parameter, could not be performed. Isoeugenol appears to degrade too quickly for the same aliquot to be processed more than once. In the course of a single sample run, the detected values of isoeugenol standards prepared to the same concentration would decrease over time. Aliquots from the same 10 mg/L prepared standard might measure 10 mg/L at the beginning of the run and 7 mg/L at the end. After a week, the standards used to create a standard curve for that run would sometimes appear as non-detects. However, by averaging the response of standards placed at the beginning and end of a sample run, standard curves consistently yielded a correlation coefficient of 97% or greater.

Through the use of pH-neutral, non-oxidizing solvents, low-temperature processing, and refrigerated or frozen sample storage, it was possible to avoid subjecting the samples to conditions that promote chemical changes in wood. The consistent identification of chemicals identified in the literature as components of poplar wood over the full course of sample processing speaks well to the fulfillment of this sampling goal. Additionally, the sets of chemicals identified during winter sampling were very similar to each other and much less similar to the chemicals appearing in samples collected during the summer. This pattern of agreement concurs with wood chemistry literature which states that the composition of the mixture of extractable chemicals present in tree tissue varies with season (Steele and Ronald, 1973).

The last goal, protecting the safety of the operator, was attained by avoiding accidental flash by maintaining both samples and solvents at relatively low temperatures and by avoiding solvents, such as benzene or toluene, which presented an unnecessary health hazard.

Summary

In the course of research, a microscale chemical method was developed that was suitable for the identification and quantification of potential PAH metabolites in woody tissue. Functioning on the microscale makes the method appropriate for studying chemical changes in living trees engaged in phytoremediation over time. Through avoiding the more toxic solvents and maintaining the solvents at room temperature or below, the procedure is safe enough to be

performed by those who possess standard laboratory safety training but are not professional chemists. By applying their analytical method, it was possible to observe a strong trend relating the concentration of isoeugenol in bark and twig tissue and the concentration of total PAH in ground water.

Since isoeugenol is not usually present in poplar tree tissue, its presence is likely a result of the uptake of PAH contaminants or microbial metabolites. Though quantification of isoeugenol was achieved through the method, quantitative results should be interpreted with caution due to the absorbance of water during removal of the first solvent.

The results from the spatial analyses comparing bark and twig tissue isoeugenol percentiles with ground water total PAH concentration percentiles reveal several trends. Overall, twig isoeugenol percentiles predict ground water contamination more accurately than bark isoeugenol concentrations. Tree tissue also tends to predict low and moderate levels of ground water contamination much more closely than high ground water contamination levels. These findings are consistent with a natural system in which isoeugenol, a PAH microbial metabolite, is taken up by the trees. The results suggest that in order to mitigate isoeugenol's toxic effects, the trees exclude the chemical from uptake after a certain critical concentration is reached. Alternately, the findings could also suggest that isoeugenol's soil/water/ tree tissue partitioning properties limit its accumulation in tree tissue, so that regardless of isoeugenol's concentration in ground water, the chemical's concentration in the trees would never exceed some chemically determined limit.

Considerations for Future Research

Future researchers may wish to consider several factors when undertaking the chemical analysis of woody tissue in conjunction with phytoremediation. Standards for the chemicals of interest were either unavailable or prohibitively expensive. Frequently the pure compounds themselves were unavailable. In this study, it was found that standards had to be prepared for each sample run, as they apparently became less accurate after storage for a week. Separate standards had to be prepared for 2,3-dihydrobenzofuran and isoeugenol, since 2,3-dihydrobenzofuran response was so much greater than that of isoeugenol that the latter's response was completely obscured below a concentration of 10 mg/L. Another consideration for

the method is that though samples may be run in batch, the processing of an entire batch through both solvents takes approximately a week and a half. Additionally, future researchers may wish to consider drying the samples under nitrogen or extracting first in the solvent for which standards are available in order to alleviate the problem of water absorption from the atmosphere.

As more field studies of phytoremediation are initiated, it becomes increasingly important to be able to measure exactly how the trees are contributing to the disappearance of the parent pollutant compounds. Currently, there are several groups of researchers using as many different methods to measure phytoremediation (Orchard *et al.*, 2000b; Burken and Schnoor, 1998; Newman *et al.*, 1997). A standardized method of measuring phytoremediation would allow results from different researchers to be compared more easily, promote regulatory acceptance of the remediation technique, aid in the development of predictive models to be used during full-scale implementation of phytoremediation, and provide researchers with another tool by which to monitor the progress of phytoremediation.

Currently, the different research methods employed by investigators makes it difficult to draw general conclusions about phytoremediation based on the results from any given set of experiments (Orchard *et al.*, 2000b). The differences in analytical technique may obscure the effects of important variables such as humidity, temperature, soil type, soil pH, fraction of organic matter, and soil moisture in the process of phytoremediation. A standard protocol on how to measure the extractable components in tree tissue would greatly aid in establishing the effectiveness of phytoremediation on a broad geographic scale, and in the elucidation of degradation pathways. These two advances would speed the development of site-specific remediation options and allow environmental professionals to have a better understanding of the fate of contaminants and their metabolites, leading to more accurate risk assessments of phytoremediation which take into account potential risks to the food chain arising from interactions among metabolites, wood-boring insects, and predatory animals (Burken and Schnoor, 1997). An improved ability to evaluate the risks and benefits of phytoremediation would greatly aid in the technology's regulatory acceptance. While this method does not purport to be a final standard, it is hoped that its dissemination will aid in the development of a standard procedure for the monitoring of phytoremediation.

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Chapter 4: Spatial Relationships Between Hybrid Poplar Tree Extractives and Ground Water Contamination

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Abstract

In this study the impact of phytoremediation on poplar tree phytochemistry at an inactive railroad tie yard was investigated. Following the discovery of creosote contamination at a site in Oneida, Tennessee, over 1000 hybrid poplar trees were planted to aid in contaminant remediation. Spatial relationships between aromatic compounds in the trees and ground water contamination at the site were investigated in order to aid in understanding the mechanism of contaminant remediation at the site and to determine if tree tissue could be used as a predictor of ground water contamination. None of the parent PAHs appeared in the tree tissue, but many other aromatic compounds were present. The aromatic content of the tree tissue was quantified based on the number of different aromatic compounds detected in extracted bud, bark, and twig tissue.

Ground water contamination was quantified based on the aqueous concentration of total polycyclic aromatic hydrocarbons (TPAH) in ground water samples. The number of unique aromatic compounds in tree tissue could not be compared directly to ground water TPAH concentrations due to the different distributions of the two datasets, so the number of aromatic compounds in the trees was compared to the ground water TPAH concentration on a percentile basis.

Percentiles were calculated for each tree and ground water multi-level sampler at the site, based on number of aromatic compounds or TPAH concentration, respectively. From the percentiles at these point locations, percentile grid themes were interpolated for the expanse of the site completely enclosed by the multilevel samplers and sampled trees. Then, a new grid was created by subtracting the ground water TPAH percentile grid from the tree aromatic compound percentile grid. A geographic information system was used to investigate whether a spatial

relationship existed between the number of aromatic compounds in the trees and the extent of ground water contamination or the ground water plume.

The number of tree tissue aromatic compounds was compared with ground water TPAH concentration during three sampling events: March 2001, July 2001, and February-March 2002. It was found that the percentiles of aromatics in the trees, based on number of aromatics, usually agreed within 20 percentiles of TPAH concentration aromatics. This finding suggests that either that trees take up TPAH directly, take up microbial metabolites of TPAH from the ground water, or both, enabling poplar trees to act as indicators of creosote contamination.

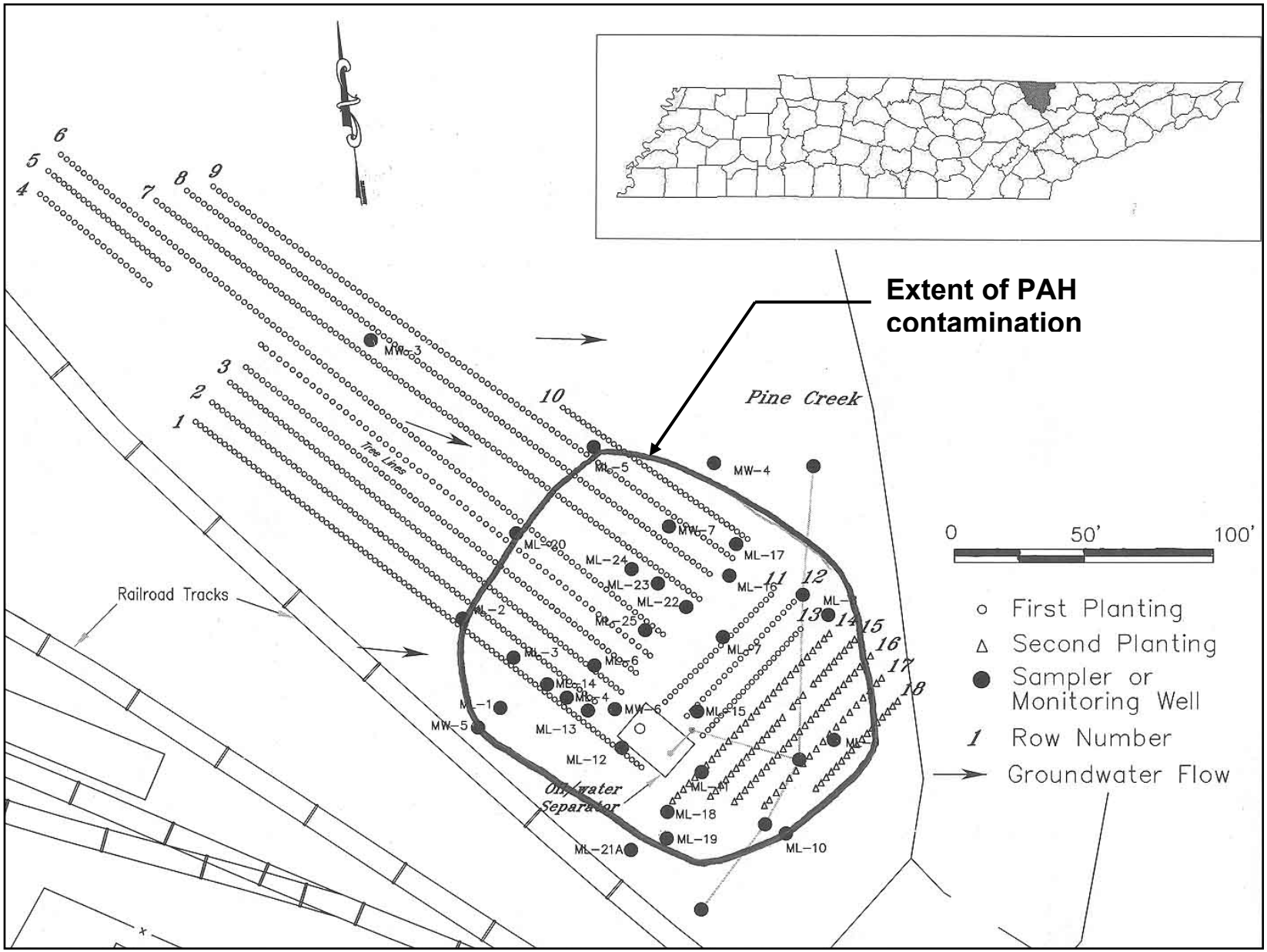
Introduction

In 1997, a creosote-contaminated railroad tie yard in Oneida, Tennessee needed remediation. The site location appears in Figure 4-1. A wood preservative and waterproofing agent, creosote, contaminated the site's soil and ground water. Though sometimes referred to as a wood-preserving chemical, creosote is actually a mixture of over 300 compounds (Dementi and Wasti, 1981; Mueller *et al.*, 1989). Creosote consists primarily of polycyclic aromatic hydrocarbons (PAH), and tends to exist as a dense, non-aqueous phase liquid (DNAPL) overlying bedrock at the site. Due to the aromatic nature of the majority of creosote's components, the mixture tends to be difficult or expensive to treat by conventional methods, such as pump-and-treat or incineration (Glass, 2000).

In order to remediate the site in the most economical way possible, an environmental consulting firm recommended the use of phytoremediation. Over 1000 hybrid poplar trees were planted, some directly over the contamination in order to promote degradation of the contaminant, others beyond the periphery of the plume to take up contaminated ground water and prevent its migration offsite.

Several characteristics of poplar trees make them particularly suitable for the remediation of ground water. Their phreatophytic nature, requiring them to take in large volumes of water, enables them to draw water from below the water table and provide hydraulic control. Hybrid poplars also grow quickly and have a reputation for hardiness. Typically, they aid in the remediation of contamination in one or more of four ways: stabilization and erosion control, hydraulic control, rhizosphere effects, and direct uptake (Schnoor, 1997).

Figure 4-1: Site Layout



Native to the southeastern United States, the trees are hardy, flood tolerant, fast-growing, and easy to propagate. Known to tolerate organic contamination, the trees have been documented to take up organic contaminants such as trichloroethylene and 1,4-dioxane (Orchard *et al.*, 2000; Burken and Schnoor, 1998).

After the tree planting, monitoring of the site for evidence of soil and ground water remediation began. Figure 4-1 shows the locations of the ground water multi-level samplers. Over time, the presence of the trees seemed to enhance contaminant reduction, but the specific mechanisms through which this enhancement took place remained unelucidated. Though it had been shown that PAH degradation was promoted via rhizosphere effects, the impact of direct uptake was unknown (Robinson, 2001). Soil bacteria from the site, when grown in culture, demonstrated the ability to utilize various polycyclic aromatic hydrocarbons as a sole carbon source, but the interactions between the trees and the soil bacteria are not well understood. The objectives of this research were to (1) determine if bud, bark, and twig tissue differed in their ability to predict ground water contamination, and (2) determine if a spatial correlation existed between the aromatic compounds in the tree tissue and the ground water total PAH plume.

In March of 2001, four years after the tree planting, tree tissue samples were collected and analyzed to aid in determining the mechanism of remediation and to attempt to quantify PAHs or their metabolites in the tree tissue. Solvent extractions were performed on the tree tissue to determine if ground water contaminants or similar compounds were present in the trees and to quantify these contaminants in the tree tissue. None of the parent PAHs appeared in the tree tissue, but many aromatic compounds did. The aromatic content of the tree tissue was quantified based on the number of unique aromatic compounds detected in extracted bud, bark, and twig tissue.

Spatial relationships between aromatic compounds in the trees and the aqueous concentration of total polycyclic aromatic hydrocarbons (TPAH) in well samples were investigated in order to aid in understanding the mechanism of contaminant remediation at the site. A correlation between the concentration of TPAH in ground water and the number of different aromatic compounds detected in tree tissue would imply that phytoremediation plays a significant role in the amelioration of creosote contamination at the site, and that the trees either take up PAHs or their microbial metabolites from the ground water.

Site History

Over many years of wood-preserving activity, a railway tie yard in Oneida, Tennessee became contaminated with creosote. The railway ties were pressure impregnated with creosote, a wood preservative, to waterproof them and extend their usable life. As a result of the treatment process or chemical storage procedures, creosote entered the soil and ground water at the site, forming a dense non-aqueous phase liquid (DNAPL) layer overlying the bedrock.

In 1990 the U. S. Army Corps of Engineers discovered creosote contamination at the site, and the Tennessee Department of Health and the Environment ordered the property owner to initiate remedial action. In 1997 a phytoremediation system consisting of over 1000 hybrid poplar trees was installed at the site, and soil and ground water monitoring for evidence of remediation began. Over time, the trees grew without inhibition, and creosote levels decreased in the site soil and ground water (Robinson, 2001).

In March of 2001, tree tissue sampling began in an attempt to gain further insight into the PAH degradation mechanisms occurring at the site. Spatial relationships between aromatic compounds in the trees and the aqueous concentration of total polycyclic aromatic hydrocarbons (TPAH) in well samples were investigated in order to aid in understanding the mechanism of contaminant remediation at the site. A correlation between the concentration of TPAH in ground water and the number of different aromatic compounds detected in tree tissue would imply that phytoremediation plays a significant role in the amelioration of creosote contamination at the site, and that the trees either take up PAHs or their microbial metabolites from the ground water. A tree tissue sampling plan was developed to ensure that the number of samples collected would be sufficient to identify spatial trends.

Sampling Plan

Based on the standard deviation of total PAH concentrations from site ground water samples, the number of trees needed to provide valid results for a significance level, α, of 0.10, within a range of 1000 ppb was selected. The trees were divided into two strata based on previous ground water data: one for those growing over areas thought contaminated, the other for

trees growing over areas thought to be uncontaminated. It was assumed that no spatial correlation existed between samples independent of ground water contamination level. Minimum necessary sample size, n, was calculated after Gilbert (1987):

$$n = \frac{z_{1-\alpha/2}^2 \sum_{h=1}^{L} W_h s_h^2 / d^2}{1 + z_{1-\alpha/2}^2 \sum_{h=1}^{L} W_h s_h^2 / d^2 N} \qquad (1)$$

where

z = cumulative probability density function for standard normal distribution

s_h = standard deviation of a given sample stratum h

$\alpha = 0.10$, significance level

d= 1000 ppb, minimum detectable difference

N = total number of population units (maximum number of samples that could be taken)

L = number of strata

N_i = number of population units in stratum i, $N = \sum_{i=1}^{L} N_i$

$W_i = N_i/N$, stratum weight

n = total number of samples taken

n_i = number of samples taken from stratum i

Based on the results from Equation (1), a minimum of 25 trees needed to be sampled, 19 in areas identified as contaminated, and 6 in uncontaminated areas. A random number generator was employed to help ensure complete coverage of the site. After the initial tree sampling trip in March 2001, the number of trees needed to be sampled was recalculated based on the new standard deviation of the number of aromatic compounds found in the tissues. According to the new calculation, only 8 trees needed to be sampled to provide statistically significant results for an alpha of 0.10, 6 in growing over contamination and 2 growing in uncontaminated areas.

Poplar Extractives

Wood chemists define the extractives as the class of chemicals within wood which pH-neutral solvents can remove. Hybrid poplar trees, as members of the family *Salicaceae*, typically contain a number of aromatic compounds among their extractives. Salicylic acid is perhaps the best-known naturally occurring aromatic compound found in this family (Tomaszewski, 1960). Upon analysis, several aromatic compounds eluted from the poplar tissue of certain trees which do not usually occur in this tree species. Since the trees at the Oneida site are genetic clones of one another, were planted within three years of each other, and were sampled during the same seasons, taxonomy, age, and season do not satisfactorily explain the presence of these unusual compounds (Steele, Ronald, and Bolan, 1973; Steele and Ronald, 1973; Browning, 1967). Environmental stress, while known to change the composition of the extractives in poplars, typically does so by changing the proportion of one extractive component relative to the others. It has not been found to promote the production of new extractive chemicals (Pelah et al., 1997; Picard, Chenault, and Augustin, 1994; Tholakalabavi, Zwiazek, and Thorpe, 1994; Constabel et al., 2000; Gebre, Brandle, and Kuhns, 1997).

While environmental stress seemed unlikely to promote the appearance of new extractive chemicals in poplar trees, exposure to creosote emerged as a plausible cause of this chemical change. Several studies have documented the ability of hybrid poplar trees to take up organic contaminants and the resultant presence of contaminant or contaminant metabolites in the tree tissue (Aitchison *et al.*, 2000; Orchard *et al.*, 2000; Newman *et al.*, 1997). Thus, exposure to environmental contamination has been found to add new chemicals to the extractive complement of hybrid poplar trees.

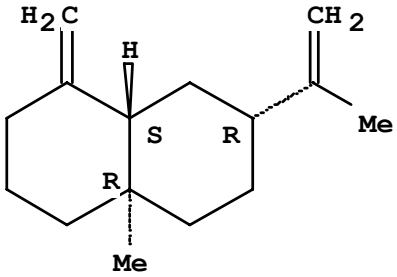
In order to investigate the chemical composition of the poplar tree tissue, samples were extracted with methylene chloride and acetone and the extract analyzed by GC/MS, allowing the quantification and positive identification of tree compounds. Details of the extraction procedure can be found elsewhere (Waters, 2003). Several naphthalenes, naphthalene-derivatives, phenols and other aromatic compounds appeared in the tree tissue. Isoeugenol, a substituted phenol not usually found in poplar tree tissue, eluted with acetone and was quantified through the use of authentic standards (Pearl and Darling, 1970; Pearl, 1969; Pearl and Darling, 1971). The results of the isoeugenol analysis appear elsewhere (Waters, 2003).

Among the aromatic extractives that eluted from the poplar bud, bark, and twig tissue, certain chemicals were classified as primary aromatics. A chemical's classification depended primarily on its structure, and primary aromatics included naphthalenes, naphthalene-derivatives, phenols, azulenes, and aromatic acids, but excluded aromatic ketones and phthalates. To be classified as a primary aromatic compound, a chemical required at least one benzene or azulene ring that did not contain a ketone as a substituent or a phthalate structure.

Excluded aromatics consisted of compounds with a benzene or azulene parent that contained a ketone as a substituent or were phthalates. Ketones were excluded from consideration in the quantification due to the large number of aromatic ketones naturally present in members of the family *Salicaceae*, to which poplars belong, while phthalates were excluded due to their ubiquitous nature and potential presence in the protective gloves used during chemical procedures (Sentsov *et al.*, 1997; Tsumura *et al.*, 2002). Example structures of both primary and excluded aromatics are shown.

Figure 4-2: Primary Aromatic Example Structures

Primary Aromatic: Naphthalene-derivative example structure

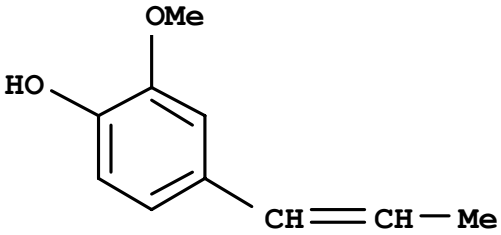


Me = Methyl group

Chemical Name: Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, (4aR,7R,8aS)- (9CI)

Alternate Names: β-Selinene (7CI); (+)-β-Selinene; β-Eudesmene; Selina-4(14),11-diene

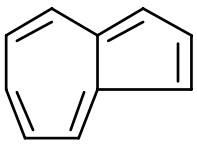
Primary Aromatic: Phenol Example Structure



Me = Methyl group

Chemical Name: Phenol, 2-methoxy-4-(1-propenyl)- (9CI)
Alternate Name: Isoeugenol

Primary Aromatic: Azulene Parent Structure

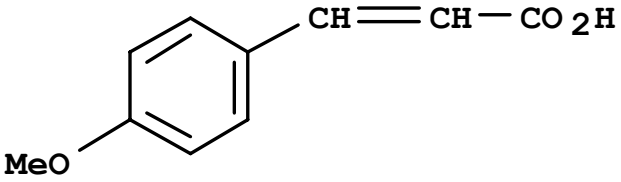


Chemical Name: Azulene

Azulene, an isomer of naphthalene, contains one 7-membered ring and one 5-membered ring.

Figure 4-3: Primary and Excluded Aromatic Example Structures

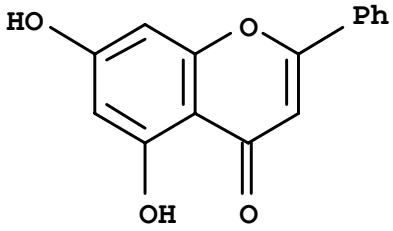
Primary Aromatic: Aromatic Acid Example Structure



Me = Methyl group

Chemical Name: 2-Propenoic acid, 3-(4-methoxyphenyl)- (9CI)
Alternate Names: Cinnamic acid, p-methoxy- (6CI, 8CI); O-Methyl-p-coumaric acid;

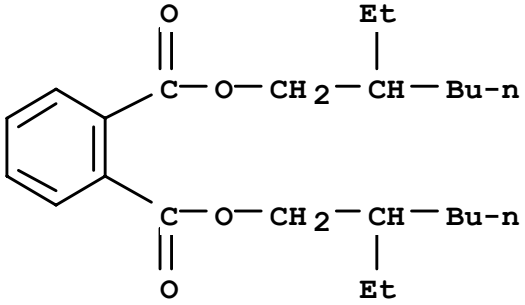
Excluded Aromatic: Aromatic Ketone Example Structure



Ph = Phenyl group

Chemical Name: 4H-1-Benzopyran-4-one, 5,7-dihydroxy-2-phenyl- (9CI)
Alternate Name: Chrysin; 5,7-Dihydroxyflavone

Excluded Aromatic: Phthalate Example Structure



Et = Ethyl group
Bu = Butyl group
n = normal (straight chain configuration)

Chemical Name: Bis (2-ethylhexyl)phthalate
Alternate Name: Bis(2-ethylhexyl) 1,2-benzenedicarboxylate

Methods and Computations

For most of the primary aromatics, neither standards nor the pure compounds themselves were available for purchase, so quantification was accomplished by counting the number of aromatics in the tree tissue. Subsequent to this quantification, a method was needed to compare levels of aromatic compounds in the trees with PAH concentrations in the ground water. The distribution of aromatics in the trees was highly symmetric, almost normal, while the distribution of PAH concentrations in the ground water was highly asymmetric, almost lognormal; therefore, tree aromatics were compared to ground water concentrations on a percentile basis.

Due to the divergent distributions of the data, the usual statistical comparisons, such as t-tests or non-parametric tests, were inappropriate. Even nonparametric statistical tests require that both data sets come from the same distribution. Calculation of percentiles, however, is a monotonic transformation. Unlike t- or z-statistics, a percentile value is equally valid whether calculated for original or log-transformed data. Both t-statistics and z-statistics rely on the means of associated datasets. In contrast, percentiles rely only on rank or order of the data, and describe the relative standing of a given data point within its dataset (McClave, Dietrich, and Sincich, 1997). This relative standing remains the same whether or not the data are log-transformed. As such, the percentile may be used to make valid comparisons between data of different distributions (Gallagher, 2001). The use of percentiles allowed the comparison of a given spatial location's relative standing within the ground water TPAH concentration dataset with the relative standing of that same spatial location within the tree tissue number of aromatic compounds dataset.

The use of geographic information systems (GIS) made possible the investigation of spatial trends in the data. In GIS, data has not only a value but also an associated coordinate location. The system unites the display and location functionalities of a map with the ability to update, analyze, and perform calculations with spatial data. GIS stores spatial data in datasets called themes. Themes are collections of objects of known coordinate location and about which the same attributes are known. The objects in a theme may be grid cells holding a value that represents a single property, such as temperature, or features on the earth, such as rivers or roads.

Themes used in this analysis were of two basic types: vector and grid. Analysts typically use vector data to represent physical objects that occupy discrete locations. Vector data comes in three basic types: points, lines, and polygons. Point themes typically represent objects whose locations but not shape or size enter into the analysis. Examples of point themes include locations of cities on a world map or stops on a bus route. Lines usually represent objects whose locations and lengths but not widths are important properties for the analysis. Typical line themes are rivers and roads. Polygons usually represent objects whose location, length, width, and shape all play an important part in the analysis. Examples of objects often represented as polygons are counties and soil types.

The second type of GIS theme, the grid theme, is used to represent properties that are continuous over an area, such as temperature or elevation. Each cell comprising a grid theme holds an associated value. Grid themes can be created by interpolation of point theme values. For example, if the user has temperature data from a set of probes at a site, and creates a point theme which stores each probe's location and temperature reading, a grid theme can be created by interpolation which contains the temperature of every coordinate location within the area of interest.

The analysis performed during this study made use of two types of themes: point themes and grid themes. Point themes represented the trees and multilevel samplers, while grid themes represented percentiles and percentile differences. Data associated with tree point themes included the trees' identifiers, coordinate location, number of aromatic compounds, and percentile ranking compared with all the trees in the data set. Data associated with the multilevel sampler theme included the sampler's name, depth-averaged ground water TPAH concentration, shallow ground water TPAH concentration, and percentile ranking for both the shallow and depth-averaged ground water collected from the sampler. Percentile grids were computed from the percentile ranking data in the tree and multilevel sampler point themes, respectively.

ArcView GIS Spatial Analyst™ was used to compare the tree and ground water percentiles. All of the computed percentiles were associated with a specific ground water multi-level sampler or tree location, and for most of the site, trees and samplers were not in close enough proximity for the percentiles to be compared directly. First two point themes were created: one containing the calculated percentiles for the number of aromatic compounds in the sampled trees, the other containing the percentiles for the TPAH concentration for the ground water in the multi-level samplers. Using Spatial Analyst™, percentile grid themes were interpolated for both the tree tissue and the ground water for the area of the site enclosed by the respective point themes. Using the Map Calculator™, these extents of these two themes were then modified to contain only the area fully enclosed by both themes. The new themes created by this latest operation now covered precisely the same area.

The inverse distance weighted (IDW) interpolation technique was used since it predicted the point values most accurately when tested against the other interpolation schemes. In the test, values of ground water TPAH concentration were interpolated over the area of interest after removing one of the wells from influencing the interpolation. The interpolation scheme that best predicted this previously-known value was then used for all further interpolations.

In order to compare the percentiles of aromatics found in tree tissue with the percentiles of TPAH concentration found in the ground water, a percentile difference theme and its absolute value theme were computed as shown. A theme containing the absolute value of the percentile differences, or percentile absolute difference (PAD) theme was computed by the following formula:

$$\mathbf{PAD} = |P_{\text{TREE-AROM}} - P_{\text{GW-TPAH}}| \qquad (2)$$

Next, the value of the difference between the two congruent percentiles themes, the percentile difference (PD) was computed and added as a new grid theme to the view, using the following formula:

$$\mathbf{PD} = P_{\text{TREE-AROM}} - P_{\text{GW-TPAH}} \qquad (3)$$

where,

$\mathbf{P_{Tree\text{-}AROM}}$ = Grid theme representing the percentiles of the number of aromatic compounds in tree tissue

$\mathbf{P_{GW\text{-}TPAH}}$ = Grid theme representing the percentile values of TPAH concentration in ground water (μg/L)

From the sequences above, four maps for each type of tree tissue (bud, bark and twig) for the methylene chloride extractives for the winter 2001 time period were created. One compared primary aromatics to shallow ground water, the second primary aromatics to depth-averaged ground water, the third total aromatics to shallow ground water, and the fourth total aromatics to depth-averaged ground water. During the summer 2001 and winter 2002 time periods the only maps created compared ground water with twig aromatic percentiles.

Results

For the March 2001 sampling event, 12 different percentile absolute difference comparisons were made: primary aromatics (PA) and total aromatics (TA) of each tree tissue type (bud, bark, and twig) were compared with shallow ground water (SGW) and depth-averaged ground water (DAGW) TPAH concentrations. The comparison table is shown in Table 4-1. A star indicates that a comparison was made for a given tissue type, extractive type, and ground water type combination.

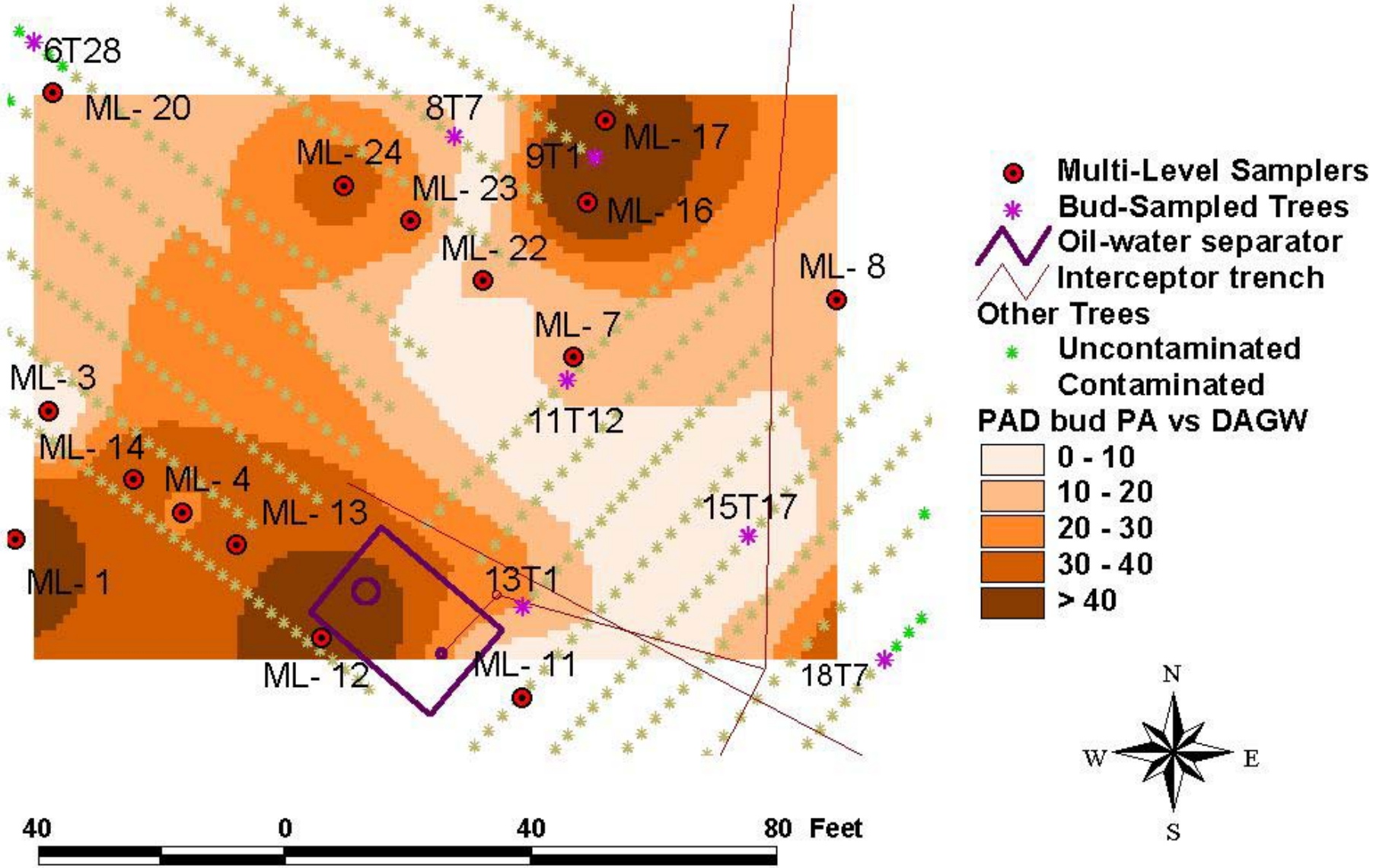
Table 4-1: March 2001 Comparisons

Tissue Type	Extractive Type		Groundwater Type
	Primary Aromatics	Total Aromatics	
Buds	✦	✦	Shallow Groundwater
	✦	✦	Depth-Averaged Groundwater
Bark	✦	✦	Shallow Groundwater
	✦	✦	Depth-Averaged Groundwater
Twigs	✦	✦	Shallow Groundwater
	✦	✦	Depth-Averaged Groundwater

Figures 4-4 through 4-8 show representative percentile absolute difference comparisons from samples collected in March 2001. Figure 4-4 shows the PAD comparison between bud primary aromatics and depth-averaged ground water TPAH concentrations. Figure 4-5 shows the PAD between bark primary aromatics and shallow ground water TPAH concentrations. Figure 4-6 shows the comparison between twig primary aromatics and depth-averaged ground water TPAH concentrations. Figure 4-7 shows the PAD between twig total aromatics and shallow ground water TPAH concentrations. In each comparison shown, a new agreement range begins at every 10^{th} percentile of agreement, with the lightest colors representing the closest agreement.

Several trends emerged from the comparisons. Buds, in general, did the poorest job of predicting ground water contamination. Only one bud comparison, primary aromatics vs. depth-averaged ground water predicted the degree of ground water contamination within 20 percentiles over 50% of the study area, at 51%, as shown in Figure 4-4. In general, bud primary aromatics predicted ground water contamination better than total aromatics, and no other trends appeared within this tissue type.

Figure 4-4: March 2001 Percentile Absolute Differences Between Bud Primary Aromatics and Depth-Averaged Ground Water Total PAH Concentrations.



Bark tissue predicted the degree of ground water contamination much better than buds. Bark percentiles fell within 20 percentiles of ground water in over half of the study area for all tissue comparisons, regardless of both ground water depth and type of aromatic compound. The highest rate of agreement appeared when comparing bark primary aromatics with shallow ground water TPAH concentrations, with 59% of the study area falling within 20 percentiles, as shown in Figure 4-5. For the bark tissue, total aromatics vs. depth-averaged ground water demonstrated the lowest predictive ability, with 53% of the study area falling within the desirable range of 20 percentiles, as shown in Figure 4-6. Within the bark comparisons, primary aromatics had greater predictive ability than total aromatics, and bark tissue predicted shallow ground water better than depth-averaged ground water, though for all comparisons, the percent of the study area agreeing within 20 percentiles was comparable.

Figure 4-5: Percentile Absolute Differences Between Bark Primary Aromatics and Shallow Ground Water Total PAH Concentrations.

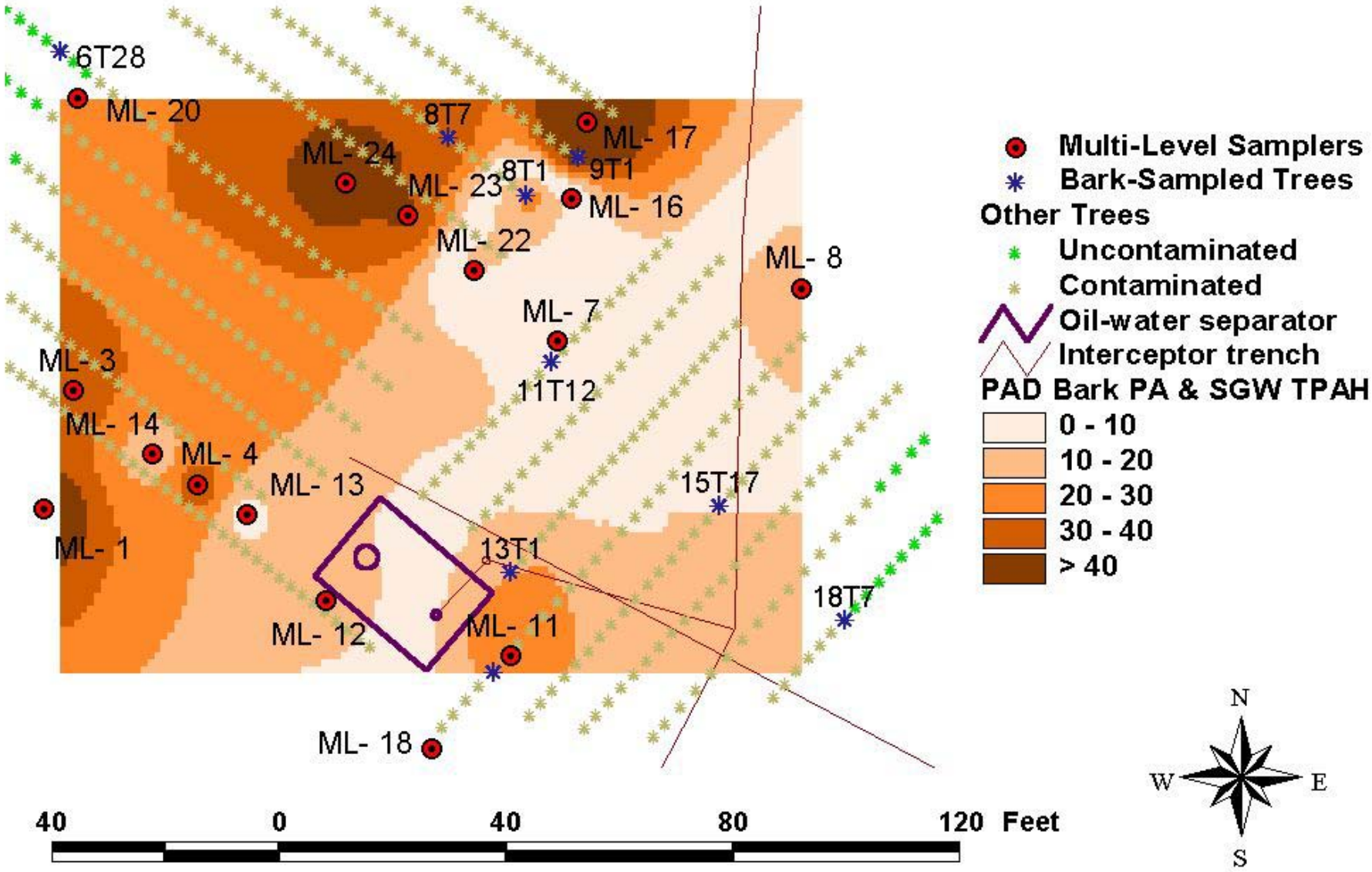
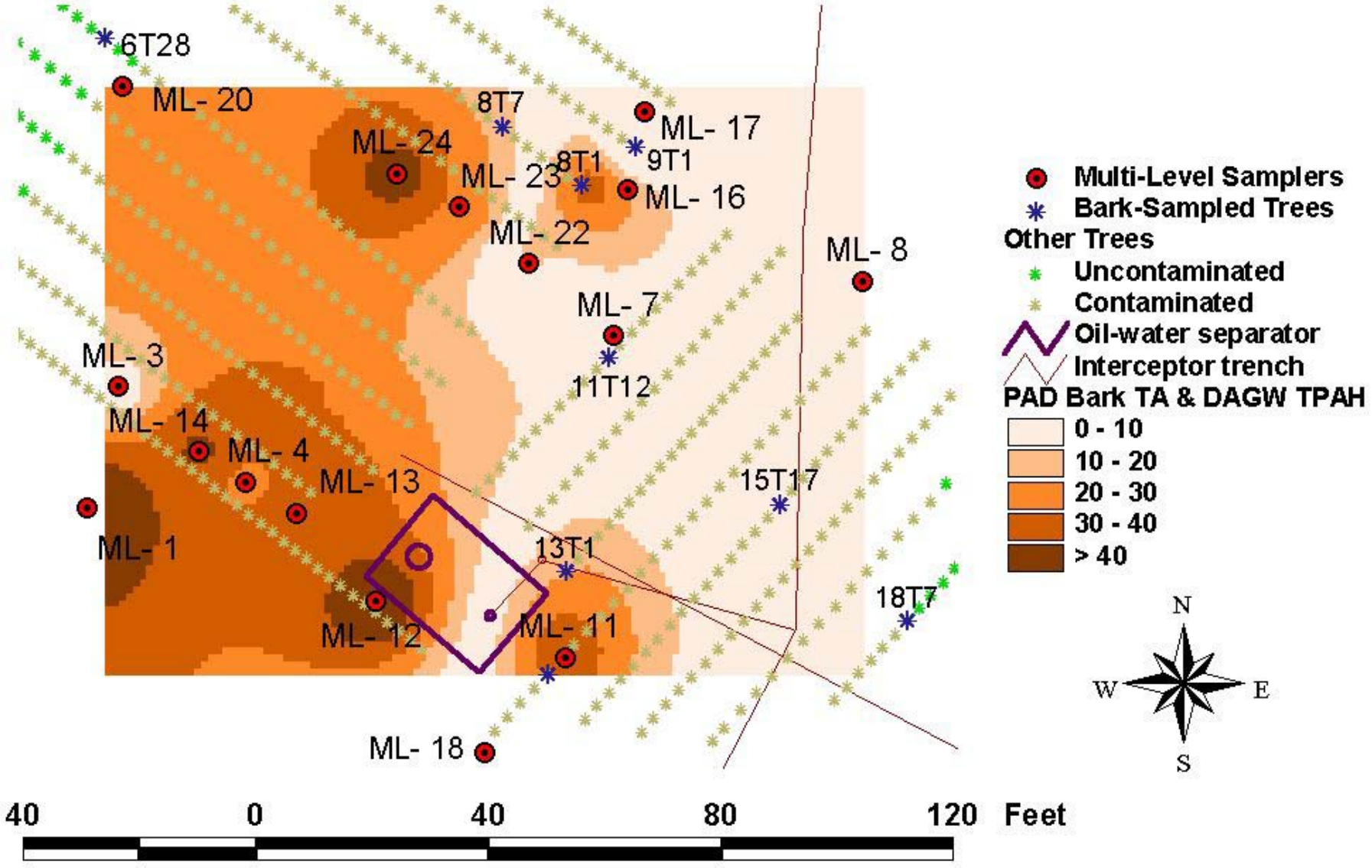


Figure 4-6: March 2001 Percentile Absolute Differences Between Bark Total Aromatics and Depth-averaged Ground Water Total PAH Concentrations.



Overall, twig tissue proved the best predictor of ground water contamination, with the highest percentages of study areas agreeing within 20 percentiles, as shown in Table 4-4. Twig tissue also had the highest proportion of study area in this range for each type of tree tissue/ground water comparison. Primary aromatics predicted the degree of ground water contamination better than total aromatics, while twigs predicted depth-averaged ground water better than shallow ground water and areas of moderate and high contamination better than areas of low contamination. Predictive ability increased with ground water contamination for depth-averaged ground water.

For twigs, the greatest percentage of the study area, 68%, fell within 20 percentiles of ground water TPAH when comparing twig primary aromatics with depth-averaged ground water, as shown in Figures 4-7 and Table 4-4. The poorest degree of agreement was between total aromatics and shallow ground water TPAH concentration, with 61% of the study area falling within 20 percentiles, as shown in Figure 4-8. Percentile agreement areal statistics for twigs of March 2001 with depth-averaged and shallow ground water are shown in Tables 4-2 and 4-3, respectively.

The trends in overall twig predictive ability can be seen in the pie charts in Figure 4-9. Charts referring to shallow ground water appear in the top row, with depth-averaged ground water charts beneath. Charts referring to primary aromatics appear in the left column, with total aromatic charts on the right. Each slice of the pie shows the proportion of the study area where the absolute value of the difference in percentiles, or percentile absolute difference (PAD) between the tree aromatic count and the ground water total PAH concentration falls within the given 10-percentile range.

Figure 4-7: March 2001 Percentile Absolute Differences Between Twig Primary Aromatics and Depth-Averaged Ground Water Total PAH Concentrations.

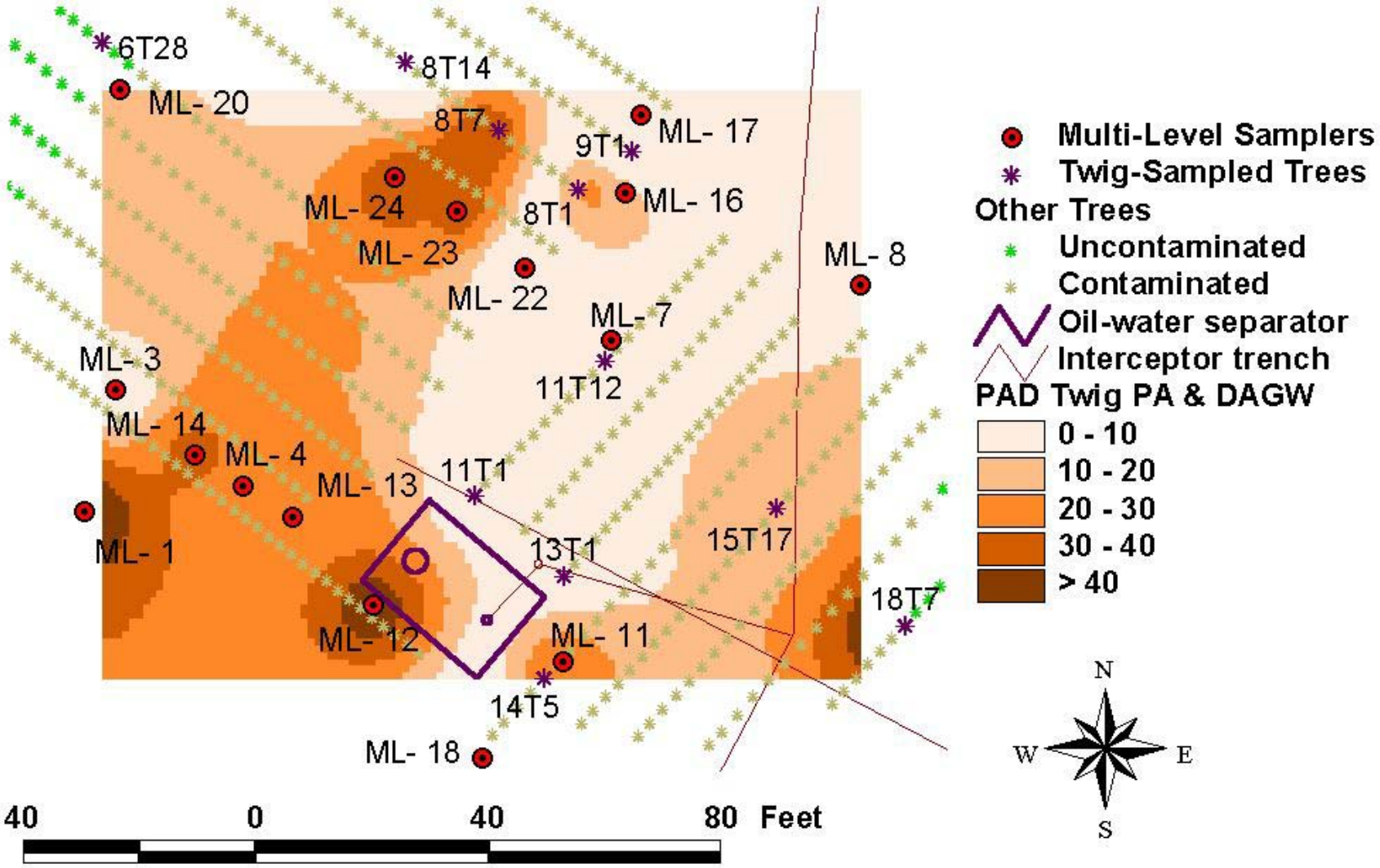


Figure 4-8: March 2001 Percentile Absolute Differences Between Twig Total Aromatics and Shallow Ground Water TPAH Concentration.

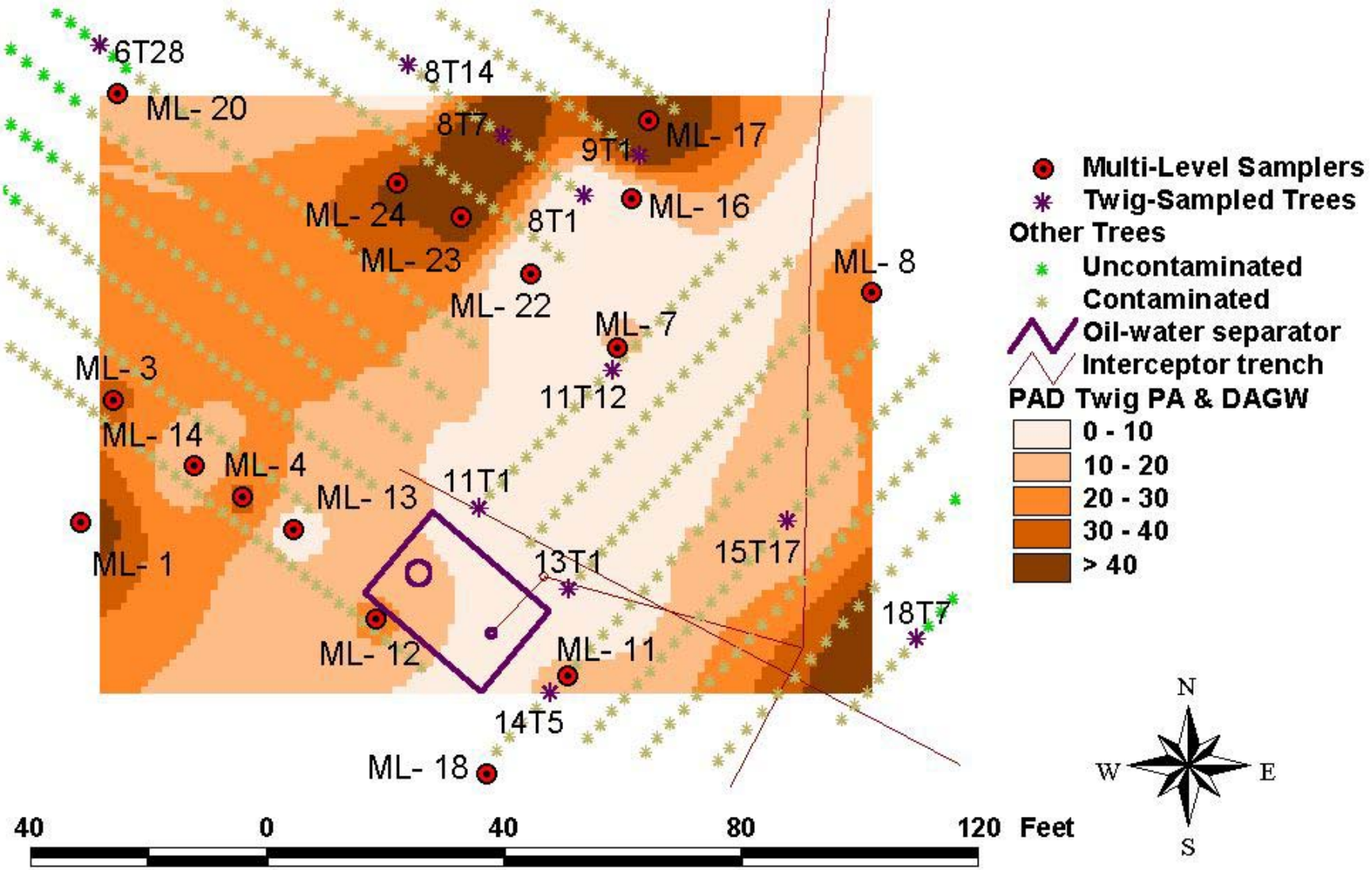
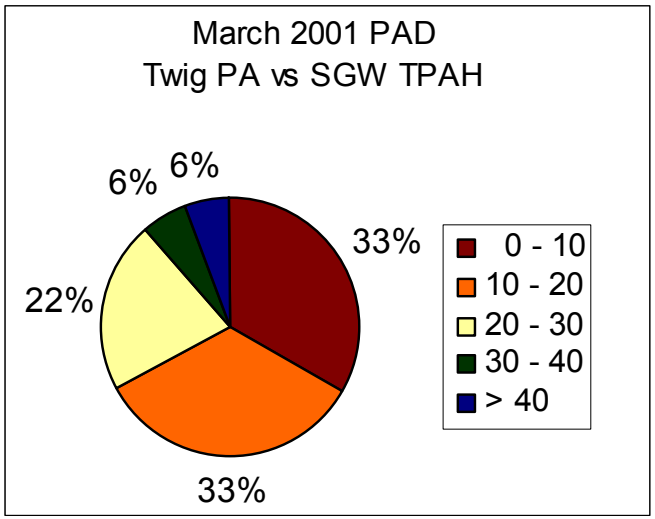
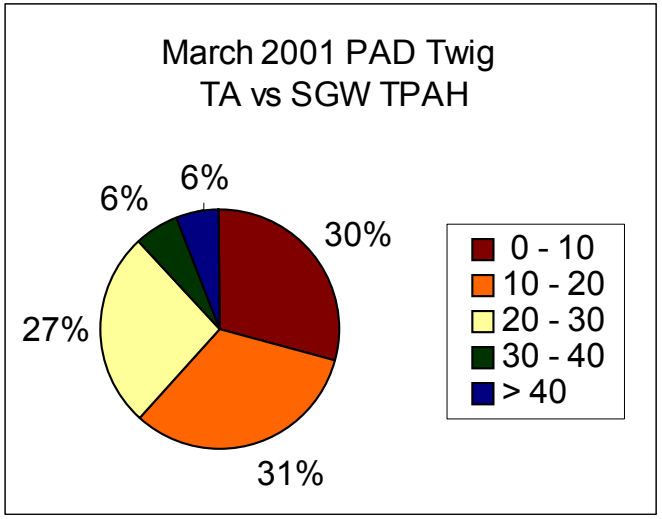
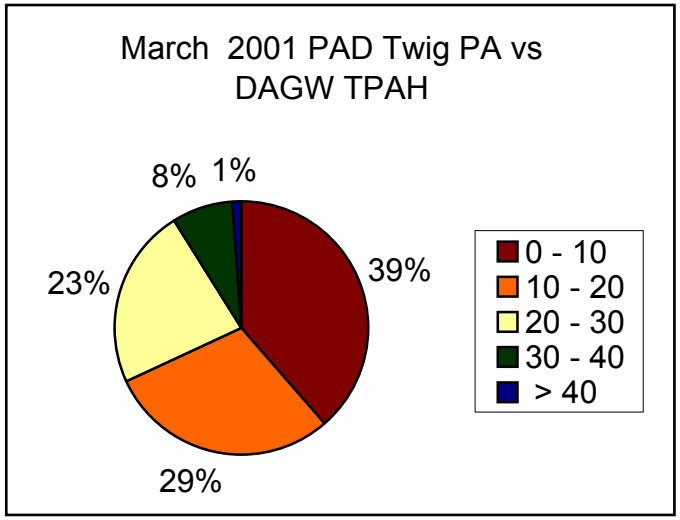


Figure 4-9: March 2001 Twig Comparison Pie Charts







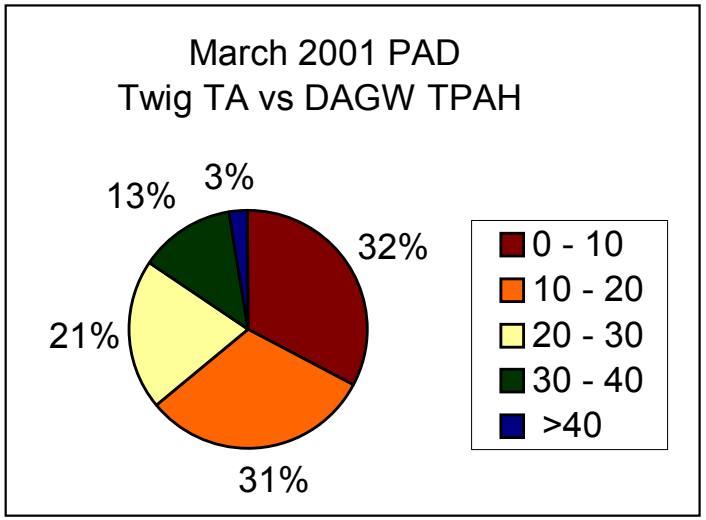


Table 4-2: March 2001 Twig Areal Statistics for Depth-Averaged Ground Water

			March 2001 DAGW		
Percentile Range	Range Area, sq. ft.	Area within 20 Percentiles, sq. ft.	Zone % within 20 Percentiles	Zone area within 10 Percentiles	Zone % within 10 Percentiles
0 - 40	4923.6	1364.7	27.7	189.0	3.8
40 - 60	2143.0	1920.8	89.6	894.2	41.7
60 - 100	6106.1	5664.6	92.8	3996.2	65.4
total	13172.7	8950.2	67.9	5079.5	38.6

Table 4-3: March 2001 Twig Areal Statistics for Shallow Ground Water

			March 2001 SGW		
Percentile Range	Range Area, sq. ft.	Area within 20 Percentiles, sq. ft.	Zone % within 20 Percentiles	Zone area within 10 Percentiles	Zone % within 10 Percentiles
0 - 40	4526.1	1268.1	28.0	40.0	0.88
40 - 60	3787.9	3518.8	92.9	1733.2	45.8
60 - 100	4858.7	4029.4	82.9	2635.6	54.2
total	13172.7	8816.3	66.9	4408.8	33.5

In comparing twig predictive ability for shallow ground water with low ($0 – 40^{th}$ percentile), moderate ($40^{th} – 60^{th}$ percentile) and high (above the 60^{th} percentile) contamination, it appeared that twigs predicted ground water contamination above the 40^{th} percentile much better than contamination below the 40^{th} percentile, though a constantly increasing relationship was not observed.

Twig tissue predicted ground water contamination most accurately, always agreeing within 20 percentiles of ground water TPAH concentrations at a 60% or greater level. Bark tissue provided the next best predictions overall, outpredicting bud tissue in each individual percentile difference category. For all three tree tissue types, primary aromatics predicted ground water contamination better than total aromatics. Twig tissue predicted depth-averaged ground water contamination more accurately than shallow ground water, while buds and bark predicted shallow ground water contamination more accurately than depth-averaged.

Table 4-4: March 2001 Percentile Absolute Difference Results Summary

	Bud PA vs SGW TPAH PAD			Bud TA vs SGW TPAH PAD	
	Difference Range	% of Area		Difference Range	% of Area
	0 - 10	21		0 - 10	14
	10 - 20	27		10 - 20	30
	0 - 20	48		0 - 20	44
	> 40	1		> 40	3
Max Difference	49.71		Max Difference	50.81	
	Bud PA vs DAGW TPAH PAD			Bud TA vs DAGW TPAH PAD	
	Difference Range	% of Area		Difference Range	% of Area
	0 - 10	20		0 - 10	12
	10 - 20	31		10 - 20	31
	0 - 20	51		0 - 20	43
	> 40	9		> 40	9
Max Difference	62.98		Max Difference	61.71	
	Bark PA vs SGW TPAH PAD			Bark TA vs SGW TPAH PAD	
	Difference Range	% of Area		Difference Range	% of Area
	0 - 10	28		0 - 10	31
	10 - 20	31		10 - 20	24
	0 - 20	59		0 - 20	55
	> 40	5		> 40	5
Max Difference	69.3		Max Difference	70	
	Bark PA vs DAGW TPAH PAD			Bark TA vs DAGW TPAH PAD	
	Difference Range	% of Area		Difference Range	% of Area
	0 - 10	42		0 - 10	41
	10 - 20	12		10 - 20	12
	0 - 20	54		0 - 20	53
	> 40	3		> 40	3
Max Difference	54.7		Max Difference	55.9	
	Twig PA vs SGW TPAH PAD			Twig TA vs SGW TPAH PAD	
	Difference Range	% of Area		Difference Range	% of Area
	0 - 10	33		0 - 10	30
	10 - 20	33		10 - 20	31
	0 - 20	66		0 - 20	61
	> 40	6		> 40	6
Max Difference	69.8		Max Difference	66.2	
	Twig PA vs DAGW TPAH PAD			Twig TA vs DAGW TPAH PAD	
	Difference Range	% of Area		Difference Range	% of Area
	0 - 10	39		0 - 10	32
	10 - 20	29		10 - 20	31
	0 - 20	68		0 - 20	63
	> 40	1		> 40	1
Max Difference	48.6		Max Difference	52.4	

While trends existed within the bud, bark and twig tissue types regarding which combination of aromatics and ground water yielded the closest agreement, the greatest differences in predictive ability were between tissue types, rather than aromatic or ground water types. The trees tend to overpredict contamination on the north and west sides of the site and underpredict on the south and east, regardless of tissue type. Since the twig tissue primary aromatics predicted the degree of ground water contamination most closely, only this tissue was analyzed for the remaining sampling events.

During July of 2001, 80% of the study area for depth-averaged ground water showed agreement within 20 percentiles, as shown in Figure 4-10. Though apparent overwhelming agreement was obtained between twig primary aromatics and depth-averaged ground water for this time period, fewer samples were available both of tree tissue and ground water, making the study area approximately 33% the size of that during the March 2001 period by area, and including the area where best agreement was obtained for March 2001. In marked contrast to the March 2001 time period, during July 2001, the highest number of primary aromatics in the twigs was four, rather than 13. This result could be caused by the drought and consequent lower moisture content of the soil changing the equilibrium of polycyclic aromatic hydrocarbon partitioning between water, soil, and tree tissue. The marked decrease in number of aromatic compounds in trees could also be due to greater biotic activity in the soil or trees due to the temperature increase from March to July. Both microbes and plants tend to be more biologically active in the summer than winter, and this increase in biological activity could have caused the more rapid disappearance of aromatic compounds via either ring-cleavage or conversion to bound residue in the tree tissue.

The July 2001 tree tissue overwhelmingly tended to underpredict ground water contamination, with the only overprediction occurring in a highly localized area near tree 11T8 and at the northwestern-most corner of the study area. No area of ground water contamination below the 40th percentile fell within in the study area due to the availability of trees for analysis. Though the study area was significantly smaller than that of March 2001, the area of moderately contaminated depth-averaged ground water was larger. The summer July twig tissue aromatics agreement within 20 percentiles of highly contaminated ground water was 71%, a significant decrease from March 2001. The chart of areal statistics for the summer of 2001 is shown in Table 4-5. Due to drought conditions, no shallow ground water was available for analysis and fewer wells yielded any ground water.

Figure 4-10: July 2001 Percentile Absolute Differences Between Twig Primary Aromatics and Depth-Averaged Ground Water Total PAH Concentration.

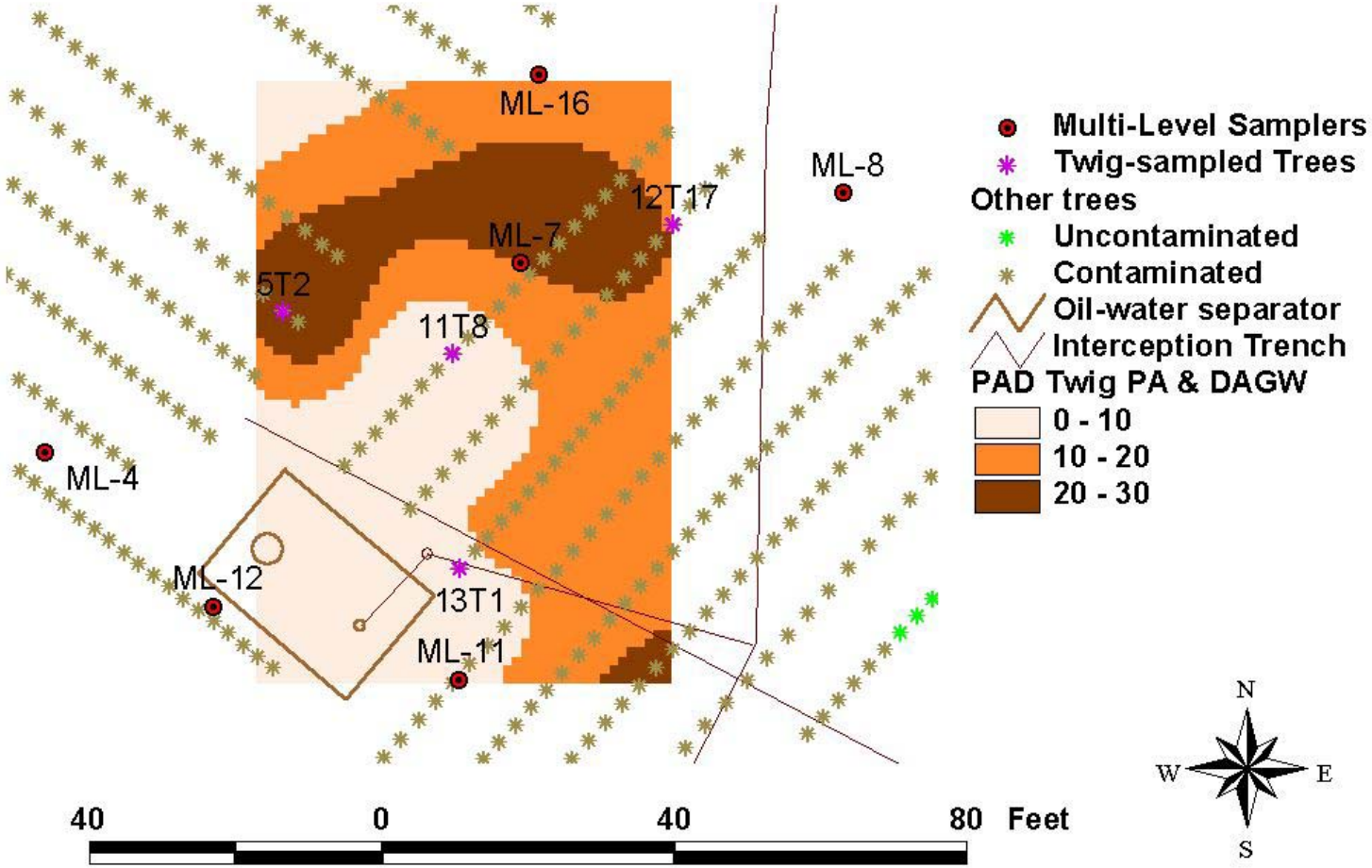


Table 4-5: July 2001 Areal Statistics

			July 2001 DAGW		
Percentile Range	Range Area, sq. ft.	Area within 20 Percentiles, sq. ft.	Zone % within 20 Percentiles	Zone area within 10 Percentiles	Zone % within 10 Percentiles
0 - 40	0.0	0.0	n/a	0.0	n/a
40 - 60	2270.0	2006.4	88.4	1305.4	57.5
60 - 100	2140.3	1520.7	71.1	327.0	15.3
total	4410.2	3527.1	80.0	1632.4	37.0
% of Mar 01	33.5				

For February-March 2002, 89% of twig primary aromatics fell within 20 percentiles of depth-averaged ground water, with 61% falling within 10 percentiles, as shown in Figure 4-11. The upper bound of the maximum PAD bin was 40 percentiles, making this the PAD map with the narrowest range. Only 1% of the area exhibited a percentile absolute difference greater than 30, occurring in two highly localized areas around multilevel samplers 25 and 17, neither of which yielded shallow ground water. The area immediately surrounding ML-23, which also did not yield shallow ground water, also had a relatively high percentile difference for this time period, falling in the 20 – 30 range, while the area surrounding ML-20, the other sampler that yielded no shallow ground water during this sampling event, showed a very low PAD, falling between zero and ten.

For this sampling event, the tree primary aromatics tended to underpredict ground water contamination over most of the site, with the overprediction only occurring in highly localized areas near ML-23 and ML-17, two samplers that did not yield shallow ground water. For this time period, the maximum number of primary aromatics was seven. The trees predicted all levels of ground water contamination well, but predicted areas of low contamination most accurately and areas of high contamination almost as well, as shown in Table 4-6. The study area for depth-averaged ground water in February-March 2002 was approximately 53%, by area, as that of March 2001For February-March 2002, 86% of twig primary aromatics fell within 20 percentiles of shallow ground water, with 58% falling within 10 percentiles, as shown in Figure 4-12. Though the study area for shallow ground water was only 66% of that for depth-averaged ground water due to a lack of shallow ground water yield at several multi-level samplers, both themes

Figure 4-11: February-March 2002 Percentile Absolute Differences Between Twig Primary Aromatics and Depth-Averaged Ground Water Total PAH Concentrations

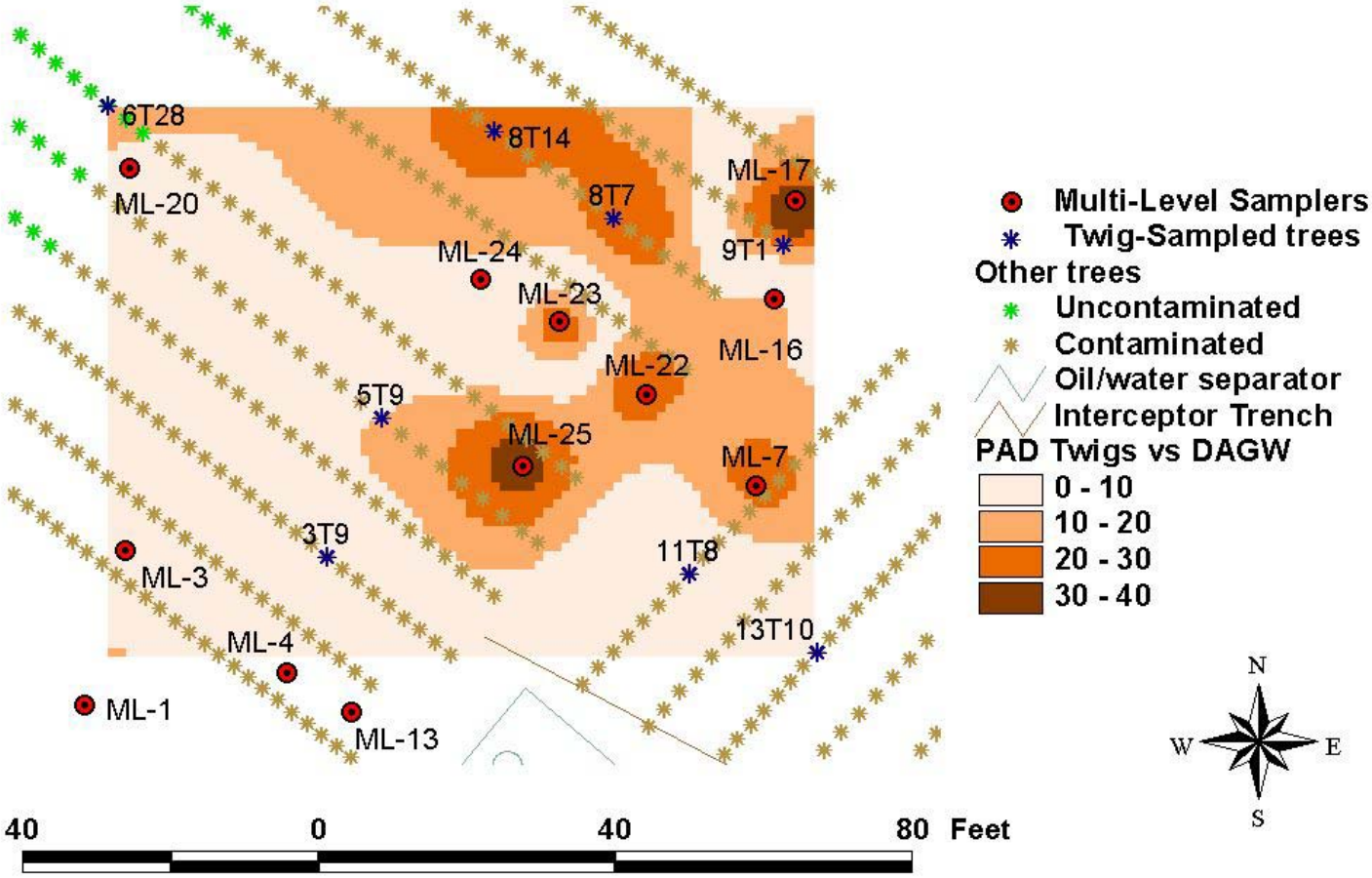


Figure 4-12: February-March 2002 Percentile Absolute Differences Between Twig Primary Aromatics and Shallow Ground Water Total PAH Concentrations

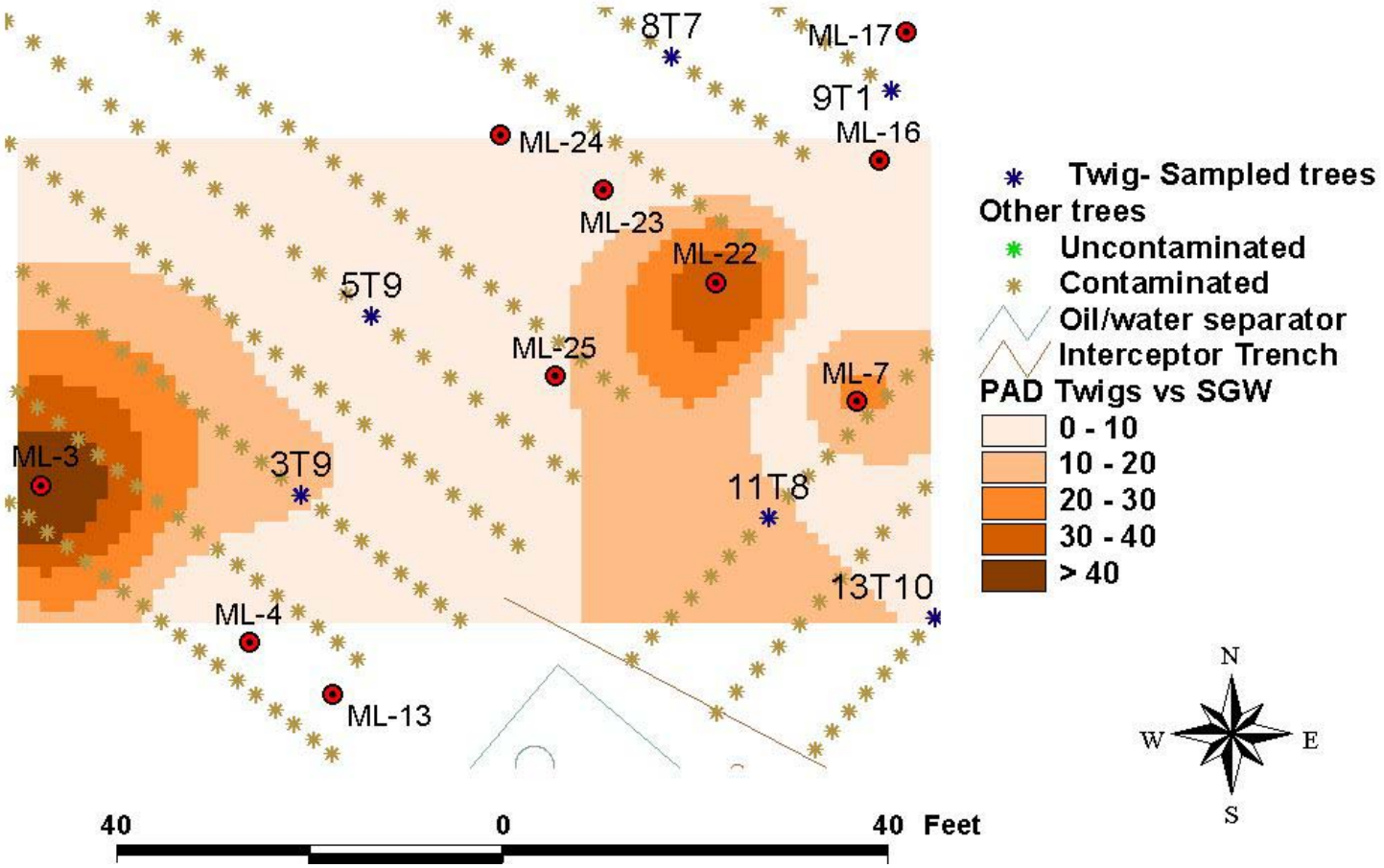


Table 4-6: February-March 2002 Areal Statistics for Depth-Averaged Ground Water

			Winter 2002 DAGW		
Percentile Range	Range Area, sq. ft.	Area within 20 Percentiles, sq. ft.	Zone % within 20 Percentiles	Zone area within 10 Percentiles	Zone % within 10 Percentiles
0 - 40	598.9	578.2	96.5	436.1	72.8
40 - 60	3549.2	3118.6	87.9	2325.2	65.5
60 - 100	2893.7	2550.1	88.1	1479.3	51.1
total	7041.7	6246.9	88.7	4240.5	60.2

showed very similar levels of percentile difference at the 20-percentile level. Areas possessing agreement within 20 units were nearly congruent for depth-averaged and shallow ground water, while areas of percentile agreement within 10 percentiles were not congruent for these two themes.

The twigs tended to predict zones of lower contamination better for depth-averaged ground water and zones of higher contamination better for shallow ground water, as shown in Table 4-7. The twigs predicted areas of moderate and high contamination extremely well, with 98.4% of both falling within 20 percentiles of agreement, but predicted areas of low ground water contamination much less well, with only 38.6% of this category agreeing within 20 percentiles.

Table 4-7: February-March 2002 Areal Statistics for Shallow Ground Water

			Winter 2002 SGW		
Percentile Range	Range Area, sq. ft.	Area within 20 Percentiles, sq. ft.	Zone % within 20 Percentiles	Zone area within 10 Percentiles	Zone % within 10 Percentiles
0 - 40	1039.1	401.6	38.6	143.5	13.8
40 - 60	2635.6	2594.3	98.4	1919.5	72.8
60 - 100	1131.5	1113.6	98.4	716.2	63.3
total	4806.3	4109.4	85.5	2779.2	57.8

The February-March 2002 depth-averaged ground water showed much better agreement within 20 percentiles for areas of low ground water contamination than its counterpart in March 2001, despite the fact that the area of low ground water contamination in February-March 2002 was much smaller than that of March 2001. The portions of the study areas exhibiting agreement within 20 percentiles for moderately and highly contaminated ground water were approximately the same. All three sampling events showed similar agreement proportions for moderately contaminated depth-averaged ground water, ranging from 87.9 to 89.6% of the respective study areas. The twigs during the winter 2002 time period followed a trend similar to that of winter 2001 in predicting shallow ground water, despite possessing an area only 35% that of winter 2001. During both sampling events, the twigs predicted moderately and highly contaminated shallow ground water much more accurately than areas of low contamination in shallow ground water.

Summary

The presence of an unusually high number of aromatics in the tree tissue correlates well with both shallow and depth-averaged ground water contamination, implying that the contaminants have a direct or indirect chemical effect on the trees. Though the exact mechanism remains unexplained, three mechanisms seem likely: the trees produce unusual aromatic compounds in response to the contamination, the trees take up some of the creosote chemicals, most likely naphthalene, directly and transform the constituent compounds, or the trees take up microbial metabolites of creosote.

For the samples collected during March of 2001 tree tissue aromatics tended to overpredict contamination on the north and west sides of the site and underpredict on the south and east, regardless of tissue type. In general, the areas of lower contamination were on the west side and higher contamination on the east.

The twigs gave the best prediction among the tissue types, perhaps due to the twigs having the most direct involvement in the poplar transpiration stream. Bark prediction was a close second, perhaps due to the fact that the xylem tissue is directly involved in the transpiration stream, while the phloem, or outer tissue, is not. Poplar trees have been shown to transpire TCE, and, similarly, could be transpiring creosote compounds at the site (Orchard *et al.*, 2000; Newman, 1997). The ability of buds to predict ground water contamination could be hampered by the large complement of aromatic compounds usually present in this tissue.

Of bark, buds, and twigs, the buds have the largest number of naturally occurring aromatic compounds. Any additional aromatics present in the buds may not be significant compared to the usual number of aromatics in the bud tissue. During the July 2001 sampling, the overwhelming majority of the site was underpredicted by aromatics in twig tissue. This finding is consistent with the drought conditions at the site in July, which could have inhibited the biotic activity of both the trees and the soil microflora. The lack of soil moisture could also have changed the equilibrium between the creosote phases of DNAPL, dissolved, and sorbed to trees or to soil. On a mass basis, more of the creosote compounds would likely have sorbed to soil surface or to the tree roots since the drought made an aqueous phase less available. The decrease in moisture at the site could also have decreased the amount of creosote that dissolved from the NAPL phase.

Similar to July 2001, most of the study area's ground water contamination was underpredicted in winter 2002. The latter season was also during a dry period, though not as dry as July. The maximum number of primary aromatics found in tissue increased with increasing moisture, with July 2001 having the smallest maximum and winter 2001 the largest, appearing to support the argument that soil moisture controls the number of primary aromatics present in the tissue.

Overall, the winter twigs tend to predict moderate or high areas of contamination much more accurately than low areas of contamination. During winter, the twigs also tend to predict depth-averaged contamination slightly better than shallow contamination, though no striking differences in predictive ability are evident. It is likely that the tree roots have penetrated to the deeper depths, where pure creosote is present. If the trees only take up microbial metabolites, the rhizosphere zone would have extended deeper into the ground water zone with the tree roots. If the trees produce unusual aromatic compounds as a stress response to chemical contamination, extending the root zone deeper would increase the exposure of the trees to creosote. While this line of reasoning would seem to suggest that the number of primary aromatics appearing in the twig tissue should increase with time, the drought conditions would reverse this trend by limiting the amount of creosote compounds in the aqueous phase that would be available to the trees.

Whether the trees produce unusual aromatics as a stress response to growing in contamination or somehow are involved in the transformation of parent pollutants or their metabolites, they could have the potential to track the progress of site remediation, perhaps even acting as biosensors. If the ability of the trees to track the progress of remediation is confirmed, researchers could even sample them during drought conditions, unlike the multi-level samplers.

Conclusions

There is good agreement between the number of primary aromatics in tree tissue and TPAH concentration in ground water. This agreement indicates that the trees produce unusual aromatics as a response to contamination, take up creosote compounds directly, or take up microbial metabolites. The spatial distribution of aromatic compounds in twig tissue acts as an indicator of the distribution of creosote contamination in ground water. Consequently, the spatial distribution of aromatic compounds in trees may be used to track the progress of phytoremediation. The trees have the potential to track creosote contamination, and by extension, the progress of phytoremediation.

Several implications arise from the results of the current study:

- Tree tissue analysis can be used to locate areas of creosote contamination and to monitor the progress of remediation.
- Of bud, bark, and twig tissue, twigs demonstrate the greatest predictive ability, followed closely by bark. Bud tissue does not have comparable predictive ability.
- Soil moisture content exerts a very strong effect on the number aromatic chemicals seen in the trees. Drought conditions reduced the number of aromatic compounds in the tree tissue by 66% from March to July 2001 and by 50% from March 2001 to March 2002. Any predictive model attempting to relate the number of tree tissue aromatics to total ground water PAH concentration would have to adjust for the effects of soil moisture content variation.

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Chapter 5: Engineering Applications

The most suitable application for this research would be use at existing phytoremediation sites, both in performing treatability studies and once the phytoremediation system has been initiated. A non-sacrificial method for chemical analysis of tree tissue samples could allow treatability studies to discover the fate of pollutants removed from contaminated soil and water by phytoremediation by tracking the appearance and disappearance of daughter products in the tree tissue. Greater knowledge of the metabolites produced through phytoremediation would allow environmental professionals to ensure that the daughter products are not more toxic than the parent pollutants and would aid in obtaining regulatory approval for phytoremediation when using risk-based remediation strategies. Such knowledge would also aid in deciding the final disposition of the trees themselves, whether they should be left in place, landfilled, or incinerated.

After a installation of a phytoremediation system at a site, the findings of this research could aid in tracking the progress of phytoremediation. Environmental monitors could measure not only the disappearance of parent pollutants from soil and ground water, but also the appearance and disappearance of metabolites in the trees, ending the remediation process when the pollutants had been transformed into their least toxic form.

Vita

Lois Diane Waters was born on December 23, 1978 in Baltimore, Maryland. She spent most of her life living in Howard County, Maryland under the care of her parents, Lois A. Waters and Gilbert W. Waters, Jr. She developed an interest in science at an early age, and became known for bringing home rock and mineral samples to add to a growing collection. While in Howard County, Diane developed interests in environmental protection, basketball, Spanish language, and choral singing. She graduated from Centennial High School in 1996 and began undergraduate studies at North Carolina A&T State University in Agricultural and Biosystems Engineering under Dr. Manuel Reyes, her advisor.

At A&T, while learning to see environmental issues on a watershed scale, Diane gained a solid knowledge of geographic information systems (GIS) hydrology, non-point source pollution control, and environmental modeling and received her introduction to the fields of hazardous waste management and phytoremediation. Diane successfully completed her undergraduate degree requirements in 2000, having earned her Engineer-in-Training (EIT) registration the previous year.

Diane came to Virginia Tech as a Via Scholar in August of 2000. While there she pursued the Natural Systems Track in the Environmental Engineering Master's degree program, selecting Dr. John T. Novak, the Nick T. Prillaman professor of Environmental Engineering, as her research committee chair.