In situ characterization and quantification of phytoremediation removal mechanisms for naphthalene at a creosote-contaminated site

Rikke Granum Andersen

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John T. Novak, Chair
Mark A. Widdowson
Linsey C. Marr
Charles Hagedorn
Steve Cox

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ABSTRACT

Phytoremediation is an attractive remediation technology due to its relative low cost and maintenance requirement. Acceptance of phytoremediation requires that the contaminant removal mechanisms are characterized and demonstrated in the field. Quantification of contributions from each mechanism to the overall remediation rate is crucial for optimization of phytoremediation systems, risk management and prediction of the total remediation time. The objective of this research was to characterize and quantify removal mechanisms for naphthalene at a creosote-contaminated site with poplar trees in Oneida, Tennessee. Groundwater monitoring for seven years in the surficial aquifer at this site demonstrated a reduction in polycyclic aromatic hydrocarbons (PAHs) with selective removal of naphthalene and three-ring compounds. Naphthalene mass loss mechanisms investigated at this site are biodegradation in the saturated zone, volatilization and biodegradation in the vadose zone and phytovolatilization. This is probably the most comprehensive field study of PAH phytoremediation mechanisms conducted to date. The significance of this research is to contribute to predictions of remediation time and end result for phytoremediation of PAHs. The understanding of in situ factors controlling each mechanism can facilitate future optimization of phytoremediation systems as well as improve risk assessment and monitoring strategies.

Biodegradation rates were determined for different conditions at this site with in situ respiration tests, laboratory soil microcosms and laboratory soil columns. The combined remediation mechanisms of volatilization and biodegradation in the vadose zone were investigated in the field and in laboratory columns. Field measurements show that lower groundwater elevations in the summer and early fall lead to elevated groundwater concentrations of naphthalene and increased volatilization. The increase in the fraction of

the porespace occupied by gas (gas saturation) in the unsaturated zone during the summer and fall further enhances the volatilization by increasing effective diffusion rates. Water consumption and interception by the phytoremediation system are believed to enhance mass transfer to the vadose zone. Column experiments and field measurements show that more than 90% of the naphthalene vapors are biodegraded within 5-10 cm above the groundwater table. The data indicate that biodegradation increases the overall volatilization flux out of the source by 10-300 times, when the source is exposed directly to the gas phase. In situ the naphthalene is generally dissolved from the source into the groundwater and then volatilezed from the groundwater to the gas phase. Under these conditions biodegradation in the vadose zone will still indirectly have an enhancing effect on the flux out of the source. This is the result of removal naphthalene from the soil gas by biodegrdation driving removal from the groundwater by volatilization, which in turn drives dissolution form the source into the groundwater.

Phytovolatilization was quantified in flux chambers mounted on trees and calculated from transpiration rates. A laboratory uptake study and analysis of tree cores from the site provided supplementary evidence for naphthalene uptake by poplar trees. Phytovolatilization was detected throughout the year and was highest in the summer and fall when the groundwater concentrations were highest and transpiration was active.

The role of biodegradation relative to physical removal mechanisms was compared for a year, for winter and summer conditions and with and without the impact of phytoremediation. Biodegradation of naphthalene in the saturated zone dominates by orders of magnitude over the removal by volatilization and phytovolatilization of naphthalene at this site. The removal of the total residual naphthalene mass was estimated to require up to 100 years with phytoremediation, but more than twice as long without phytoremediation. The estimated removal of naphthalene was three times larger in the summer than in the winter due to slower biodegradation in the saturated zone and smaller rates of volatilization to the vadose zone in the winter. The research shows that phytoremediation enhances the overall naphthalene removal, mainly by stimulating faster biodegradation in the rhizosphere and promoting mass transfer of naphthalene to the vadose zone followed by rapid vadose zone biodegradation. In the future, phytoremediation research focusing on the capillary zone is desirable.

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CHAPTER 1

Introduction

Phytoremediation is the use of plants and their rhizosphere ecosystem for remediation and control of contaminants. Phytoremediation can be a cost effective, low impact and aesthetically pleasing alternative to traditional remediation technologies, especially for large areas with low concentrations of dispersed contamination. Natural attenuation of polycyclic aromatic hydrocarbons (PAHs) can be accelerated by phytoremediation, but the contribution from individual mechanisms has not yet been delineated in full scale systems. Regulatory and public acceptance of phytoremediation requires that the combined remediation mechanisms occur at a rate that prevents further spreading of the contamination. Remediation time and end result must be predictable and exposure risks must be understood. In order to promote acceptance of phytoremediation applications the active mechanisms must be identified and quantified in situ and demonstrated in long term full scale applications.

Phytoremediation of PAHs with poplar trees has been studied by our research group for 8 years at a creosote-contaminated site in Oneida, Tennessee. The overall objective of the Oneida research has been to identify and quantify the mechanisms responsible for PAH removal, investigate the role of the phytoremediation system in natural attenuation and provide long term monitoring data of the remediation progress. The significance of this research is to contribute to predictions of remediation time and end result for phytoremediation of PAHs. The understanding of in situ factors controlling each mechanism can facilitate future optimization of phytoremediation systems as well as improve risk assessment and monitoring strategies.

The specific objectives for the research presented in this dissertation are to:

1. Investigate removal mechanisms for naphthalene and quantify their rates in situ.
2. Investigate the phytoremediation impact on the natural attenuation of naphthalene.
3. Quantify the total removal by the combined effect of all mechanism and compare the contributions from the different mechanisms

Biodegradation in the saturated zone, volatilization coupled with biodegradation in the vadose zone, and plant uptake coupled with phytovolatilization are the active removal mechanisms for naphthalene identified at the Oneida site. Biodegradation in the upper saturated zone and lower vadose zone in the area of fluctuating groundwater dominates the overall mass removal by orders of magnitude. In the saturated zone, biodegradation removes naphthalene year round, whereas volatilization to the vadose zone followed by rapid biodegradation in the vadose zone only is significant in the summer and fall months. Release of naphthalene to the atmosphere by phytovolatilization and volatilization occurs mainly in the summer and fall, but the mass removed by these processes is small and presents no significant exposure risk. The phytoremediation system enhances all these mechanisms, but in particular has the potential to enhance mass transfer out of the groundwater followed by rapid biodegradation in the aerobic and rhizosphere-impacted vadose zone. The estimated overall remediation time for remaining naphthalene at this site is reduced significantly by phytoremediation.

Figure 1.1 shows a map of the research site in Oneida TN. The site is highly contaminated with creosote spilled during rail road tie treatment in 1950-1973. Free phase creosote resides on top of the confining bedrock 3 m below ground in thicknesses up to 0.3 m, and PAHs are continuously leaching into the shallow aquifer above. The maximum extent of the DNAPL creosote and the dissolved PAH plume is shown on Figure 1.1. The creosote was discovered leaching into a creek downstream from the site in 1990 and an interception trench and oil water separator were installed to deter plume migration. In 1997 a phytoremediation system of poplar trees was planted on the site to contribute to the hydraulic control of the dissolved PAH plume while enhancing natural attenuation processes for contaminant mass removal.

Since the installation of the phytoremediation system in 1997, a team of Virginia Tech researchers has conducted research and monitored the remediation progress of PAHs at the site. Groundwater monitoring has demonstrated a reduction of the extent of the PAH plume (Figure 1.2) and of the total naphthalene mass (Figure 1.3) in the surficial aquifer.

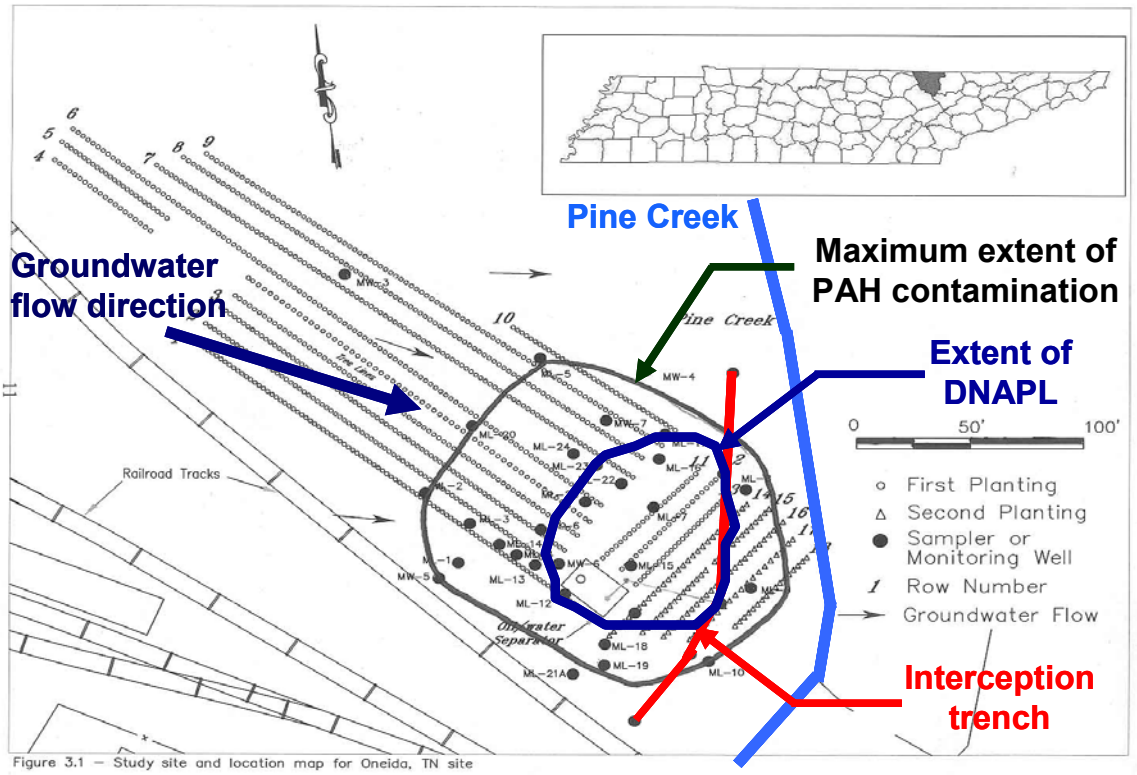

FIGURE 1.1 Map of the research site in Oneida, Tennessee

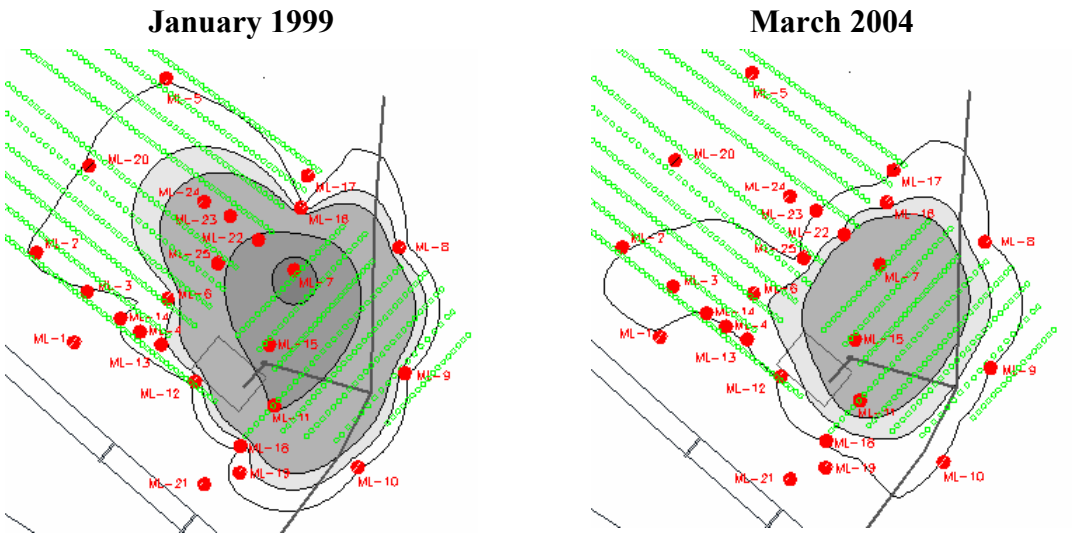

FIGURE 1.2 Groundwater PAH concentration profiles 1-2 m above bedrock

Naphthalene is the primary contaminant mobilized from the creosote into groundwater at the site. Fortunately naphthalene undergoes preferential remediation over PAHs with three or more rings as illustrated in Figure 1.4, which shows concentrations of pyrene and

naphthalene 1 m above the bedrock in the center of the plume. Naphthalene is being removed over time whereas pyrene remains unchanged. Enhanced removal rates of naphthalene were observed after three growing seasons coinciding with the tree roots reaching the groundwater table in 1999 (Figure 1.4). A year later the naphthalene concentration stabilized at a lower level. The new steady state naphthalene concentration is believed to be due to equilibrium between dissolution of naphthalene from the creosote source and removal by the active remediation mechanisms.

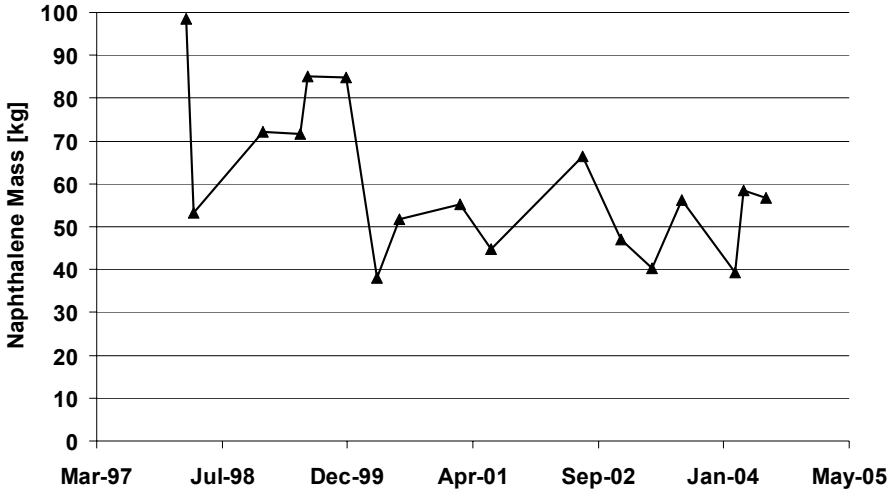

FIGURE 1.3 Total naphthalene mass in the saturated zone above the creosote

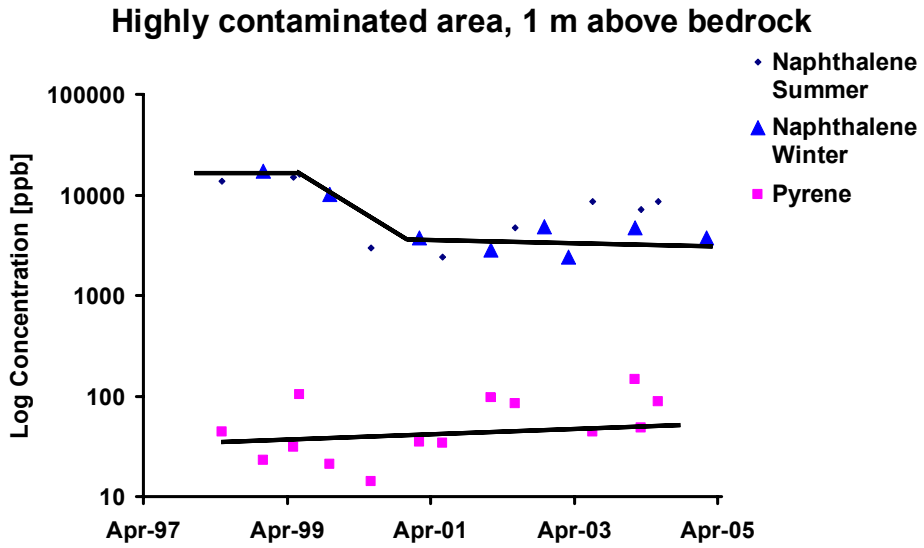

FIGURE 1.4 Naphthalene and pyrene concentrations in the center of the plume, 1 m above bedrock from 1997 to 2005.

The monitoring data supports the hypothesis that phytoremediation accelerates natural attenuation processes when the roots reach the water table, while providing plume control for PAHs at this site. Figure 1.5 illustrates some of the mechanisms that can be active in natural attenuation impacted by phytoremediation.

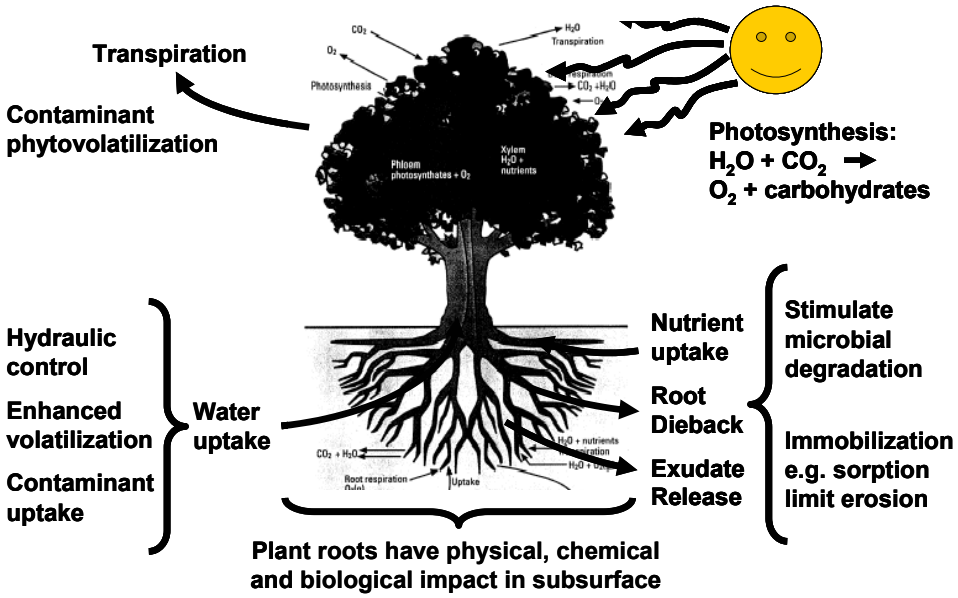

FIGURE 1.5 Potential phytoremediation mechanisms for PAHs, drawing adapted from Burken and Schnoor, 1998 (2).

Direct plant uptake of contaminants followed by phytodegradation of and/or accumulation of contaminants in the plants or volatilization of contaminants to the atmosphere has been found to be important for many different contaminants (1,2). Naphthalene uptake and phytovolatilization are active at the Oneida site, but the naphthalene detected in above ground biomass and released to the atmosphere is relatively small. Transpiring plants can reduce leaching and contain plumes of dissolved contaminants (3,4,5). Field studies have shown that mass transfer to the vadose zone can be a significant removal process for volatile contaminants (6, 7, 8*)*. Transpiration extracts water from the unsaturated zone and lowers the groundwater table, which increases volatilization to the unsaturated zone (9, 10). The transpiration and interception of water by the poplar trees contributes to hydraulic control of the contaminant plume at the

Oneida site. Furthermore, the water consumption contributes to lowering the groundwater table and lowering the moisture content in the unsaturated zone in the summer and fall months (Chapter 3). Field research indicates that the fluctuation of the groundwater table and drying out of the unsaturated zone enhances mass transfer of naphthalene to the vadose zone where biodegradation is rapid (Chapter 3).

Many studies have shown increased biodegradation of PAHs in rhizosphere soil compared to unplanted soil (11, 12, 13, 14, 15, 16,). Field data indicates that aerobic and anaerobic biodegradation is active at this site (17) and enhanced biodegradation of PAHs in the rhizosphere and in the presence of trees have been shown by in situ respiration tests and in laboratory microcosms for this site (14, 18, 19). Rapid aerobic biodegradation is demonstrated above the water table in the field and in a controlled laboratory study (Chapter 3). The steeper concentration gradient created by the biodegradation promotes faster flux out of the source. Mass transfer of oxygen is faster in the vadose zone than in the saturated zone due to faster diffusion in gas than in water. Enrichment of the naphthalene biodegradation potential with long term exposure to naphthalene vapors was demonstrated. The research indicates the fluctuating water table and induced mass transfer create a zone of elevated mass transfer and biodegradation potential. Assessment of the in situ rate of vadose zone biodegradation is highly dependent on accurate estimation of the mass transfer rate to the vadose zone.

The relative importance of biodegradation in the upper saturated zone compared to the vadose zone is an important area for future research in order to optimize natural attenuation and phytoremediation for volatile and semi volatile biodegradable contaminants. Based on this research the impacts of plant transpiration and the plant rhizosphere on biodegradation in the vadose zone are believed to be key system parameters for optimization of phytoremediation.

The principal concept of each of these mechanisms has been proven before in laboratory and small scale applications for various contaminants, but few projects attempt to quantify and compare the relative contributions from all the mechanisms in situ. To my knowledge this is the first in situ quantification and comparison of naphthalene removal by these different phytoremediation mechanisms. Geochemical and biological parameters vary seasonally and spatially at actual sites, which complicate full scale quantitative

comparison of the mechanisms In spite of these limitations, the final chapter of this document pursues such a comparison and at the very least, presents lower and upper bounds for each mechanism.

The overall remediation time for naphthalene at this site is estimated to be up to 100 years with phytoremediation, but up to 240 years without it. The long remediation time is not an issue at this specific site since there is no immediate exposure risk and the contamination is contained by the hydraulic action of the trees and the interception trench. Based on this research, phytoremediation with poplar trees can be a useful tool for remediation of dissolved naphthalene plumes after removal of the majority of the source, but phytoremediation is generally not appropriate as a stand alone remediation method for sites with substantial residual creosote DNAPL.

Chapter 2 contains brief introductions to PAHs, monitored natural attenuation and phytoremediation followed by a detailed literature review of different mechanisms involved in natural attenuation of PAHs impacted by phytoremediation.

Chapter 3 presents the research results for the combined mechanisms of volatilization and biodegradation in the vadose zone at the site. Naphthalene concentration profiles in the soil gas were evaluated in the field and a laboratory experiment was used for determination of flux and biodegradation rates. Lower groundwater elevations in the summer and early fall lead to elevated groundwater concentrations of naphthalene and increased volatilization. The phytoremediation system is believed to enhance mass transfer to the vadose zone by contributing to lowering the water table and drying out the unsaturated zone. The results show that more than 90% of the naphthalene vapors are biodegraded within 5-10 cm above the groundwater table and that aerobic biodegradation rates increase as a result of long term exposure to naphthalene vapors. First order biodegradation rates of 3-26 day^{-1} were obtained in a laboratory column study. The column data indicate that biodegradation significantly increased the overall volatilization flux. This chapter is proposed for publication.

Chapter 4 discusses the research results for the mechanism of uptake and phytovolatilization of naphthalene by poplar trees at the site. In situ measurements of the phytovolatilization of naphthalene were obtained directly on tree trunks in the field. The rates exhibited large seasonal and spatial variability. A laboratory uptake study and analysis of tree cores from the site provided supplementary evidence for naphthalene uptake by poplar trees. Naphthalene phytovolatilization rates and concentrations in plant tissue decrease with the height of the tree. Naphthalene phytovolatilization rates of 4.7 - 46 μg/day per tree were determined based on the field measurements and tree sizes. Phytovolatilization appears to play a minor role in the loss of naphthalene from the site. This chapter is proposed for publication.

Chapter 5 is a Battelle conference proceedings paper containing a preliminary discussion and comparison of vadose zone biodegradation relative to volatilization and phytovolatilization induced by the poplar trees at the site.

Chapter 6 contains an overall estimate for the mass removal of naphthalene at the Oneida site by all the investigated mechanisms. The comparison is conducted for winter and summer conditions as well as with and without the phytoremediation system. This chapter is a first attempt to quantitatively assess naphthalene removal mechanisms for the entire site and the main purpose is to identify the most important mechanisms for future research. Sensitive assumptions and knowledge gaps are discussed for recommendation of future site assessments and research that can enable more accurate quantification estimates. This chapter is proposed for publication.

Appendix A contains a comprehensive site description and long term monitoring data for soil and groundwater at the Oneida site.

Appendix B is a paper published in ES&T presenting comprehensive results and discussion for the long term groundwater and soil monitoring at the Oneida site. The data shows reduction in the overall plume size and PAH concentrations. Preferential removal of naphthalene and three ring PAHs and enrichment of the larger PAHs are observed. The removal rate increased after the third growing season. Nearly steady state conditions

during the last three years of the study suggest that the phytoremediation effectiveness is limited by the dissolution rate from the DNAPL.

Appendix C, published in ES&T, describes a field study at the Oneida site using respiration tests in the saturated zone to assess in situ aerobic biodegradation rates. The first order rates were obtained by applying push-pull tests across the site throughout a year. The rates were 3-5 times higher in areas with trees than in areas with no trees and rates were up to 4 times higher in the summer than in the winter.

The remaining appendices contains supplementary data and calculations

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CHAPTER 2

Literature review

Phytoremediation can enhance or supplement natural attenuation of contaminants. The focus of this chapter is the mechanisms involved in phytoremediation of polycyclic aromatic hydrocarbons. The origin, risk and remediation options for PAHs are discussed in section 2.1. Section 2.2 and 2.3 introduce monitored natural attenuation (MNA) and phytoremediation. Section 2.4 is dedicated to a literature review of the processes involved in natural attenuation of PAHs impacted by phytoremediation. Due to the broad scope the review is not comprehensive but instead aims to discuss key features of each mechanism.

2.1 Introduction to Polycyclic Aromatic Hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) have been part of the Earth's biosphere for millennia. PAHs can originate from natural sources such as volcanic eruptions, combustion of biomass in forest fires or aged, compressed organic material that eventually can turn into crude oil. PAHs are formed during incomplete combustion of organic materials and since the industrial revolution the production and spreading of PAHs into air, soil, water and biota has dramatically increased as a result of human activities. Examples of sources of PAH contamination are soot emissions to the air from incomplete combustion of fossil fuels in power plants and transport systems, road run-off, industrial wastewater discharge and spills into the subsurface from oil and gas industry. Old manufactured gas plant sites for example are typically heavily contaminated with PAHs from waste coal tar (Thomas and Lester, 1993). Creosote, consisting of mainly PAHs, has found widespread use for impregnation of wood in the industrial countries. In coastal regions waste coal gas tar has been applied for preservation of fishing nets which are then laid out to dry, leading to large areas with PAH contaminated topsoil. These are just some examples of the many different anthropogenic sources, which have led to numerous sites with PAH contaminated soil and groundwater as well as ubiquitous global distribution of diffuse PAH contamination in sediments, on topsoil, on vegetation and bioaccumulated through the food chain.

Creosote is a dense non-aqueous phase liquid, which means that it is a viscous liquid mixture at relevant subsurface temperatures, does not mix with water and is denser than water. Creosote has historically been used for wood preservation. It has been spilled into the subsurface soil and groundwater during handling or from unlined storage ponds and leaking tanks at numerous wood treatment facilities. One common use of creosote has been treatment of rail road ties, as is the case at a creosote contaminated site in Oneida, Tennessee (Appendix A). Creosote consists of 85% PAHs by weight, 10% phenolics and 5% N-, S- and O-heterocyclics (Nestler, 1974). Saturated unsubstituted PAHs are most abundant in creosote and naphthalene is the dominating PAH (Prince and Drake, 1999).

2.1.1 Risk associated with PAHs

PAHs become a concern when large amounts or high concentrations are found in the environment, because some are known or suspected to have toxic, mutagenic or carcinogenic properties (IARC, 1983). PAH are lipophilic and therefore have a high potential for biomagnification through the food chain (Kanaly and Haryama, 2000). The U.S. Environmental Protection Agency (USEPA) has classified 17 of the PAHs as priority pollutants listed in Table 2.1 along with their carcinogenity classification and Toxic Equivalency Factors (Nisbet and LaGoy, 1992, ORD, 1993). Benzo(a)pyrene is considered the be most toxic of these priority pollutant PAHs and is 1 of 12 target compounds or groups defined in USEPA's new strategy for controlling persistent, bioaccumulative and toxic contaminants (Kanaly and Haryama, 2000).

In general the larger the PAH is the more genotoxic it is (Table 2.1), and the less mobile it is due to low solubility and volatility (Table 2.2). The risk associated with a contaminant is a function of its toxicity and persistence in the environment as well as its mobility along exposure pathways and potential for spreading (Peters et al, 1999). Sites contaminated with PAHs must therefore be addressed based on a site specific risk assessment considering the contamination distribution and composition in order to effectively protect human health and the environment from risk (Peters et al, 1999). The larger most toxic PAHs will typically be the major concern for PAH contaminated top soil, due to the exposure routes from direct ingestion of soil, inhalation of soil dust in air or through the food chain via consumption of vegetation with PAH deposits. The smaller

PAHs are of relatively less concern in topsoil compared to the larger PAHs, since they are less toxic and can be expected to be volatilized to the air, diluted in rainwater and/or readily aerobically biodegraded or photo-oxidized. In deeper subsurface soil the smaller PAHs may present the most immediate exposure risk due to their mobility via dissolution into groundwater, volatilization into the soil gas and potential uptake to aboveground biomass. The larger more toxic PAHs persist, but are not a direct risk if they reside relatively immobile deep in the subsurface. Here, they remain out of reach for exposure to humans and unlikely to spread further in the environment. In the latter case it is important to consider possible future exposure to the larger persistent PAHs due to dynamic environmental changes or change in site use. It is also often considered socially and environmentally un-ethical by local communities and environmental advocates to simply leave contaminants as a potential problem for future generations.

The production, use and disposal of hazardous materials have endangered human health and the environment. In 1976 the US Congress passed the Resource Conservation and Recovery Act (RCRA) to regulate the production, use, transport and disposal of hazardous materials. The Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) was enacted in 1980 ("Superfund") to promote remediation of hazardous waste contaminated sites that pose a risk to human health or the environment. Sites across the US are inspected to determine which pose a serious threat to human health and the environment under the direction of USEPA. The sites that are in greatest need of remediation are then inventoried and prioritized by EPA on the National Prioritiy List (NPL). A CERCLA list of Priority Hazardous Substances was compiled by EPA and the Agency for Toxic Substances and Disease Registry in 1999 (Olson et al, 2003). The list identified and ranked 275 toxic substances that pose the greatest risk to health and the environment.

The risk to human health and the environment from the widespread and persistent PAH contamination in the global environment is evident as recognized on EPA's priority pollutant list. PAHs are ranked as ninth priority and creosote has a rank of 21. Numerous individual PAHs and mixtures containing PAHs are also ranked on the priority list.

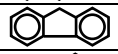
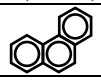
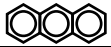
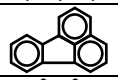
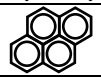
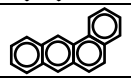
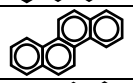
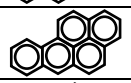
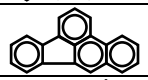
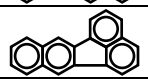
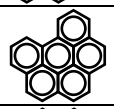
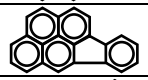
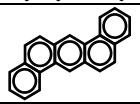
The 30-year remediation cost for contaminated sites in the USA was estimated to be 1700 billion US dollars in 1996 (Timian and Connolly, 1996). It is estimated that up to 352 billion US dollars may be spent in cleanup costs for contaminated sites in Europe in the next 20-25 years (Glass, 1999).

The growing concern about pollution and the enormous costs associated with cleanup of contaminated sites has led to demand for alternative remediation technologies. A risk-based approach to the problem can ensure optimal use of economic resources for pollutant control. There is a need to develop more efficient and economical remediation strategies for PAH-contaminated sites.

TABLE 2.1 Toxicity classification reported Toxic Equivalency Factors (TEF) of the PAHs included in EPA priority pollutants *(*Nisbet and LaGoy, 1992, ORD, 1993)**.**

	Carcinogenity			**Toxicity (TEF)**	
	USEPA group B2	NTP	IARC Group 2	USEPA	Nisbet 1992
Naphthalene					0.001
2-Methylnaphthalene					0.001
Acenaphthylene					0.001
Acenaphthene					0.001
Flourene					0.001
Phenanthrene					0.001
Anthracene					0.01
Flouranthene					0.001
Pyrene					0.001
Benz[*a*]anthracene	X	X	X	0.1	0.1
Chrysene	X			0.001	0.01
Benzo[*a*]pyrene	X	X	X	1	1
Benzo[*b*]flouranthene	X	X	X	0.1	0.1
Benzo[*k*]flouranthene	X	X	X	0.01	0.1
Benzo[*g,h,i*]perylene					0.01
Indeno[1,2,3-*c,d*]pyrene	X	X	X	0.1	0.1
Dibenz[*a,h*]anthracene	X	X	X	1	5

TABLE 2.2 Physical and chemical characteristics of PAHs, T=25ºC=298 K *(*Kjeldsen and Christensen, 1996, Mackay et al, 1992*)*.

Name	Formula	Structure	Molecular weight [g/mole]	Vapor Pressure [Pa]	Aqueous solubility [mg/L]	Water-gas distribution coefficient, K_H (Mackay et al, 1992)	Octanol-water distribution coefficient, log K_{ow}	Water-organic carbon distribution coefficient, log K_{oc} *(usually proportional to water-soil distribution coefficient, K_D)*		
								Laboratory	Field	Estimated
Naphthalene	$C_{10}H_8$		128.2	10.4	31.0	0.017	3.36	3.11	5	2.65
2-Methylnaphthalene	$C_{11}H_{10}$		142.2	9.0	25.4	0.018	3.86	3.93	-	3.17
Acenaphthylene	$C_{12}H_8$		152.2	0.90	3.93	$4.8 \cdot 10^{-3}$	4.1	3.56	-	3.42
Acenaphthene	$C_{12}H_{10}$		154.2	0.30	3.42	$6.0 \cdot 10^{-3}$	3.92	3.66	5.38	3.24
Flourene	$C_{13}H_{10}$		166.2	0.09	1.98	$4.1 \cdot 10^{-3}$	4.18	-	5.47	3.51
Phenanthrene	$C_{14}H_{10}$		178.2	0.016	1.2	$1.6 \cdot 10^{-3}$	4.57	4.36	6.12	3.91
Anthracene	$C_{14}H_{10}$		178.2	$1.4 \cdot 10^{-3}$	0.041	$2.4 \cdot 10^{-3}$	4.54	4.42	5.76	3.88
Flouranthene	$C_{16}H_{10}$		202.3	$1.3 \cdot 10^{-3}$	0.21	$5.0 \cdot 10^{-4}$ *	5.22	-	6.38	4.59
Pyrene	$C_{16}H_{10}$		202.3	$6.1 \cdot 10^{-4}$	0.14	$4.4 \cdot 10^{-4}$	5.18	4.92	-	4.55
Benz[*a*]anthracene	$C_{18}H_{12}$		228.3	$2.7 \cdot 10^{-5}$	0.014	$3.3 \cdot 10^{-4}$	5.61	-	6.3	4.99
Chrysene	$C_{18}H_{12}$		228.3	$8.4 \cdot 10^{-7}$	$2.0 \cdot 10^{-3}$	$5.0 \cdot 10^{-4}$ *	5.91	-	6.27	5.31
Benzo[*a*]pyrene	$C_{20}H_{12}$		252.3	$7.3 \cdot 10^{-7}$	$3.8 \cdot 10^{-3}$	$3.9 \cdot 10^{-4}$ *	6.5	-	6.26	5.92
Benzo[*b*]flouranthene	$C_{20}H_{12}$		252.3	$5.0 \cdot 10^{-7}$	$1.5 \cdot 10^{-3}$	$3.0 \cdot 10^{-5}$ *	6.57	-	-	5.99
Benzo[*k*]flouranthene	$C_{20}H_{12}$		252.3	$1.3 \cdot 10^{-8}$	$8.0 \cdot 10^{-4}$	$1.6 \cdot 10^{-6}$ *	6.84	-	5.99	6.27
Benzo[*g,h,i*]perylene	$C_{22}H_{12}$		276.3	$1.3 \cdot 10^{-8}$	$2.6 \cdot 10^{-4}$	$5.6 \cdot 10^{-6}$ *	6.90	-	-	6.34
Indeno[1,2,3-*c,d*]pyrene	$C_{22}H_{12}$		276.3	-	$6.2 \cdot 10^{-2}$	-	7.66	-	-	7.13
Dibenz[*a,h*]anthracene	$C_{22}H_{14}$		278.4	$3.7 \cdot 10^{-10}$	$5.0 \cdot 10^{-4}$	$8.3 \cdot 10^{-8}$ *	6.5	6.31	-	5.92

2.1.2 Remediation of PAH-contaminated soil and groundwater

Traditional remediation practice for PAH contaminated soil is excavation and landfilling or composting. This approach is expensive, energy and transport intensive, disrupts the site and creates exposure risk during the remediation. Landfilling rather than destroying the contaminants or detoxifying the soil, means the contamination remains a problem for future generations to inherit. Conventional pump-and treat based methods to clean PAH contaminated groundwater will usually lead to prolonged remediation times due to the slow release of PAHs from soil and DNAPL. This requires continuous energy input and cost-intensive above ground treatment over many years. In recent years the search for less expensive, less energy intensive and less intrusive remediation options for PAH contaminated soil and groundwater has ignited the interest for in-situ strategies.

In-situ remediation leaves the contaminated soil and groundwater in place and thus does not involve above ground treatment of groundwater or invasive excavation of soil for treatment or disposal. The integrity of the site is maintained and minimal disturbance is involved during in-situ treatment. The exposure risk for humans due to handling and transport of contaminated soil and groundwater is avoided. Furthermore the energy consumption involved in excavation and pumping of large quantities of soil and groundwater as well as energy for above ground treatment and/or transport to disposal in a landfill is eliminated. In-situ remediation strategies, which destroy or detoxify contaminants in place, can involve both abiotic, such mechanical, thermal or chemical, and biological remediation of soil and groundwater.

Biological remediation processes have the advantage of removing the contaminants while maintaining or restoring soil quality properties, such as organic matter content, texture and soil ecosystem. The preservation of soil quality is crucial for sustainable management of soil as a natural resource. MNA and phytoremediation are both low cost and low impact biological in-situ treatment technologies that benefit from the inherent ability of ecosystems to degrade, detoxify or immobilize contaminants. In-situ phytoremediation can enhance and supplement the remediation processes occurring in natural attenuation.

Common for all in-situ treatment technologies is that it is crucial to understand the processes that govern the fate and transport of the contaminants under the site specific

geochemical and biological conditions. The site specific knowledge can be integrated into a conceptual model. The conceptual model for the site is used to perform a sound risk assessment and to design appropriate remediation strategy and monitoring systems. Computer modeling based on the conceptual model and monitoring data can be applied to predict the time frame and end result of the cleanup process. At this point the scientific knowledge of the mechanisms involved in phytoremediation is still at an early stage. Computer models and validation of principles in long-term full scale field applications are still scarce for phytoremediation. This is a major problem, since proving how the technology works, evaluating the risks, predicting how long it will take and what the end result will be are all required to achieve public and regulatory acceptance.

2.2 Introduction to Monitored Natural Attenuation (MNA)

This focus of this review is phytoremediation of PAHs, but natural attenuation is an integrated part of an in-situ phytoremediation system. The natural attenuation processes are affected by the incorporation of plants in the remediation strategy. Since plants add a layer of complexity it is beneficial to describe the natural attenuation processes for PAHs first and then discuss how plants can enhance and supplement these processes.

MNA has found widespread application and is usually an integrated part of in situ treatment of hazardous waste sites. MNA is often suitable for large areas with moderate contamination, such as brownfields. Brownfields are industrial areas contaminated by activities in the past, but with economic value for redevelopment if the contamination is removed or controlled (Licht and Isebrand, 2005). At sites where the contaminant source has been removed with a more aggressive technology, the residual contaminated soil and groundwater contaminant plume can often be cleaned by MNA over time.

MNA has been subject to controversy since some environmental advocates have argued that it is a “do-nothing” technology. On the other hand the technology has been embraced by industry as a means to save money on clean-up of low risk sites with widespread diffuse contamination. The truth about MNA is somewhere in between these two extremes. Depending on the specific site and contamination, MNA is potentially a low cost alternative requiring less aggressive treatment, but it is not the same as doing nothing. Proving that natural attenuation confines and remediates contamination over

time, while not posing an unacceptable risk to humans or the environment, is a key part of implementation of this technology.

USEPA stated in a 2002 guidance document (USEPA, 2002) that MNA refers to *"an approach to clean up environmental contamination by relying on natural processes and monitoring. Natural attenuation processes include a variety of physical, chemical and biological processes that, under favorable conditions, act without human intervention to reduce the mass, toxicity, mobility, volume or concentration of contaminants in groundwater".* USEPA highlighted the destruction and immobilization processes as being essential for the success of MNA and said that MNA is likely to be acceptable to regulators when

1) it is demonstrated that MNA is likely to achieve cleanup goals

2) source control measures are in place,

3) the dominant natural attenuation processes are those that cause destruction of contaminants rather than merely diluting and preventing them from spreading.

A committee under the National Research Council (NRC) issued a comprehensive assessment of natural attenuation in 2000 (NRC, 2000). The NRC committee advocated that processes such as dilution and dispersion are unacceptable as primary ways to manage contamination. In other words, it is not sufficient to show that contaminant concentrations become low or undetectable over time; the underlying removal mechanisms must be understood and documented. The chairman of the NRC natural attenuation committee, Bruce Rittmann of Northwest University, said; *"natural attenuation is a valid concept…under the right circumstances, but only when the mechanisms responsible for the destroying or immobilizing the contaminant is scientifically recognized, documented to be working at the specific site and sustained for as long as the source is present"* (NRC, 2000). The NRC also stresses that natural attenuation processes always are site specific dependent on present hydrogeology, biogeochemistry and contamination. The committee recommended a three step evaluation process for natural attenuation, which would work for phytoremediation applications as well: 1) providing a conceptual model of the site,

2) looking for "footprints" of the expected natural attenuation processes

3) monitoring the site.

In order to provide the conceptual model, look for footprints and decide on an appropriate monitoring program it is necessary to understand how the contaminants are transported and distributed in the subsurface and what natural attenuation processes are active and most important at a specific site. In the following discussion, "removal mechanism" is used as a synonym for irreversible immobilization or destruction of the contaminant to render harmless degradation products. Processes such as volatilization and dissolution, which transfer the contaminants from one phase to another, are not considered removal mechanisms.

2.3 Introduction to Phytoremediation

Phytoremediation is the use of plants, algae and fungi to remove or control contamination or to stimulate waste breakdown by the microbial community in their rhizosphere (Meharg, and Cairney, 2000). A number of studies show that phytoremediation holds promise for accelerating natural attenuation of PAHs (Aprill et al, 1990, Banks et al, 1999, Joner et al, 2001, Reilly et al, 1996, Robinson, 2001, Robinson et al 2003, Schnoor et al, 1995).

Phytoremediation, like MNA, can often be an affordable remediation strategy for large areas with diffuse contamination and for secondary remediation of diffuse contamination at sites where the source has been removed. Phytoremediation is developing into a primary remediation strategy for some contaminant specific applications and under some conditions source removal is not necessary. Already plant stimulated rhizodegradation of PAHs and petroleum hydrocarbons is the primary remediation at some sites (Hutchinson et al, 2003). In addition phytoremediation can be used proactively in green technology to prevent accumulation of contaminants over time or control spreading of contaminants e.g. from landfills' leachate and agricultural runoff.

Plants on contaminated sites have traditionally been considered as a potential pathway for increased exposure of contaminants from soil and groundwater to the above ground environment, wildlife and humans via uptake in the food chain (Landmeyer, 2001). Plants have also been used as ecological indicators at contaminated sites (Landmeyer, 2001). In recent years accumulating evidence of plants ability to enhance

natural attenuation processes had led to a shift in the risk perception and plants are now being included in the remediation strategy at many sites.

Bioremediation and biodegradation in natural attenuation typically rely on heterotrophic microbial communities present in the natural ecosystem. These communities usually do not operate in isolation, but are affected by other partners in their ecosystem. An important mechanism in phytoremediation is the plants' ability to spur greater heterotrophic biodegradation in their rhizosphere.

Plant metabolism is fundamentally different than metabolism of heterotrophic microorganisms used in bioremediation. Plants are photoautotrophic and use sunlight and atmospheric CO_2 to produce organic matter, whereas heterotrophic microorganisms require energy and carbon from water or soil. Phytoremediation applications are solar powered and more sustainable compared to enhanced bioremediation and mechanical remediation strategies.

Plants and microbes both require water and nutrients from their environment, but vascular green plants have the ability to exert some control over their rhizosphere. The rhizosphere is defined as the zone of soil directly influenced by plant roots (Curl and Truelove, 1986). To some degree plants can "self-engineer" the local microclimate, and biochemistry as well as availability of nutrients and water in the rhizosphere (McCutcheon and Schnoor, 2003). Plants have developed remarkable abilities to survive in hostile environments and these trades are the basis for phytoremediation. Plants can tolerate high concentrations of some contaminants and use others as nutrients and therefore be pioneer species in recovering damaged ecosystems. Plants also exude simple organic compounds, enzymes and fertilize soil with root dieback. This can spur growth of richer and more diverse microbial communities in the rhizosphere. Plants can also change redox and pH conditions to favor these symbiotic microbes. Furthermore plants can stabilize, precipitate and bind contaminants in the rhizosphere (Davis et al, 2002).

At sites where PAH contaminants do not represent an immediate risk to humans or the ecosystem, phytoremediation may offer a lower impact and less expensive alternative than conventional remediation technologies.

A major advantage of using MNA or phytoremediation is that the biological viability of the soil and ecosystem can be restored during the process. Phytoremediation

can also meet secondary objectives such as site maintenance, erosion control and providing biomass for energy or as raw material for processing (Davis et al, 2002, Marmiroli and McCuthcheon, 2003, Licht and Isebrand, 2005).

Like natural attenuation, in situ phytoremediation of PAHs can be relatively slow; sometimes decades are required for satisfactory clean up goals to be reached (Trapp and Karlson, 2001, Karlson et al, 2001). Mass transfer may limit availability and therefore cause the slow remediation, or sources such as residual NAPL can be present and continue to leach contaminants into the groundwater and soil gas. Traditionally it has been recommended to remove as much of DNAPL source areas as possible before installing phytoremediation or relying on natural attenuation for plume remediation at contaminated sites (USEPA, 2002). The reason is that the relatively slow processes of natural attenuation and phytoremediation must be able to prevent spreading of the contaminant plume. The rate of release of contaminants from NAPL sources must therefore be proven to be less than the remediation rate. There is currently sparse long term data on the final endpoints and time frame for cleanup with phytoremediation (Schnoor et al, 1995).

Phytoremediation is not applicable for all contaminants and phytotoxicity may occur for high concentrations or specific chemicals. Furthermore phytoremediation is limited to contamination within the depth of the rhizosphere or the depth of influence from evapotranspiration depending on the removal mechanisms most important in the specific phytoremediation application.

In summary some main advantages and disadvantages of phytoremediation are:

Advantages:	**Disadvantages / limitations:**
Presumed lower costs at some sites	Relatively slow for many applications
Applicable to extensive areas with diffuse contamination	Depth of treatment limited and mass transfer may limit availability
Solar driven, sustainable energy	Complex knowledge based system
Site maintenance and erosion control	Phytotoxicity to many contaminants
Secondary biomass production	Risk for uptake in foodchain
Attractive as a green technology and aesthetically pleasing	Some regulatory policies not consistent with use of phytoremediation

The scientific understanding of phytoremediation is interdisciplinary in nature (Landmeyer, 2001). There are a multitude of physical, chemical and biological interactions between plants and the soil ecosystem, which can affect the natural attenuation processes for PAHs in soil and groundwater (Davis et al, 2002, McCuthceon and Schnoor, 2003). Sorption to roots and the increased organic matter from plants can retain PAHs in the root zone. Transpiring trees can be viewed as solar driven pumps that decrease infiltration of clean rain water, which decreases the dissolution of PAHs into the groundwater as well as aids in hydraulic control of contaminated groundwater containing dissolved PAHs (Matso, 1995). The lowering of the groundwater table may also decrease DNAPL migration, since the DNAPL tends to pool in areas with less hydraulic pressure from above. Even though these mechanisms do not reduce the overall contaminant mass, they can be important factors in stabilizing the contaminated area for the time needed for other phytoremediation processes to occur. Transpiration can enhance the volatilization of contaminants to the unsaturated zone and aeration of the subsurface (Landmeyer, 2001), which along with various interactions in the rhizosphere of plants can stimulate enhanced biodegradation (Anderson et al, 1993). Finally plants can take up certain contaminants and either degrade and/or store these in plant tissue or release them to the atmosphere (Burken and Schnoor, 1998).

Potential mechanisms in natural attenuation impacted by phytoremediation are:

- Sorption to soil and roots
- Immobilization in soil
- Hydraulic control
- Plant uptake and accumulation
- Volatilization
- Plant uptake and phytovolatilization
- Biodegradation
- Rhizodegradation
- Plant uptake and phytodegradation

Each of these mechanisms are reviewed and discussed in the following section.

2.4 Mechanisms in MNA of PAHs impacted by phytoremediation

2.4.1 Mass transfer and distribution processes

When a DNAPL source of PAHs, as for example creosote, is spilled on soil, it sinks down through the subsurface until it reaches confining layers as for example clay lenses or bedrock. Residual globules of the DNAPL are trapped in soil pores as the DNAPL travels downward. Natural weathering processes leads to distribution of components of the creosote into the different phases in the subsurface according to their chemical and physical properties. Table 2 shows some key parameters governing the mass transfer of the ten PAHs most prevalent in creosote. The smaller PAHs with higher vapor pressure and water solubility will volatilize into the soil gas and dissolve into the groundwater to a greater extent than the larger PAHs. Likewise the larger and more hydrophobic PAHs have stronger sorption tendencies and smaller solubility and therefore sorb to the soil phase and remain in the DNAPL phase to a greater extent than the smaller PAHs.

Over time the contaminant source is enriched in the more recalcitrant heavy compounds, while the fraction of the lighter more mobile and degradable compounds decreases. This was observed in a long-term monitoring study for PAHs dissolved in groundwater at a creosote-contaminated site in Oneida, Tennessee. Furthermore the contaminants left behind in the source migrate deep into soil pores and become more incorporated into the soil matrix over time, which makes them even less mobile and bioavailable (Burgos et al, 1996, Hatzinger and Alexander, 1995). These processes are called “aging” and it is important to consider these dynamic changes of the contamination composition and mobility.

In systems with plants some additional distribution pathways must be considered. Contaminants can sorb to the surfaces of plant roots (Schwab et al, 1998). Contaminants can also be taken up directly via the plants’ transpiration of contaminated groundwater (Briggs et al, 1982). Passive diffusion of contaminants in solid, gas or liquid phase in and out of plants also occurs due to concentration gradients and/or affinity for sorption to biomass (Ma and Burken, 2003). Upon entering the plant, contaminants can undergo transformation processes, accumulate in plant tissue, be transferred to the atmosphere via phytovolatilization or be subject to some combination of these processes.

Mass transfer and transport of contaminants in soil, NAPL, groundwater, soil gas and biomass do not reduce the overall mass of the contaminants even though they may appear to if dilution and spreading occur to a degree where the PAH concentrations become undetectable. However mass transfer and transport can indirectly facilitate or limit remediation by making the contaminant more or less exposed to removal mechanisms. For example volatilization from the anaerobic saturated zone to the aerobic unsaturated zone may result in increased biodegradation. Sorption to organic carbon and root tissue may result in contaminants being less bioavailable and thus decrease removal by biodegradation (Schwab et al, 1998). Volatilization of PAHs to the atmosphere may result in removal by photochemical reactions such as photoxidation and ozonolysis (Finlayson-Pitts and Pitts, 2000).

In order for natural attenuation impacted by phytoremediation to be an acceptable remediation strategy the overall mass must be decreased or detoxified over time. Mass transfer, dilution and dispersion of the contaminants are not sufficient. Possible transformation processes can include complete mineralization, transformation to less toxic degradation products or irreversible immobilization into soil or organic matter.

2.4.2 Dissolution

The degree of dissolution of PAHs into the groundwater is a function of groundwater flux through the contaminated area as well as contaminant solubility. The groundwater flux depends on vertical infiltration as well as horizontal groundwater flux into the contaminated area from upstream areas of higher hydraulic pressure. The infiltration is a function of precipitation subtracted evapotranspiration from the ground surface and vegetation. Due to weather (e.g. rain and temperature) and yearly vegetation growth cycles the groundwater table is generally higher in the winter than in the summer. Thus the amount of groundwater that comes in contact with subsurface contamination, and therefore the contaminant mass dissolved, can be expected to be larger during winter than during summer. The dominating PAH in creosote is naphthalene and the solubility is also largest for the smaller PAHs, so naphthalene will typically be the dominating PAH found dissolved in groundwater in contact with creosote. Typical solubilities in water for PAHs are listed in Table 2. Groundwater concentrations of the larger PAHs beyond their low

solubilities can occur when relatively high concentration of naphthalene is present due to co-solvent effects (Widdowson et al, 2005).

The solubility of contaminants can also be increased by enzyme activity resulting in oxidation or reduction. Oxidation is an important reaction for increasing solubility of lipophilic compounds. A common example is increased solubility by hydroxylation (adding OH^-) induced by P-450 enzyme activity (Burken, 2003).

Surfactants and biosurfactants can increase the solubility of hydrophobic compounds such as PAHs by reducing their surface tension or incorporating them into micelles. Increased solubilization of PAHs treated with surfactants has been shown from the sorbed phase in soil (Tiehm et al, 1997) and from NAPL (Hill and Goshall, 2002). The increased dissolution enhances the bioavailability of the PAHs. The biodegradation was found to increase significantly by surfactant treatment of an aged PAH contaminated soil from a manufactured gas plant site (Tiehm et al, 1997).

2.4.3 Sorption

Due to the high hydrophobicity of PAHs, sorption to soil and organic matter is a significant process with increasing importance for larger PAHs. Organic matter content and type is usually the single most important factor controlling sorption of PAHs in soil (Kjeldsen and Christensen, 1996) but the number of active sorption sites on mineral surfaces affects sorption as well. The sorption coefficient for a PAH to soil usually correlates well with the organic carbon content of the soil. Thus the sorption coefficient for a specific soil (K_d) can be estimated based on sorption coefficients for the PAH to organic carbon (K_{oc}). Typical K_{oc} values for PAHs are listed in Table 2.

Plants increase the soil organic matter content and humus by sloughing as root tips penetrates the soil, exudation of organic compounds and root die back in the winter (Anderson et al, 1993, Leigh et al, 2002). Hence the number of organic sorption sites is increased in soil impacted by plant roots. The roots themselves can also serves as sorption sites for pollutants (Briggs et al, 1982). The sorption coefficient for PAH to roots were found to be similar to sorption coefficients to organic matter in soil in a study by Schwab et al (1998). Roots must be close to the soil sorbed PAH for transfer to occur, so plants with extensive mass of fine roots or mychorrizae fungi are advantageous for contaminant

control by sorption. Heavy PAH will only move to roots if roots come close, whereas naphthalene may be accumulated as water containing naphthalene flows past root surfaces. Sorption of contaminants on or in roots is characterized by the root concentration factor (RCF) defined as the concentration in the roots relative to the concentration in the surrounding water (Briggs et al, 1982).

Desorption is a function of the flux of clean groundwater through the contaminated area. Groundwater chemistry such as the presence of co-solvents, biosurfactants or emulsifiers, pH, and salt concentration also influences the degree of desorption. Organic compounds often undergo an initial rapid desorption followed by a much slower desorption. Physical aging processes in PAH contaminated soil can involve diffusion of compounds deeper into micropores in the soil particles (Wu and Geschwend, 1986), partitioning into organic matter (Brusseau et al, 1991, Hatzinger and Alexander, 1995) and stronger surface adsorption. The aging results in increasingly slow desorption rates retarded by diffusion limitations, partitioning out of the organic matter and desorption from surfaces, which can be mistaken for irreversible immobilization if the mass transfer is only evaluated for a short time period. The rate of desorption is a general limitation common to all water based remediation technologies such as pump-and-treat and biodegradation, which primarily takes place in the water phase. The most desorption limited PAHs are often persistent in natural environments (Hatzinger and Alexander, 1995). Desorption and dissolution rates were found to be limiting for the biodegradation of PAHs at a creosote contaminated site in Oneida, Tennessee (Smartt, 2002). In addition to sorption of contaminants, chemical or biological transformation coupled with immobilization into soil matrix or organic matter can be important mechanisms for PAHs. Immobilization can be considered a removal mechanism given that the soil is truly detoxified and that the contaminants are irreversibly bound.

2.4.4 Irreversible immobilization

Strong chemical bonding of contaminants to the soil matrix can lead to immobilization of contaminants (Berry, 1999). The immobilization can make the soil less toxic to living organisms, but at the same time makes the contaminant unavailable for biodegradation. Often the strong chemical binding is extremely inert, but some may become labile for

example due to the action of enzymes (Hsu and Bartha, 1974). Irreversible incorporation of organic contaminants, such as phenolic compounds, to humic substances and activated carbon (sooth), is well documented (Burgos et al, 1996). Generally this process is thought to be due to covalent binding via oxidative coupling into soil organic matter (Novak et al, 1998, Berry, 1999). The organic matter and humus available for immobilization by incorporation are increased in soil impacted by plant roots. Parrish et al showed that the presence of plant roots and time passage reduces the bioavailability of target PAHs (Parrish et a,l 2005).

Saturated PAHs are not believed to be subject to oxidative coupling, but metabolic substituted intermediates such as hydroxylated PAHs produced during aerobic biodegradation may undergo oxidative coupling (Banks et al, 1999, Burgos et al, 1996). Irreversible binding of naphthalene intermediates into soil humus as a result of oxidative coupling has been documented (Burgos et al, 1996). The oxidative coupling can be mediated biologically by enzymes (Bollag, 1992) or catalyzed by clay minerals (Wang et al, 1978) or metal oxide surfaces in the soil (Stone, 1987). Some biological enzymes that have been shown to induce oxidative coupling are peroxidase, lacchase and tyrosinases (Novak et al, 1998). In some cases microbes have been shown to decouple and biodegrade humus incorporated contaminants over time (Bollag, 1992). Some plants or fungi and bacteria associated with their roots produce lacchase and peroxidase (Donelly and Entry, 1999, Wolfe and Hoehamer, 2003) believed to activate oxidative coupling of hydroxylated PAHs produced from aerobic biodegradation (Novak et al, 1998). Banks et al (1999) found an increase in the degradation of ^{14}C marked benzo(a)pyrene in soil planted with tall fescue compared to unplanted controls, but more than 90% of the ^{14}C label remained in the soil, showing that the major sink for the degradation products was the soil matrix. Complete mineralization to CO_2, volatilization and plant uptake were found to be minor pathways for this 4-ring PAH.

In risk assessment and determination if a site is clean it is important to distinguish the irreversibly bound contaminants from those slowly desorbing (Novak et al, 1998). However if the desorption is sufficiently slow compared to simultaneous degradation and immobilization processes it may be sufficient to protect against spreading of the contaminants to protect human health and the environment.

2.4.5 Hydraulic containment by transpiration

Plants with high water consumption have the potential to alter the site hydrology by evapotranspiration of large quantities of precipitation, soil moisture and groundwater. Plants decrease infiltration of rainwater and can for example be applied as vegetative caps on landfills to limit leaching (Jordahl et al, 2003), prevent groundwater contamination from sources in the unsaturated zone (Landmeyer, 2001) and decrease runoff from agricultural areas in vegetative stream buffer zones. Plants and trees can be viewed as solar driven pumps. "Pump-and–tree" is a low maintenance, low cost alternative to pump-and-treat under appropriate conditions (Al-Yousfi et al, 2000).

Groundwater levels, flow direction and fluxes can be altered by plants and used for hydraulic control of dissolved contaminant plumes in the groundwater (Landmeyer, 2001). Phreatophytes, such as poplar and willow, that develop tap roots penetrating downward to the groundwater table, can have natural root depths as deep as 50 meters when they do not find sufficient water in the surface soil (Negri et al, 2003). Root growth manipulation techniques are being developed to achieve deeper roots to contaminated depth in temperate regions with where roots do not grow deep due to presence of sufficient transpiration water in shallow depths (Negri et al, 2003).

Transpiration is affected by meteorological factors such as relative humidity, wind speed, temperature and radiation for photosynthesis (Landmeyer, 2001). Furthermore transpiration can be constrained by site specific bioavailability of the water such as soil moisture, conductivity and depth to groundwater table (Landmeyer, 2001). It can be difficult to monitor hydraulic control effects from plants due to the dynamic climatic factors causing seasonal fluctuations in groundwater table, but Landmeyer (2001) has discussed various strategies for monitoring the hydraulic control effects in phytoremediation systems.

Plant species and density are naturally important factors in transpiration rates (Al-Yousfi et al, 2000). All plants exposed to the atmosphere having C-3 and C-4 photosynthetic pathways of photosynthesis consume water, but C-3 plants (e.g. alfalfa) consumes roughly twice as much water as C-4 plants (maize) (Kramer and Boyer, 1995).

Transpiration rates are generally proportional to the biomass production so fast growing plants are preferred candidates for maximum water uptake (Davis et al, 2002).

The total leaf surface area is also proportional to the transpiration (Al-Yousfi et al, 2000), so a closed tree canopy is ideal (Kjeldsen and Christensen, 1996). Deep-rooted fast growing phreatophytes such as poplar, mulberry, willow and alfalfa are good candidates for hydraulic control of shallow to moderate depth groundwater (Al-Yousfi et al, 2000). Even though alfalfa is not a tree it has considerable transpiration and extensive root systems that can reach 10 m below ground (Narayanan et al, 1999b).

In general direct evaporation from rain is greater from a tree canopy than from shorter crops, but the transpiration may be less (Davis et al, 2002). Poplar trees can act as low-flow pumps and remove 20 liters/day for young trees and 120 liters/day for 5 year old trees under optimal conditions (Newman et al, 1997). Willow trees can transpire up to 200 liters/day (Gatliff, 1994). In a field study in Australia by Cochis et al (2000) evaporation of alfalfa and poplar trees were compared. The peak alfalfa crop water usage was 255-256 mm in July and August, while for 3 year old poplars it was 231 and 173 mm in the same months. The tree crop canopy was closed at the third growing season, and the site is located in an area with a long growing season, so the estimated water usage for the polar trees is believed to be close to maximal. The implication of the study is that the water usage by poplar trees is unlikely to be larger than that of alfalfa at the specific site.

Plants can draw up water containing dissolved contaminants from the saturated zone to the vadose zone (Zhang et al, 2001, Davis et al, 1993), where the contaminants can either volatilize out of the ground surface or be biodegraded (Narayanan et al, 1999a). Transfer of water to the unsaturated zone has been observed for poplar seedlings, maize and alfalfa (Hansen and Dickson, 1979, Zhang et al, 2001).

2.4.6 Plant uptake of contaminants

Uptake of pollutants by plants can occur in different ways. Contaminants can partition into the lipophilic root solids or be taken up dissolved in water with the transpiration stream (Briggs et al, 1982, Burken and Schnoor, 1998). Uptake can also occur by diffusion from soil gas in the unsaturated zone (Ma and Burken, 2003) or by uptake of pollutants from the atmosphere **(**Jeffers and Liddy, 2003**).**

Plant uptake in the transpiration stream is usually described the transpiration stream concentration factor (TSCF) defined as the concentration in the transpiration stream

relative to the concentration in the surrounding water (Briggs et al, 1982). Briggs et al found that the plant uptake varied with the octanol-water coefficient for different compounds in a bell shape curve function with an optimum log K_{ow} of 1.78, and Burken(1998) later found the maximum TSCF at a log K_{ow} of 2.5 (Briggs et al, 1982, Burken and Schnoor, 1998). Compounds with log K_{ow} between 1 and 3.5 have been demonstrated to be taken up along with water (Burken and Schnoor, 1998). Compounds with log K_{ow} higher than the optimum (more hydrophobic) can cross lipid membranes and enter the roots, but will then tend to be bound in the roots rather than taken up in the xylem. Compounds with log K_{ow} lower than the optimum (more hydrophilic) have more difficulty passing through the lipid membranes in the roots, but are then more readily translocated to stem and leaves via the xylem (Burken and Schnoor, 1998). The only PAH in this range is naphthalene with a log K_{ow} of 3.36 (Table 2.2), but metabolites from biodegradation of PAHs may also fall within the range.

Compounds with log K_{ow} greater than 3.5 become bound in the roots (Burken, 2003). The affinity of organic compounds to bind to root tissues is expressed by the root concentration factor (RCF), which is the concentration in the root tissues relative to the concentration in the groundwater (Burken and Schnoor, 1998). RCF has also been described as a function of low K_{ow} (Briggs et al, 1982): Log RCF = 0.77 K_{ow} – 1.52.

After uptake the contaminants can either diffuse out through roots in the unsaturated zone, diffuse out of aboveground biomass to the atmosphere or can be biodegraded and/or stored within the plant. Plant accumulation, phytodegradation and phytovolatilization are described in more detail later.

2.4.7 Volatilization

Volatilization of contaminants present as free phase, sorbed to the soil or dissolved in the groundwater is a potentially significant contaminant transport process. Studies have documented volatilization of hydrocarbons coupled with aerobic biodegradation in the vadose zone (Baerh 1987, Lahvis et al, 1999). Volatilization of hydrocarbons has been shown to be the primary mechanism for fresh crude-oil spills, while biodegradation becomes more dominant as the plume ages (Chaplin et al, 2002). Few numerical groundwater models incorporate volatile losses and this may result in over prediction of

biodegradation rates (Chaplin et al, 2002). In particular the role of volatilization has been largely overlooked in consideration of phytoremediation of PAHs. This is warranted by the relatively low vapor pressures and gas-water phase distribution coefficients characteristic for PAHs compared to volatile organic compounds such as for example BTEX, MTBE and chlorinated solvents. However, in the light of the low solubility of PAHs, volatilization may be relatively important for the fate and transport especially for the 2-3 ring PAHs. Volatilization of PAHs to the unsaturated zone could be coupled with biodegradation in the more oxygen rich unsaturated zone or volatilization to the atmosphere where photodegradation can take place (Finlayson-Pitts and Pitts, 2000).

Measurements of volatilization and biodegradation of semi-volatiles have been carried out under induced venting in laboratory experiments or in the subsurface to test requirements for bioventing systems, but little work has been reported on volatilization under natural conditions (Narayanan et al, 1999b, Smith et al, 1996). Park et al. (1990) found that 20-30% of the measured loss of naphthalene was due to volatilization in a laboratory experiment with a high air-replacement rate. In a study of adsorption of radioactive labeled naphthalene to fescue and alfalfa roots, up to 45% of the radioactive labeled naphthalene was volatilized (Schwab et al, 1998). Up to 38% of the total naphthalene was volatilized in 28 days (Hickey and Paek, 1996). Although air replacement rates in-situ can be expected to be lower and the depth to the location of the contamination under field conditions, these studies suggests that volatilization may be an important mechanism in the phytoremediation of aromatic hydrocarbons.

Diffusion of contaminants in soil gas depends on the concentration gradient as well as the available pore space for gas transport. Since diffusion is several orders of magnitude faster in air than in water, volatilized PAHs will spread much faster in the soil gas than in the groundwater. Temperature affects volatilization, since the effective diffusivity is temperature dependent. The Henry's constant for naphthalene was found to be 0.018 at 10ºC and 0.031 at 25ºC (Dewulf et al, 1998).

Soil moisture affects gas diffusion in the unsaturated zone due to the change in air filled porosity available for volatilized contaminants in the soil gas (Washington, 1996). A dry soil will have higher volatilization and higher aeration than a wet soil, which can promote aerobic biodegradation of the contaminants in the unsaturated zone. On the other

hand biodegradation occurs primarily in the water (Kjeldsen and Christensen, 1996, Alexander, 1994) and vertical contaminant transport can be retarded by partitioning of the contaminants into the soil water (Davis et al, 2001). The degree of volatilization and biodegradation in the unsaturated zone is therefore likely to be correlated to the moisture level. There is believed to be an optimal soil moisture level that allows volatilization and aeration but also ensures sufficient soil moisture for biodegradation and retardation of the contaminants before they reach the ground surface.

Pressure differences between the atmosphere and the subsurface can drive advective transport of soil gas and its constituents up through the unsaturated zone or advective transport of clean surface air down into the unsaturated zone in systems with open ground surface (Massman and Farrier, 1992). Results of soil gas surveys can therefore show lower concentrations of contaminants in the soil gas when the barometric pressure is high and higher concentrations when the barometric pressure is low. Relatively small pressure gradients can result in advective gas fluxes that are much larger than diffusive gas fluxes (Massman and Farrier, 1992). Choi et al (2002) found that pressure differences in a shallow aquifer frequently resulted in advective flux larger than the diffusive flux of TCE, but most of the time the advective flux was small compared to the diffusive flux. Advective transport driven by atmospheric pressure pumping is therefore likely to be negligible in shallow unconfined aquifers.

The influences from soil moisture, temperature and pressure differences on the vertical profiles of naphthalene in the soil gas all change according to weather and transpiration. The seasonal changes are therefore important to monitor in order to assess the overall removal of naphthalene through coupled volatilization and biodegradation in the unsaturated zone. The dissolution of contaminants into the groundwater is also dependent on infiltration. It is therefore important to monitor the seasonal changes in PAH concentrations in order to assess the overall phase distribution and fate of PAHs in groundwater and soil gas. Infiltration can be reduced by transpiration by vegetation, which for example is utilized in vegetative covers of landfills. The vertical upward movement of water caused by the evapotranspiration or downward movement from rainfall can affect the vertical distribution of naphthalene in the unsaturated zone (Davis et al, 2001).

Volatilization may be a relatively important mechanism involved in the phytoremediation of lighter PAHs. Plants increase the porosity of soil and form macropores from decaying roots and increased diffusion and advection of soil gas result from this. Phytoremediation can indirectly enhance volatilization by lowering the groundwater table and decreasing the water saturation in the unsaturated zone. Diffusivities are about 4 orders of magnitude faster in air than in water; thus dewatering the subsurface automatically leads to enhanced volatilization. Laboratory experiments with TCE have shown that the relative water saturation greatly affects volatilization and volatilization was limited by aqueous phase diffusion in the moist vadose zone (McCarthy and Johnson, 1995, Narayanan et al, 1999a). Plants may in some cases lower the groundwater so much that DNAPL becomes exposed (Davis et al, 2002). Since the vapor pressure is much higher than the water-gas distribution coefficient, exposed DNAPL will lead to a dramatic increase in the volatilization flux.

A planted area with an active rhizosphere can shorten the half life of biodegradable contaminants considerably. At the same time plants decrease infiltration and can lower the groundwater table by transpiration. Due to the faster diffusion in air than in water these processes will decrease the residence time available for biodegradation of a contaminant before it might escape to the atmosphere (Davis et al, 2001). It is necessary to assess the combined effect of volatilization and removal in the vadose zone in order to evaluate the actual volatilization to the atmosphere.

2.4.8 Phytovolatilization

In addition to direct volatilization, contaminants can be transported from contaminated areas in gas or water within the plant roots and diffuse out of the roots into less contaminated areas of the subsurface, (Davis et al, 2002).

Live trees may act as ventilation air ducts for volatilized contaminants present in the soil gas in the unsaturated zone and diffusion into the roots and out of aboveground biomass (Nietch et al, 1999, Struckhoff et al, 2005). Diffusive loss out of the roots has also been shown to occur during passage through to the unsaturated zone (Struckhoff et al, 2005).

The xylem, which transports water and nutrients from the subsurface, flows deeper in the tree underneath the active growth region, while phloem, the sap located right under the bark, transports building materials produced by photosynthesis in the leaves to the active growth areas of the tree. Naphthalene may diffuse radially out from the xylem to the atmosphere, creating a diffusion profile out through the tree trunk (Ma, and Burken, 2003, Struckhoff et al, 2005).

Failure to detect contaminants in plant extremities such as leaves, buds and petioles does not necessarily mean that the compound was not taken up or has been phytodegraded; the contaminant may simply have diffused out through roots and stems (Zhang et al, 2001).

The coupled pathway of plant uptake and phytovolatilization has been explored in several laboratory experiments and shown to be significant for volatile organic compounds such as BTEX, chlorinated solvents, 1,4-dioxane, herbicides, MTBE (Briggs et al , 1982, Burken and Schnoor, 1997, Burken, 2003, Davis et al, 1999, Kelley et al, 2001, Orchard et al, 2000, Rubin and Ramaswami, 2000). Hydroponic poplar seedlings were applied in many of these studies, but full grown plants under field conditions might behave differently. Field tree core data supports the significance of the uptake pathway for chlorinated solvents (Ma and Burken, 2002, Vroblesky et al, 1999) and concentrations decline with the sample height on the trunk (Vroblesky et al, 1999). Phytovolatilization of naphthalene has to our knowledge not yet been reported under field conditions prior to the research in this dissertation. It is crucial for a sound risk assessment and evaluation of the system performance for phytoremediation to assess how much of the volatilized PAHs enter the atmosphere along these routes without being biodegraded.

2.4.9 Other abiotic transformation processes

Abiotic transformation processes such as photolysis and chemical oxidation, reduction or hydrolysis can occur, but are generally not important mechanisms for PAHs in subsurface soil (Hatzinger and Alexander, 1995). Photochemical degradation of PAHs can be induced by sunlight (Finlayson-Pitts and Pitts, 2000), but is only relevant for PAHs in the topsoil or for degradation upon release of volatized PAHs from the subsurface to the atmosphere. The coupled removal mechanism of volatilization to the atmosphere

followed by rapid photo-oxidation may be an acceptable component of a remediation system, given that the photo-oxidation is relatively fast compared to the volatilization flux. This requires that there is no risk for unacceptable human or ecosystem exposure from levels of PAHs in air above the regulatory limits.

2.4.10 Biodegradation

Natural ecosystems have developed numerous biodegradation strategies for PAHs and the most important PAH degradation mechanism in the soil environment is probably biodegradation (Banks et al, 1999, Juhasz and Ravendra, 2000). Microbial PAH degraders have been found in a broad range of different habitats and under aerobic as well as anaerobic conditions (Prince and Drake, 1999). Aerobic biodegradation of PAHs is more common and generally much faster than anaerobic biodegradation (Prince and Drake, 1999).

Aerobic biodegradation of PAHs is well documented (Smith 1990, Cerniglia 1992, Sutherland et al, 1995). The initial reaction of all aerobic degradation pathways for PAHs is the insertion of O_2 (Prince and Drake, 1999). Four distinct initial activation processes for aerobic biodegradation of PAHs have been found to occur in the environment:

1. Activation of dioxygenases to produce cis-dihydrodiols catalyzed by bacteria and green algae
2. Activation of methane monooxygenase to produce phenols catalyzed by methanotrophic bacteria
3. Activation of cytochrome P450 monooxygenases to produce arene oxides catalyzed by many fungi and a few bacteria
4. Activation of lignin-degrading enzymes to produce quinines activated by lignolytic fungi

The activation with the enzyme dioxygenase carried out by bacteria and algae leads to insertion of both O-atoms from an oxygen molecule into the substrate. Even though the overall reaction is thermodynamically favorable, this initial step requires reducing power (Prince and Drake, 1999). In other words a significant amount of metabolic energy is

invested in this first step of metabolism of PAH compounds by activation with dioxygenase. The energy investment is repaid in the following degradation steps, but this initial energy input may explain why intermediate compounds from this pathway are often hard to detect in the environment, since the bacteria tries to conserve these within the cell (Prince and Drake, 1999). The dioxygenase-pathway has been demonstrated for naphthalene, acenaphthylene, flourene, dibenzothiophene, anthracene, phenanthrene, flouranthene, pyrene, chrysene, benzo[a]anthracene and benzo[a]pyrene (Prince and Drake, 1999).

It has been found that the enzymes that act as dioxygenases on some substrates can act as monoxygenase on others (Selifonov et al, 1996). It is not certain what the range of substrates utilization is for dioxygenase, but in many cases it has been found that bacteria capable of degrading larger PAHs can degrade smaller ones as well, but the opposite is not generally the case. Some organisms do however exhibit specificity for individual substrates. In a study by Tongpim and Pickard for example it was found that three isolates that could grow on anthracene could not grow on naphthalene, phenanthrene, flourene, flouranthene, acenaphthene, pyrene or chrysene (Tongpim and Pickard, 1996). PAH degradation is probably most effective when there is a larger diversity of degrading organisms in the environment (Prince and Drake, 1999).

Methanotrophic bacteria use methane monooygenase as the activating enzyme for the initial step of their metabolism. The initial enzyme methane monoxygenase (MMO) inserts a single O-atom into the substrate. The MMO enzyme has very broad substrate specificity even though the methanotrophs use methane or methanol as their sole substrate for growth. This pathway has been used for cometabolic biodegradation of chlorinated solvents. Dalton et al, 1981 showed that MMO can transform naphthalene to 1- or 2-napthols, but it is uncertain whether this is an important pathway for PAH biodegradation in nature (Dalton et al, 1981).

Cytochrome P450 monooxygenase is an enzyme found in most eukaryotes, especially fungi and many bacteria as well as in the liver of higher organisms and in plants. This enzyme is responsible for activating some PAHs to carcinogenic compounds in animal livers, but some bacteria and plants are able to use the cytochrome P450 system to detoxify PAHs (Burken, 2003). The initial step in the cytochrome P450 pathway

converts an unsaturated bond in the PAH to an arene oxide. Subsequent steps convert the oxide to trans-dihydrodiol and different organisms convert this compound to for example sulfate, xyloside or glucoside, which then again are substrates for other compounds. This pathway benefits from a cooperation of a diversity of different organisms to degrade the PAH.

Lignin is a polyphenolic structural compound of plants, which is not readily available for biodegradation. White rot fungi is known as the primary degrader of lignin utilizing a range of extra cellular enzymes including lignin- and Mn peroxidases, which use hydrogen to create powerful oxidizing agents. These agents can introduce oxygen into a wide variety of substrates and generate quinines, which are then further biodegraded. White rot fungi has been found capable of degrading a wide variety of contaminants including PAHs (Davis et al, 1993, Field et al, 1992, Canet et al, 2001, Andersson et al, 2000, Bumpus, 1989). Inoculation with white rot fungi along with a lignin source for biodegradation of creosote or PAHs in soil has been carried out in the lab or on-site. In a 56 day long field study Davis et al found that depletion of 3- and 4- ring PAHs was faster in treatments with lignin-degrading fungus compared to plots without the fungus, whereas PAHs with 5 or more rings persisted (Davis et al, 1993). In some studies incomplete degradation and accumulation of dead-end products have been observed and it has been proposed that both the actions of white rot fungus and capable indigenous microorganism to carry out subsequent degradation steps are needed (Broedkorp and Legge, 1991, Andersson and Henrysson, 1996). Kottermann et al was able to show this cooperation between native bacteria and introduces white rot fungi (Kottermann et al, 1998). In a microcosm study, Canet et al observed some problems of achieving successful thrive of white rot fungi introduced along with straw into soil highly contaminated with PAHs. In this study the control microcosms containing only native microorganisms along with straw actually had the greatest losses of acenapthene, flourene, phenanthrene and anthracene (Canet et al, 2001). This implies that white rot fungi can not always be expected to succeed in competition with the naturally occurring microorganisms. Similar findings are common in attempts to bioaugment natural ecosystems with a specific contaminant degrading microorganism species.

Anaerobic biodegradation of PAHs is less well understood. In general anaerobic biodegradation of PAHs is slower, less widespread and less complete than aerobic biodegradation. At many sites anaerobic biodegradation is relevant nevertheless due to the depletion of oxygen in the source areas and groundwater plume. PAH biodegradation has been demonstrated under nitrate-, sulfate- and iron-reducing conditions. Biodegradation of naphthalene, acenaphthene, acenaphthylene, flourene, anthracene, phenanthrene and pyrene has been demonstrated under NO_3^--reducing conditions (Mihelcic and Luthy, 1988, Mc. Nally et al., 1998, Durant et al, 1995, Rockne, 1998). Naphthalene, acenaphthene, phenanthrene, flourene and flouranthene have been shown to biodegrade under sulfate-reducing conditions (Coates et al, 1996). Sulfate-reduction combined with aerobic processes led to biodegradation of a PAH mixture in laboratory microcosms (Brauner, 2000). Naphthalene was observed to be degraded in the Fe(III)-reducing conditions in the Bemidji aquifer (Anderson and Lovely, 1999). Robinson showed PAH biodegradation under sulfate- and Fe(III)- reducing conditions in laboratory microcosms with soil from a creosote contaminated site (Robinson, 2001). Godsy et al. observed active methanogenesis in an aquifer contaminated with creosote, but direct correlation between biodegradation of PAHs and methanotrophs was not demonstrated (Godsy et al, 1992). Methanotrophic degradation was not active in biodegradation of PAHs in a creosote contaminated aquifer (Robinson, 2001).

Generally the biodegradability of PAHs decreases with increasing number of benzene rings. Biodegradation of low molecular weight PAHs is well established (Kastner and Mahro, 1996, Banerjee et al 1995, Mueller et al 1991), while the high molecular weight (HMW) PAHs are more recalcitrant to biodegradation (Park et al 1990, Cerniglia, 1992). The decreasing bioavailability results from increasing hydrophobicity and thus decreasing solubility and increasing sorption of HMW PAHs (Prince and Drake, 1999, Heitkamp and Cerniglia, 1988). The increasing recalcitrance of HMW PAHs also stems from higher electrochemical stability due to resonance energies in their structures (Heitkamp and Cerniglia, 1988). In a long term groundwater monitoring study, we observed the same tendency of increased recalcitrance to biodegradation with increased number of aromatic rings in PAHs (Widdowson et al, 2005).

PAHs can be biodegraded either as the sole source of carbon and energy or by co-metabolism. Co-metabolism is the "unintended" degradation of a contaminant induced by enzymes produced for metabolism of a structurally similar compound, which is utilized as source of carbon and energy. Biodegradation of naphthalene, acenaphthene, anthracene and phenanthrene as the sole source of carbon and energy has been well documented (Walter et al, 1991). Observations of utilization of 4- ring PAHs as the sole source for carbon and energy is less common. Flouranthene has been observed to be biodegraded by a pure culture (Weissenfels et al, 1990) and a seven member bacterial community (Mueller et al, 1989). Flouranthene, pyrene and chrysene have been demonstrated to be degraded as the sole source of carbon and energy by an isolated Rodococcus species (Walter et al, 1991). PAHs with 5 or more rings have not been demonstrated to be biodegraded as the sole source of carbon and energy. Co-metabolic biodegradation appears to be required for PAHs with 5 or more rings, and it is likely that a supply of co-metabolite is a required source of energy and carbon in order to reach low levels of PAHs with fewer rings as well (Prince and Drake, 1999). In addition to biodegradation enhancement by co-metabolism from presence of organic carbon, the cooperation by a diversity of organisms in the natural ecosystems can lead to more efficient biodegradation than by a single species.

2.4.11 Aeration

The supply of oxygen to soil is considered the most important limiting factor for biodegradation of the majority of organic contaminants in the soil environment. Indeed aerobic biodegradation of PAHs is faster, more widespread and more complete than anaerobic biodegradation (Prince and Drake, 1999).

Aeration occurs naturally via oxygen diffusion down through the unsaturated zone. Plants that transpire large amounts of water can increase the depth of the unsaturated zone and lower the moisture content in the unsaturated zone and thereby increase the oxygen supply by downward diffusion (Davis et al, 1993). Together the lowering of groundwater, drying out of the unsaturated zone and increase in porosity can lead to a significant increase in the aeration of the subsurface facilitated by plants and trees. Plants

also increase the porosity and thus the permeability of soil and the number of macropores due to root-die back.

Plants need oxygen supply for respiration required for growth in the root zone and most terrestrial plants have roots surrounded by the same concentrations of oxygen as in atmospheric air (Conrad, 1995) Plants have therefore developed the ability to transport oxygen internally to the root zone in addition to the natural aeration through the soil (Davis et al, 1993). Plants may utilize diffusion or advection driven by pressure or temperature differences for the internal oxygen transport (Davis et al, 1993). The ventilation efficiency varies greatly amongst plant types (Landmeyer, 2001). Non-wetland species can not supply enough oxygen to prevent development of anaerobic in the root zone during flooding events and will not thrive under these circumstances. Woody wetland plants, such as willow and poplar, have moderate ventilation capabilities and can survive flooding by increasing the internal oxygen transport to the root zone. Most efficient are the herbaceous wetland plants, which have their roots submerged under water most of the time and supply all the oxygen necessary via internal transport (Landmeyer, 2001).

2.4.12 Rhizodegradation

Perhaps the most important remediation process involved in phytoremediation is biodegradation in the rhizosphere. The rhizosphere is the zone of increased microbial activity at the interface of root and soil, where physical, chemical or biological processes are modified by the plant root (Joner et al, 2003). The rhizosphere is only a few mm thick, but depending on the root density and numbers of fine root-hairs on a plant the rhizosphere can have a substantial impact on the soil ecosystem. Many studies show enhanced biodegradation in the rhizosphere (Anderson et al, 1993) and enhanced PAH biodegradation has been demonstrated in rhizosphere soil in several studies (April and Sims, 1990, Reilley, 1996, Joner et al, 2003). There are speculations and indications on the reasons for this phenomenon, based on knowledge from plant physiology and microbiology, but the complex cooperation between plants and rhizosphere microorganism in biodegradation of pollutants is still not well understood. This is in part caused by the difficulty in separating the individual causes and effects without altering

the symbiotic plant-microbial system. The function of the system as a whole is more than the sum of the functions of the individual parts. Laboratory studies can use radioactive labeled contaminants to separate microbial degradation in the soil from immobilization into soil, volatilization and plant uptake and phytodegradation.

Cooperation in degradation of contaminants by plants and their rhizosphere microorganisms is probably a common mechanism involved in phytoremediation. Plants coexist in their ecosystem with the microbiological communities of bacteria and fungi in the rhizosphere (Anderson et al, 1993). The plant facilitates enriched microbial ecosystems in their rhizosphere by providing organic matter from exudates and decaying roots, aeration, pH and surface for attachment for the bacteria and fungi (Joner and Leyval, 2003. Anderson et al, 1993). The benefit for the plant is enhanced nutrient and water uptake assisted by their microbial partners and may also include microbial protection against plant-pathogens and toxic levels of contaminants.

It is well established that the microbial community is altered in the rhizosphere of plants compared to surrounding soil. Increased activity and diversity of the microbial community in rhizosphere soil is documented (Anderson et al, 1993, Davis et al, 2002). Tree rhizospheres are for example often associated with population consortia richer in mycorrhizae fungi compared to the non-rhizosphere soil. The enhanced microbial activity in the rhizosphere may enhance the rate of biodegradation of PAHs. Most studies have looked at culturable microorganisms and only few studies have been carried out to compare the microbial communities of uncultured rhizosphere soil compared to bulk soil (Davis et al, 2002). Since a large fraction of microorganisms are not culturable it is therefore impossible to generalize about how plants alter the microbial diversity, but there appear to often be a larger fraction of fungi associated with plant roots. Microbial enumerations showed one to two orders of magnitude higher bacterial numbers in soil samples collected from the phytoremediation system relative to control locations, with much higher levels of actinomycetes (47-78% compared to 0-19%) and increased PAH degradation rates (Robinson, 2001).

The plants can modify the physical and chemical properties of soil by increasing aeration, changing pH and improving porosity (Joner and Leyval, 2003). Root turnover in

winter contributes to formation of macro pores which increase aeration (Leigh et al, 2002).

Plants provide carbon as exuded carbohydrates, amino acids and alcohols (Anderson et al, 1993, Schnoor et al, 1995) as well as from root turn-over (Leigh et al, 2002) to the fungi and bacteria in their rhizosphere. In a study of root turnover from mulberry trees, Leigh et al found that 58% of the fine roots died at the end of the growing season (Leigh et al, 2002). In return for the large investment of readily available carbon, the plant benefits from enhanced availability of nutrients and water provided by the microorganisms in the rhizosphere. Mycorrhizae can provide 200,000 times the length of the roots, resulting in a large increase in surface area and thereby dramatically increase the volume of soil explored for nutrients and water (Davis et al, 2002). In fact many trees need mycorrhizae in order to thrive (Davis et al, 2002).

All the enzymes involved in degrading a particular contaminant may not be produced by a single organism, but rather by a complex consortium of microorganisms present in the rhizosphere (Meharg and Cairney, 2000). Rhizosphere microorganisms may degrade contaminants through direct metabolism or through co-metabolism as a consequence of non-specific enzymes targeted to degrade plant derived cyclic compounds (Joner et al, 2001).

Symbiotic associations between mycorrhizael fungi and higher plants are common (Anderson et al, 1993, Davis et al, 2002, Joner et al, 2001, Meharg and Cairney, 2000). Contrary to the white rot fungi, mycorrhizael fungi do not attack the plant roots, but rather there is a mutual beneficial interaction between fungi and host (Donelly and Entry, 1999). Mycorrhizael fungi may play an important role in degradation of PAHs in the environment and will be described in more detail later in the rhizosphere effect section of phytoremediation of PAHs

Bacteria and fungi are not only found in the bulk soil and adsorbed to the surface of the roots but also inside or partly inside the plant roots (Donelly and Entry, 1999). This complicates the study of the contributions from the plant versus microorganisms to the overall biodegradation.

A range of non-specific enzymes has been detected in mycorrhizae soil (Donelly and Entry, 1999), but it is often not possible to distinguish between enzymes produced by the mycorrhizae, the plant or by changes in the microflora due to the mycorrhizae (Meharg and Cairney, 2000). Joner et al. (2001) used phospholipids fatty acid profiles acid in the examination of rhizosphere effects on the microbial community and found indication that mycorrhizae associated microflora was responsible for the observed contaminant reductions (Joner et al, 2001).

White rot fungi occupy the same ecosystems as mycorrhizae, but instead of living in symbiosis with the plant, they degrade dead plant-derived material. White rot fungi produce a range of powerful extracellular lignin and humic acid degrading enzymes (e.g. peroxidase), which have also been found to attack many contaminants (Meharg and Cairney, 2000).

The microbial community in the rhizosphere may serve as protection against pathogens by competition and protection against contaminants by detoxification (Davis et al, 2002). It is possible that plants alter their rhizosphere microbial ecosystem in response to contaminants. Siciliano et al used gene markers to measure microorganisms (entophytes) within the plant tissue that carried the catabolic genes targeting a certain contaminant (Sicilian et al, 2001). This work suggests that phytodegradation inside plants can be carried out by entophytic bacteria and that plants may be able to select specific bacteria. Plants may also provide non-specific enzymes involved in transformations in the rhizosphere. It is possible that the mycorrhizae may stimulate the plants to produce more of certain enzymes (Davis et al, 2002).

Investigations of the different partners in this integrated ecosystem in isolation will often give a skewed picture of the overall function of the natural system. The complexity of the plant-mycorrhizae-bacteria interactions empathize the need for work with symbiotic systems (Meharg and Cairney, 2000). The function of the system as a whole is more than the sum of the functions of the individual pieces. “The plants, fungi and bacteria act as a responsive ecosystem” (Davis et al, 2002).

2.4.13 Phytodegradation

Plants can take up contaminants and metabolites (2.4.6). After uptake contaminants may be phytovolatilized directly to the atmosphere (2.4.8) or accumulate directly into plant tissue or undergo transformation them into less toxic metabolites, which are then incorporated into plant tissue or released to the atmosphere.

Plants are photoautotrops, which means they use photosynthesis to harvest energy for catabolism from the sunlight and fix carbon dioxide from the atmosphere for anabolism. Since plants do not rely on organic compounds for energy and carbon, they do not have as extensive enzymatic pathways for catabolism and anabolism as heterothrophs and fungi. Other than transformation of the photosynthates, plant metabolism generally is concerned with transformation and isolation processes to avoid build-up and potential toxicity to their organelles from compounds. Plant metabolism of xenobiotics is similar to mammal liver functions in several ways and has lead to an overall description of this process by the "green liver concept", which is discussed in more by Burken (Burken, 2003). The green liver concept has three general steps:

1. Transformation
2. Conjugation
3. Storage or elimination

Plant metabolism of many compounds has been shown in phytoremediation studies and agrees with the green liver concept. The negative impacts from bioaccumulation in the food chain are well documented for compounds such as dioxin, DDT and PCBs (Burken, 2003). The uptake of contaminants by plants therefore raises concern for uptake in the food chain and due to the metabolism it is clearly not sufficient to show the disappearance of the parent compound upon uptake by plants. Storage as bound residues and transformation and conjugated derivates are causes for concern. The potential toxicity of metabolites is not yet well understood, although the metabolism generally appears to reduce toxicity. The translocation of contaminants from being confined in the subsurface and incorporation into aboveground biomass may greatly increase exposure to the ecosystem and humans through ingestion, even if the toxicity is reduced. Bound residues can persist in plant biomass for long periods of time and they may or may not be bioavailable. Generally toxicity has not been observed from ingestion of bound residues

even when a fraction if the residue becomes bioavailable. Conjugated products on the other hand are vulnerable to cleavage in the animal digestive system, which can lead to release of potentially toxic transformation products or even the parent compound (Sandemann et al 1990 and 1992).

No one wants to be responsible for future ecosystem exposures, and therefore the food chain uptake concern has led to resistance to accept phytoremediation as a more widely applied remediation strategy for contaminated sites. Lack of knowledge about the interactions between plants and contaminants is the major barrier. It is important to consider transformation products, conjugates and bound residues, in addition to the parent compound, when exploring the interactions. At this point little direct evidence shows that plant uptake and metabolism raise significant toxicological concern for the ecosystem foodchain (Burken, 2003).

2.4.14 General benefits of vegetation

Besides the direct phytoremediation influence on rate and extend of natural attenuation, there are various general benefits for the overall site restoration by incorporating plants in a remediation design.

Plants improve the quality of the soil and stabilize the ecosystem by decreasing soil erosion, aerating the soil, controlling pH, improving the soil texture and increasing the organic matter content of the soil. Plants can in many cases tolerate higher levels of contamination than microorganisms. Spoiled soils and contaminated areas can be colonized by native plants over time followed by ecological succession leading to increased diversity of plants and microbes (Davis et al, 2002).

Planted areas allow infiltration of rainwater, which will decrease the flood effect of rain events compared to paved areas. Planted areas can improve the water balance in groundwater and streams in a watershed by replacing runoff released fast in one point to the recipient water body with water slowly being released over time through evapotranspiration and subsurface groundwater flow. Furthermore trees can provide shade and cooling by transpiration in hot climates and insulation and wind breakage in cold climates. Plants and trees can improve our quality of life: landscapes and city-scapes incorporating vegetation are generally perceived as aesthetically pleasing. Sociology

studies have actually showed that the crime- and divorce rates are significantly lower in urban neighborhoods with green areas (conference "Living within nature", Nov. 21-22, 2002, Roanoke).

In addition to the local climate and greening of urban areas plants can sometimes help clean the air by absorption of pollutants (Jeffers and Liddy, 2003). In light of global warming, another air pollution benefit of plants is their CO_2 fixation into biomass.

Plants used in phytoremediation can be harvested and used as biofuels or materials for new wood products (Licht, 2005). In this scenario phytoremediation truly is a sustainable green technology; as contaminated land is restored and recycled back into productive use, the treatment system is itself a sustainable resource.

Brief overview

This review shows that many mechanisms can be active in complex interactions during phytoremediation of PAHs. Most of the prior research focused on biodegradation and immobilization of PAHs in the rhizosphere or by uptake into the plant roots. The relative importance of biodegradation compared to the physical mass transfer processes of phytovolatilization and volatilization has not yet been quantified in situ.

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CHAPTER 3

Volatilization and biodegradation of naphthalene in the unsaturated zone impacted by phytoremediation

Rikke G. Andersen, Elizabeth C. Booth, Linsey C. Marr, Mark A. Widdowson, John T. Novak

The Charles E. Via Jr. Department of Civil and Environmental Engineering, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0105

Abstract

The combined remediation mechanisms of volatilization and biodegradation in the vadose zone are investigated for the removal of naphthalene at a creosote contaminated site with 7 year old poplar trees. Field measurements show that lower groundwater elevations in the summer and early fall lead to elevated groundwater concentrations of naphthalene and increased volatilization. The fraction of the pore volume which is occupied by gas increases in the unsaturated zone during the summer and fall. This trend further enhances the volatilization due to higher effective diffusion rates. Water consumption by transpiration and interception of the phytoremediation system is believed to enhance contaminant mass transfer to the vadose zone. Column experiments and field measurements show that more than 90% of the naphthalene vapors are biodegraded within 5-10 cm above the groundwater table. First order aerobic biodegradation rates for unsaturated soil from this site were quantified in columns with no oxygen limitation and found to be 2-26 day^{-1}. The column results show that aerobic biodegradation rates increase as a result of long term exposure to naphthalene vapors. The maximum rates were obtained after acclimatization in the lab and higher rates were observed in soil previously exposed to naphthalene in the field. The data indicate that biodegradation increased the overall volatilization flux out of the source by 10-300 times.

3.1 Introduction

Phytoremediation is a process that uses plants and the microbial ecosystem in their rhizosphere for control and removal of contaminants (1). Phytoremediation holds promise for accelerating natural attenuation processes while providing hydraulic plume control (2). Phytoremediation of PAHs using poplar trees has been studied by our research group for the last 7 years at a creosote-contaminated site. Naphthalene is the primary contaminant mobilized from the creosote present as a dense non-aqueous phase liquid (DNAPL) into groundwater at the site. Naphthalene undergoes preferential remediation over the PAHs with three or more rings at this site (3). Enhanced aerobic and anaerobic biodegradation in areas impacted by poplar trees has been demonstrated in prior research on this site (3,4,5). The focus of this paper is to determine if direct volatilization of naphthalene from the groundwater to the soil gas in the vadose zone, coupled with biodegradation in the vadose zone, is active and to assess the significance of this mechanism. This study also investigates how phytoremediation may enhance naphthalene removal by promoting mass transfer of naphthalene to the vadose zone.

3.1.1 Naphthalene volatilization and vadose zone biodegradation

Volatilization and rapid biodegradation in the vadose zone immediately above the groundwater table can act as a significant mass removal mechanism for volatile organic compounds (VOCs) (6,7,8,9). Active biodegradation creates a steeper contaminant concentration gradient and this increases diffusion driven volatilization out of the saturated zone (8). A microbially enriched and aerated vadose zone is likely to have faster biodegradation rates than the oxygen limited saturated zone. Lahvis et al (8) quantified aerobic biodegradation and volatilization of hydrocarbons by analyzing vapor transport in the unsaturated zone at a gasoline spill site. In their study 68% of the volatilized mass of total hydrocarbons was biodegraded and mass losses were greatest within the capillary zone. Park et al. (10) found that 20-30% of the measured naphthalene loss was due to volatilization in an induced venting laboratory experiment. In a study of adsorption of radioactive labeled naphthalene to fescue and alfalfa roots, up to 45% was volatilized (11).

3.1.2 Phytoremediation impact on volatilization and vadose zone biodegradation

Phytoremediation may enhance biodegradation by promoting mass transfer of VOCs from the saturated zone to the unsaturated zone. The vertical upward movement of water caused by evapotranspiration and downward infiltration can affect the vertical distribution of naphthalene in the unsaturated zone (12). These seasonal trends are enhanced by transpiring plants (13). In the case of naphthalene, solubility and diffusivity in water are limiting factors for loss by volatilization if a contaminant source is covered by a layer of water (14). The upward migration of VOCs is proportional to the thickness of the water layer due to the much slower diffusion of naphthalene in water compared to diffusion in gas (15). Lowering the groundwater table will enhance the volatilization from sources below the groundwater table. Soil moisture affects gas diffusion in the unsaturated zone due to the change in air-filled porosity available (15). Macro pores from decaying roots also facilitate faster transport of volatilized contaminants to the atmosphere and replacement with fresh air into the subsurface (16).

The objective of this study is to evaluate and quantify the coupled mechanism of volatilization and biodegradation of naphthalene in the unsaturated zone. Extensive field measurements of parameters in soil gas and groundwater along with monitoring of weather data were carried out throughout a year at different locations across the site. The data was applied to evaluate the seasonal variations and the impact of transpiring phytoremediation systems on the combined volatilization-biodegradation mechanism. Column experiments were conducted parallel to the field measurements to determine rates for volatilization with and without biodegradation in the unsaturated zone. The column study also provides information on enrichment of the naphthalene degrading microorganisms in the vadose zone by long-term exposure to naphthalene.

3.2 Materials and Methods

3.2.1 Site description

The study site located in Oneida, Tennessee, is contaminated with creosote from a railroad tie operation active from the early 1950s to 1973. Creosote was discovered in a creek alongside the site in the early 1990s. An interception trench and oil-water separator

were installed to prevent off site contaminant migration. In 1997 a system of 1,146 hybrid poplars, *Populus deltoids x nigra DN34*, were planted on the site to provide hydraulic control of the dissolved contaminant plume and to stimulate enhanced natural attenuation. Prior to planting, excavated soil containing coal was spread on the surface of the site in thicknesses up to 0.5 m. The aquifer underlying the site consists of sand and sandy clay to a depth of 3.0-3.5 m below ground with dense shale underneath. Following planting, a detailed site characterization led to the discovery of creosote present as a dense non-aqueous phase liquid (DNAPL) pooled on the confining bedrock present at the base of the aquifer, varying from 0 to 30 cm in vertical thickness. The site has been subject to monitoring and research since installation of the system in 1997. The groundwater concentration and plume size of PAHs has decreased over time (3). Increased removal rates were evident after three growing seasons and coincided with penetration of the tree roots to the groundwater table. The plume size and concentration have been stable since 2001 due to equilibrium between the rate of mass transfer from the DNAPL source and the rate of naphthalene remediation (3).

3.2.2 Field measurements

Five clusters of **s**oil gas probes were established to enable measurement of soil gas concentrations of naphthalene, oxygen and carbon dioxide in the unsaturated zone. The locations of the soil gas probe clusters are shown in Figure 3.1. The soil gas probes (SV) were constructed of galvanized steel pipes sealed with rubber stoppers and outfitted with fittings and clamps for sampling. Soil gas probes were installed in different depths to investigate the vertical concentration profiles in the unsaturated zone. Initially, 15 soil gas probes were installed in October 2003 in clusters of 3 depths with a hand auger, 15-30 cm of sand was filled around the intake of the probe and the hole was backfilled with a slurry of bentonite and concrete to the ground surface. The depths of the intake depended on location of highly permeable sandy layers and varied from 0.7-1.1 m below ground for the shallow sampling points, 1.1-1.5 m below ground for the medium depths and 1.5-2 m below ground for the deepest points. As the groundwater table retreated during the summer, additional soil gas probes were installed with a fencepost hammer down to depths of 2.15 m below ground. Existing probes were replaced when measurements

indicated clogging or leakage. The supplementary probes were sealed loosely while hammered into the ground and the seal broken upon reaching the appropriate depths by pulling the probe up 5-15 cm with a pipe wrench.

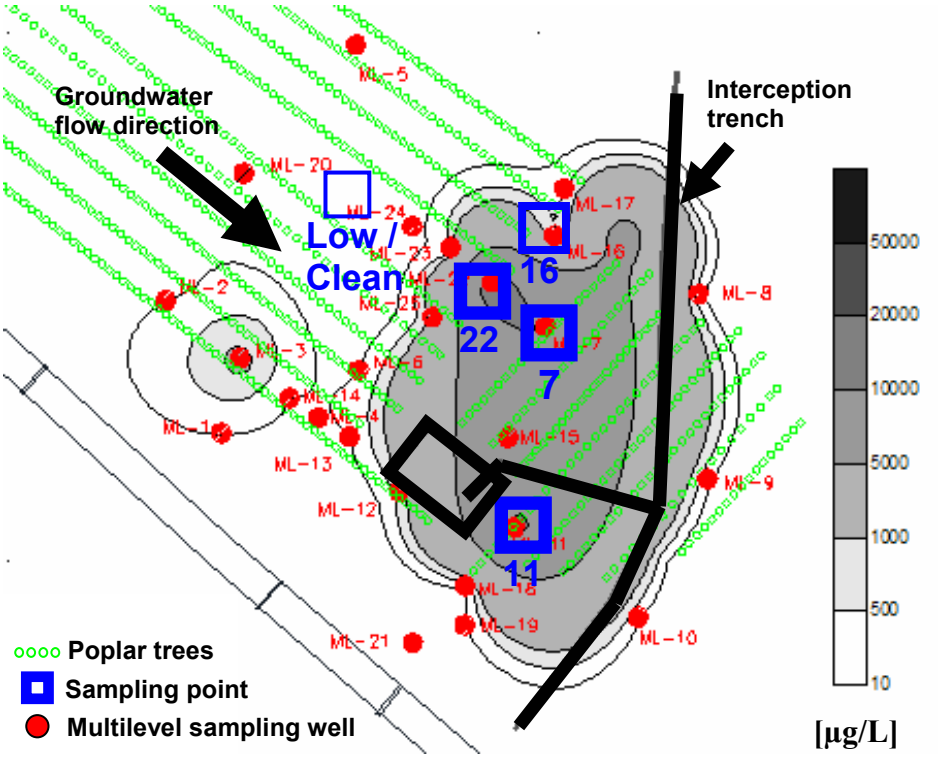


FIGURE 3.1 Deep (0-0.8 m above bedrock) naphthalene groundwater concentrations July 2004 (µg/L) and data sampling locations.

The gas contents of the soil gas probes were evacuated prior to sampling using a vacuum pump or an SKC pump. Soil gas was sampled by pumping 1 liter of soil gas onto sorbent tubes every 30-60 minutes until at least 15 liters had been collected. This ensured a limited radius of influence for the sample (7-25 cm) and allowed time for the naphthalene present in the soil gas to be replaced. The naphthalene content in the soil gas was collected onto XAD-2 sorbent tubes with a 1 L/min flowrate using SKC pumps. Direct sampling of the soil gas with low flow SKC pumps was used when the permeability of the soil was high (dry sandy soils). At low permeability, a high flow vacuum pump was used to sample soil gas via a vacuum box containing a Tedlar bag for soil gas collection (wet or clayey soils). Subsequently the naphthalene in the soil gas in

the Tedlar bag was transferred to sorbent tubes at 1 L/min with a SKC pump. The sorbent tubes were capped, wrapped with aluminum foil to prevent photodegradation, and stored at 4°C for no longer than 24 hours before analysis.

Oxygen and carbon dioxide concentrations in the soil gas were measured directly from the soil gas probes on the site with a handheld GasTech gas monitor. The GasTech could measure O_2 up to 30% and CO_2 up to 5%. Most of the soil gas samples had CO_2 above 5%, so dilution with clean air in Tedlar bags was necessary. The dilution results in inaccuracies of 6 0.05 % in the CO_2 reading in the diluted sample or up to 25% of the total CO_2 value after multiplication by the dilution factor.

The moisture content in the unsaturated zone was measured with probes (Watermark Moisture Sensors, Riverside, CA) installed in the same locations and depths as the initial soil gas probes. The moisture measurements were converted to gas saturation percentages. Groundwater elevations were recorded using a manual gauge located in piezometers and groundwater samples for PAH analysis and were collected from multilevel sampling wells (ML) near where the soil gas and surface flux measurements as were made.

Naphthalene volatilization out of the ground surface was measured with stainless steel flux chambers in the locations as the soil gas probe clusters (Figure 3.1). The chambers were installed on the ground surface and designed to trap naphthalene vapors escaping from the soil surface to the atmosphere. Naphthalene collected in the chamber was trapped on XAD-2 sorbent tubes and the clean air recycled back into the other end of the chamber. The method is described in more detail by Booth (17).

3.2.3 Laboratory column experiments

In order to investigate the fate and transport of naphthalene vapors through the unsaturated zone a column experiment was conducted. At the end of April 2004, soil was collected from both contaminated and clean locations at the phytoremediation site, located at SV7 and northwest of SV16 respectively (Figure 3.1). The soil was obtained from sand layers directly above the groundwater table, at a depth of 0.9 to 1.5 m below ground surface at the contaminated location and a depth of 1.5 to 2.1 m at the clean location. Soil parameters such as moisture content, density, and porosity were determined

from intact field samples. Because equipment was not available to obtain undisturbed soil samples at those depths, topsoil was used for the density and porosity measurements. The moisture content was 0.11 g g^{-1}, the bulk density was 1.6 g cm^{-3}, and the porosity was 37%.

A portion of each soil type was autoclaved over a period of 40 days more than 15 times for abiotic controls. The water content of the autoclaved soil was adjusted back to 0.11 g g^{-1} to account for water lost during autoclaving, and the live soil (not autoclaved) was air dried and brought back up to a water content of 0.11 g g^{-1} with distilled water. Autoclaved and live contaminated soil was packed into columns 1 and 2, respectively. Autoclaved and live uncontaminated soil was packed into columns 3 and 4, respectively.

The column design shown in Figure 3.2 is 71 cm in height and 5 cm in diameter and is constructed of PVC (polyvinyl chloride) pipe. Male coupling PVC socket fittings and threaded caps were placed at the ends of each column. Metal mesh on top of a metal strainer plate was placed in the bottom of each column to prevent soil from falling into the source cap. The columns were maintained at constant temperature (23.1°C) and humidity (38.4%) and monitored over a period of 231 days.

Five grams of naphthalene crystals (Fisher Scientific) were placed in aluminum bowls in the bottom of each column. Teflon tape and PTFE paste sealer were applied to the bottom and top socket fittings, and both caps were tightened and sealed with epoxy that contains no naphthalene to prevent air from entering the columns. The interface between the steel tubing of the sampling ports and the column was also glued with epoxy.

Sampling ports 3 mm in diameter were drilled along the length of the column and a 0.05 m long piece of 3 mm outer-diameter, stainless steel high pressure tubing was inserted 2.54 cm into each sampling port. The steel tubes were outfitted with brass compression fittings and gas chromatograph septa. Via these ports soil air was sampled with a gastight syringe to obtain vertical concentration profiles of naphthalene, oxygen and carbon dioxide. The column has seven sampling ports spaced 5 cm apart at the bottom and 10 cm apart at the top as displayed in Figure 3.2. The spacing of the sampling ports is smaller at the bottom of the column to account for the expected fast biodegradation of naphthalene.

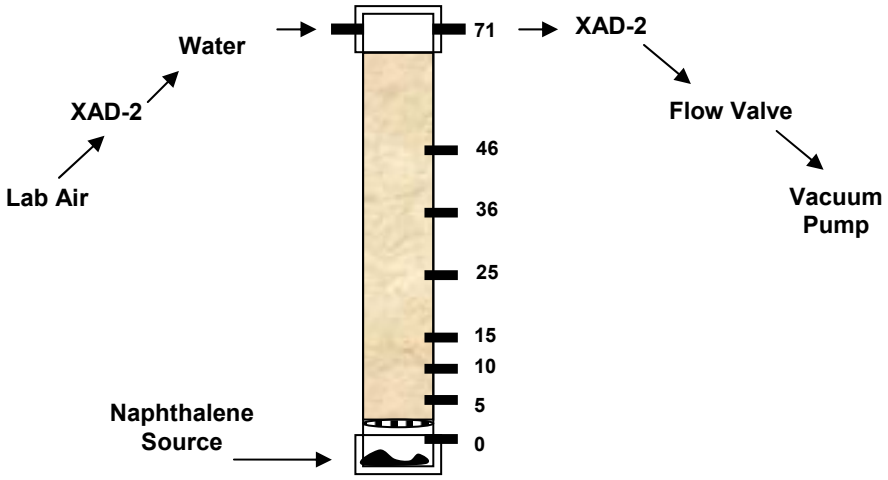


FIGURE 3.2 Column design with sampling port locations and experimental setup.

Lab air entered the column system, as illustrated in Figure 3.2, and flowed through a sorbent tube to remove background contaminants. The clean air then traveled through a water bath, which consisted of a 125 mL Erlenmeyer flask filled with 100 mL of distilled water, to humidify the air and prevent the soil from drying out. Air entered (and exited) the column headspace through 3 mm diameter steel tubes outfitted with compression fittings and Teflon tubing. The contaminated air exiting the column was pulled out of the column and through two XAD-2 sorbent tubes in series. The second sorbent tube served to capture any breakthrough from the first. Clean air leaving the sorbent tubes flowed through a valve and then into a vacuum pump at a flow rate of 0.5 L/min. This flow rate takes into account recommendations from the NIOSH 5515 procedure (18) and the manufacturer for the sorbent tube efficiency. Used sorbent tubes were capped, wrapped with aluminum foil, and stored at 4°C for no longer than 24 hours before analysis.

3.2.4 Analytical methods

Sorbent tubes from the field soil gas measurements and from the columns were extracted following the NIOSH 5515 extraction procedure with toluene. Microcosms and column soil were extracted with methylene chloride as described by Robinson et al (5) and groundwater samples were extracted as described by Widdowson et al (3). The extracts were analyzed on an automated Hewlett-Packard 5890 gas chromatograph with flame

ionization detection (GC-FID) a DB-5 capillary column along with external liquid standards.

Vertical gas concentration profiles in the columns were obtained biweekly by using a gastight syringe to pull 100-200 μL samples of air from each column sampling port. In order to acquire a representative sample, 20 μL of stagnant air were pulled out of the port and expelled before the actual sample was taken. The syringe was then reinserted into the port and slowly pumped to gently mix the soil gas and obtain a uniform sample. Samples were taken from each port until the standard deviation of at least two measurements fell below 20%. Samples were manually injected into the GC-FID. The injector, detector, and oven temperatures were set to 250°C, 310°C, and 80°C, respectively, with a temperature ramp of 10°C min^{-1}. Liquid naphthalene standards were used for the calibration curve.

Carbon dioxide and oxygen gas were sampled biweekly alternating with weeks when naphthalene was sampled using the same sampling method as described above for naphthalene. Oxygen samples of 100-200 μL were analyzed on a GOW-MAC GC Series 580 instrument (Bridgewater, N.J.) with a Thermal Conductivity Detector (TCD) and Propaq Q packed column. Carbon dioxide samples of 100-200 μL were analyzed on a Shimadzu GC-14A (Kyoto, Japan) with a TCD detector and Propaq Q packed column.

3.3 Results and Discussion

Naphthalene concentrations in the soil gas and surface flux of naphthalene out of the ground surface were measured over a year to examine seasonal effects on direct volatilization. Soil gas parameters were measured at least once a month from March 2004 to March 2005 using soil gas probes installed in several depths at four locations (Figure 3.1). The surface flux of naphthalene was monitored at the same 4 locations and at a clean control location (Figure 3.1) once a month from October 2003 to October 2004. The sampling locations are located in the northern (7), northwestern (22) and southern (11) end of the highly contaminated area as well as in a medium contaminated area north of this (16).

3.3.1 Field measurements

The concentrations of naphthalene in the soil gas are shown in Figure 3.3 along with the average groundwater concentrations of naphthalene in the top two ports sampled for the four sampling locations (SV7, SV16, SV11 and SV22). The groundwater concentrations represent the levels in the top of the saturated zone at the individual sampling events and the sampling depth is moving up and down with the groundwater. The soil gas concentrations shown are the maximum concentration measured at each location on the particular date. Naphthalene levels in the soil gas were significant only in the first couple of sampling ports above the water table. The data presented in Figure 3.3 is from the sampling ports closest to the depth of the water table, which is moving over time. Generally at this site, soil gas concentrations of naphthalene and the surface flux of naphthalene out of the ground surface increase in the late summer and early fall (Figure 3.3, 17). This coincides with the lowest groundwater table elevation and the highest gas saturation in the unsaturated zone.

The highest soil gas concentrations of naphthalene of up to 30 µg/L were measured in deep soil gas probes reaching depths closer to the NAPL source residing on top of the bedrock. These probes could only be sampled when the groundwater table was at the lowest level and the gas saturation in the sampling depths was high enough to recover air samples from July to September 2004. All sampling events except August-September 2004 show naphthalene concentrations below 0.2 µg/L in the soil gas. This is likely due to removal mechanisms including sorption to soil, biodegradation and plant uptake.

Figure 3.3a shows concentrations of naphthalene in the groundwater and the highest concentration in the soil gas in the northern end of the highly contaminated area (SV7). The soil gas concentrations rise from below the detection limit in early May 2004 and to a peak of 13 µg/L in August 2004 corresponding to increasing concentrations in the groundwater. The flux of naphthalene out of the ground surface peaked in August as well (2, Appendix Marr). The soil gas concentrations decrease from August 2004 to November 2004 as the groundwater concentrations drop. The soil gas concentrations are below the detection limit from March 2004 to early May 2004 and from November 2004 to March 2005. A similar correlation between naphthalene concentrations in soil gas and in the top of the groundwater was observed in the northwestern end (SV22/MLS22) and

southern end (SV11/MLS11) of the highly contaminated area (Figure 3.3b and c). In these locations the flux of naphthalene out of the ground surface also peaked in August (17).

The groundwater concentration of naphthalene along with the highest naphthalene concentrations in the soil gas in a medium contaminated area north of the highly contaminated area (SV16/MLS16) is shown in Figure 3.3d. The soil gas concentrations are generally low in this location and do not appear to be directly correlated to the groundwater concentration. However the highest concentrations, occurring in August 2004, coincide with the highest concentrations in groundwater and soil gas in the highly contaminated area south of this location. Diffusion in gas is orders of magnitude higher than in water, so soil gas in this area could be affected by the groundwater contamination south of this location.

Throughout most of the year when the groundwater table is high, the highest observed soil gas concentrations are close to the detection limit and the surface fluxes are similar in all sampling locations, ranging from 1 to 6 $\mu g\ m^{-2}\ hr^{-1}$ (17), regardless of whether they are located above highly contaminated groundwater (location 7, 11, and 22) or medium contaminated groundwater (16). Due to faster diffusion of contaminants in gas than in water, it is possible that these results simply reflect that the diffuse soil gas plume is spread out in a larger area than the groundwater plume. Naphthalene diffusion out of tree roots following uptake of naphthalene from the saturated zone may also contribute to wider spreading of naphthalene in the soil gas.

The highest soil gas concentrations and surface fluxes are measured in areas with the highest groundwater concentrations (by MLS7, MLS11 and MLS22). The highest soil gas concentrations of 13 and 30 µg/L were measured in August 2004 in SV7 and SV22 respectively. The highest naphthalene flux of 23 $\mu g\ m^{-2}\ hr^{-1}$ was measured at MLS7 (17). There is a difference between high groundwater concentrations in the MLS11 well and low soil gas concentrations in SV11 probes located a 0.5-1.5 m away. There is a localized residual DNAPL at this groundwater well resulting in the high groundwater concentrations, but low-permeable claylayers are believed to provide a diffusion barrier for naphthalene vapors in the 1 m distance between MLS11 and the soil gas probes at SV11.

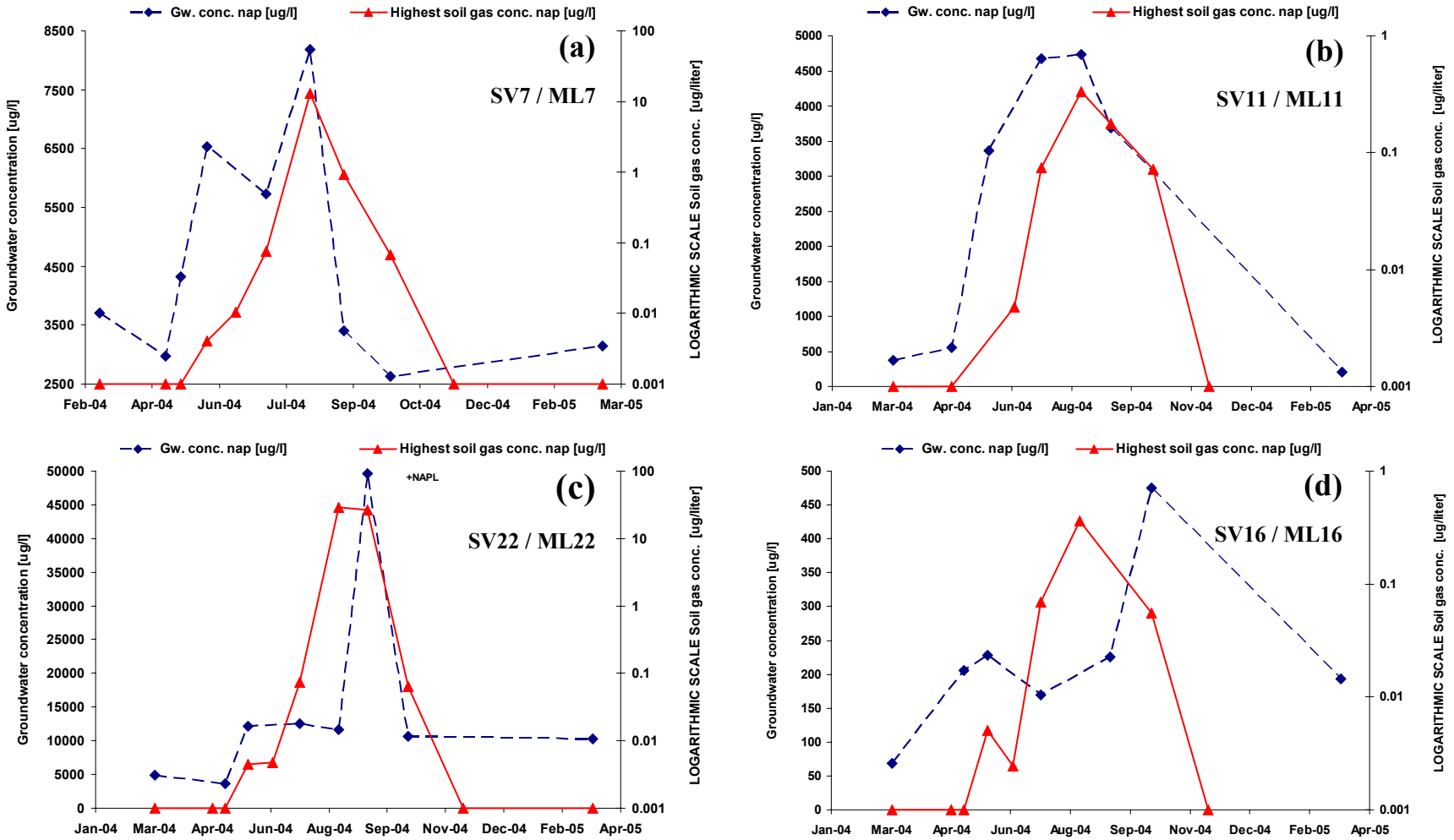
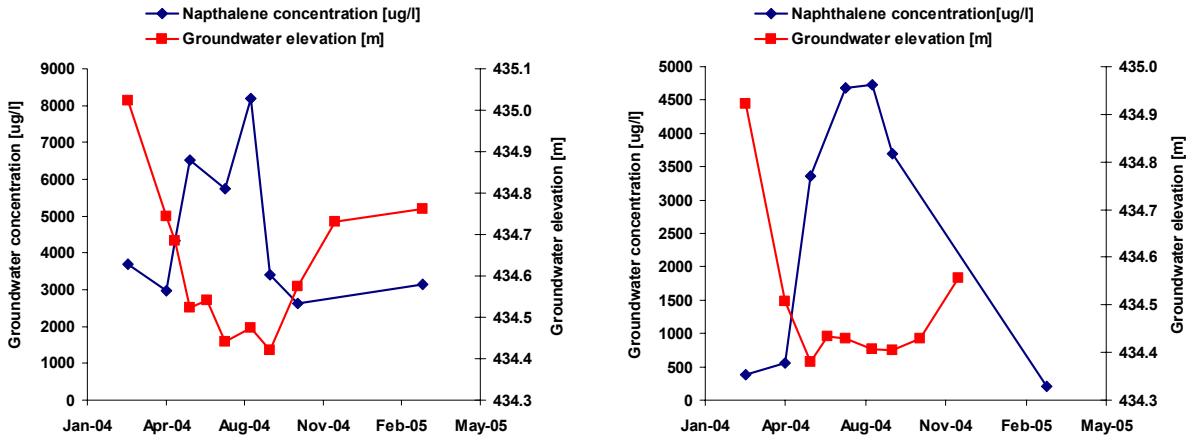


FIGURE 3.3. Concentration in the top 0.6 m of groundwater and highest concentrations in the soil gas of naphthalene from March 2004 to March 2005. Soil gas concentrations are shown on the right y-axis on a logarithmic scale.

The elevation of the water table at the site is highest in the winter, between 0.5 to 1 m below ground (19). Throughout the spring and summer the elevation of the groundwater table decreases and reaches the lowest level around 3 m below ground in the early fall. The retreat of the water table is due to decreased precipitation and increased loss of water by evapotranspiration during the warmer months. Figure 3.4 shows that the groundwater table declined about 0.6 m from March 2004 to July 2004 at the northern end of the highly contaminated area and stayed low until October. Similarly the groundwater table elevation dropped around 0.5 m from March 2004 to June 2004 in the southern end of the highly contaminated area and remained low until increasing in November 2004 (Figure 3.4).



Northern end, ML7 **Southern end, ML11**

FIGURE 3.4 Concentration of naphthalene in the top 0.6 m of the groundwater and groundwater table elevations in the Northern (left graph, ML7) end the Southern (right graph, ML11) and of highly contaminated area from March 2004 to March 2005.

The groundwater concentrations of naphthalene increase in the summer and fall months as the elevation of the groundwater table declines. The concentration of naphthalene in the top 0.6 m of the groundwater in both locations clearly increases as the groundwater table drops (Figure 3.4). In ML7 the concentrations increase from below 4000 µg/L in the winter to a peak of around 8200 µg/L in August and in ML11, the increase is from winter concentrations below 1000 µg/L to around 4700 µg/L in July-August.

The soil gas oxygen and carbon dioxide were monitored along with naphthalene in the soil pores. The most complete time series for the soil gas data was obtained 1.3-1.6 m below ground and at these shallow sampling depths, the highest soil gas concentrations of naphthalene occurred in August 2004 (Figure 3.5) corresponding to high concentrations in August in the deeper sampling depths (Figure 3.3). Figure 3.5 shows naphthalene concentrations in the soil gas 1.3 m below ground in the northern end of the highly contaminated area (SV7) and in soil gas 1.3-1.5 m below ground in the northwestern end of the highly contaminated area (right side, SV22) from March 2004 to November 2004. The concentrations in both locations begin to increase in June 2004 and peak in August 2004 after which they decrease in the fall.

The gas saturation of the soil pores and increased naphthalene concentrations in the soil gas in SV7 follow the same pattern (Figure 3.5, left side). This location contains sand from 1 m below ground to the bedrock. The soil gas saturation 1.3 m below ground in SV7 increased from 2% to 28% and remained high through September 2004, which is consistent with the highest soil gas concentrations in September 2004. The relationship between soil gas concentrations and gas saturation is less pronounced in SV22 than in SV7.

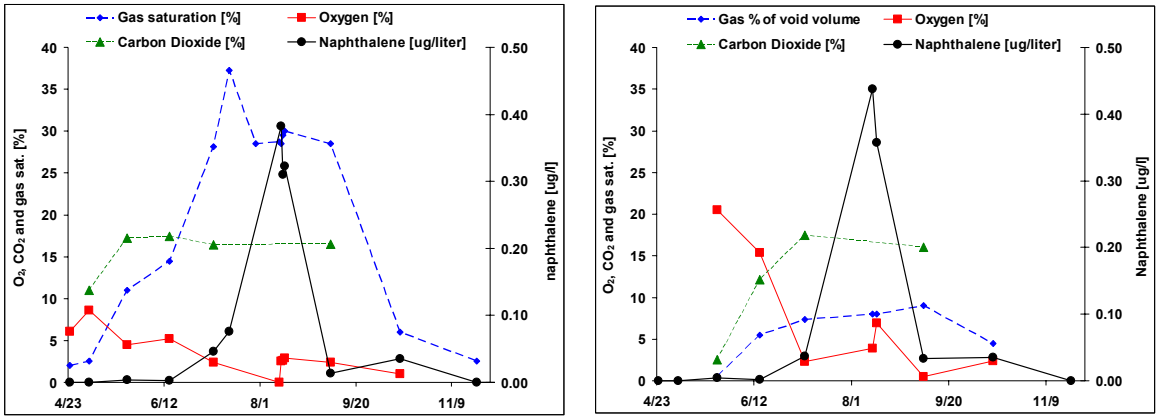


FIGURE 3.5. Soil gas data in the northern end of highly contaminated area (SV7), 1.4 m below ground (left side) and northwestern end of highly contaminated area (SV22), 1.3-1.5 m below ground (right side), March 2004 to November 2004.

Aerobic biodegradation in the subsurface can leave a footprint of oxygen consumption and carbon dioxide generation. Data in Figure 3.5 shows a typical time series of oxygen

and carbon dioxide concentrations in the soil gas at this site. The oxygen percentage measured 1.3 m below ground in SV7 declines from 6-8 % in April and May to below 2% in August (Figure 3.4, left side). The oxygen measured 1.3-1.5 m below ground in SV22 similarly decreased from 20% in May to 2% in July and remained below 6% through October 2004 (Figure 3.4, right side). The carbon dioxide percentage in the soil gas at both SV7 and SV22 exhibits an increasing trend in the spring of 2004 and remained elevated through September after which no more carbon dioxide data was collected. The consumption of oxygen and generation of carbon dioxide concentrations indicates increased aerobic biodegradation in the summer. The data does not indicate whether biodegradation occurs in the unsaturated zone or the saturated zone or both.

Typical vertical profiles of naphthalene, oxygen and carbon dioxide in the soil gas, along with gas saturation of the soil pores, are shown in Figure 3.6 in two highly contaminated locations for samples collected on September 7. 2004. At both locations the oxygen content declined with depth while the carbon dioxide content increased with depth. This indicates that oxygen is being consumed and carbon dioxide produced deeper in the subsurface indicating aerobic biodegradation in the vadose zone or in the groundwater.

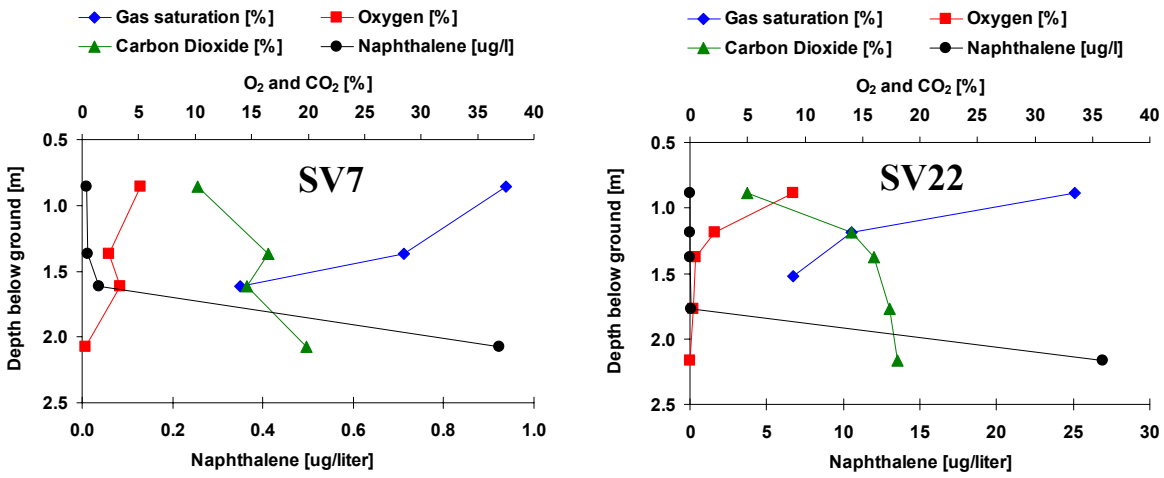


FIGURE 3.6. Vertical profiles of soil gas data in northern (SV7) and northwestern (SV22) end of highly contaminated area, September 7, 2004.

Flux estimates based on the concentration profiles in the groundwater and in the soil gas, ignoring loss mechanisms, indicate that the flux should be 1-2 orders of magnitude larger

than what is measured exiting the ground surface. The difference between the measured flux and the estimated flux is due to loss of naphthalene in the unsaturated zone. Possible loss mechanisms include biodegradation, sorption and plant uptake. Biodegradation in the saturated and unsaturated zone complicate the estimation of volatilization. The removal of naphthalene by biodegradation would result in steeper naphthalene concentration profiles, which would impact the mass transfer rate. Sampling of soil gas closer than 5-10 cm to the groundwater table was not practical because some water was also collected by pumping. The deepest soil gas probes installed and successfully sampled were 2.15-2.2 m below ground, 5-10 cm above the groundwater table. The vadose zone immediately above the groundwater table is thought to be characterized by rapid biodegradation. The field measurements were supplemented with laboratory column experiments to examine details of the process of naphthalene volatilization and biodegradation in the vadose zone.

3.3.2 Column data

In order to quantify the rates of volatilization and biodegradation in the vadose zone immediately above the source a laboratory soil column study was conducted.

Typical vertical concentration profiles of naphthalene in soil columns are shown in Figure 3.7 for live and autoclaved soil from the contaminated area. The shape of the profiles did not differ substantially over time once steady state conditions were obtained.

Maximum field soil gas concentrations measured in a highly contaminated area on August 14, 2004 are similar to the concentrations measured in the live column at the same distance from the source (larger labels on Figure 3.7). The nearly linear profiles in the autoclaved soil suggest that diffusion is the dominant process affecting naphthalene transport. The difference between the autoclaved and live profiles shows that significant biodegradation occurs in the live column and very little biodegradation occurs in the autoclaved soil. Low oxygen levels in the active columns generally suggest oxygen consumption by aerobic bacterial activity while the two autoclaved columns maintained oxygen levels close to background level between 20% and 20.9% throughout the length of the column. Carbon dioxide is a good indicator of active bacterial metabolism, and both autoclaved columns had very low levels of carbon dioxide ranging between 0.135 and 0.046%. The majority of the biodegradation occurs in the first 5 cm above the source

where over 90% of the total naphthalene flux out of the source is removed. Similar differences between naphthalene profiles in live and autoclaved soil were evident in the columns with uncontaminated soil, except that the loss rate was less for the uncontaminated soil than for the contaminated soil (Figure 3.8).

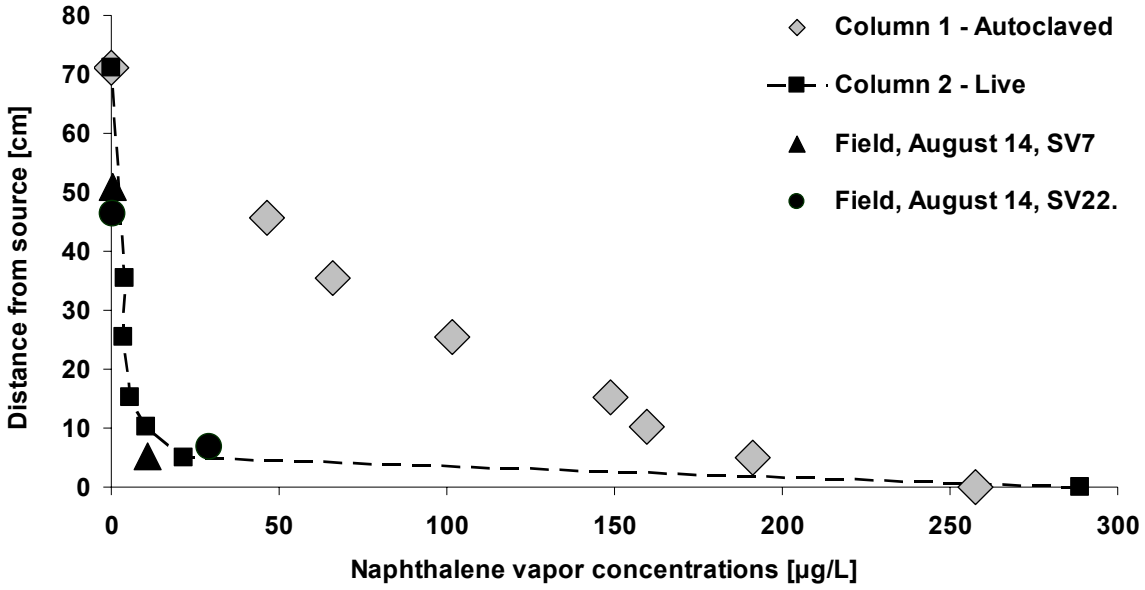


FIGURE 3.7 Naphthalene vapor concentration profiles in the contaminated soil columns (autoclaved and live) after 89 and 151 days, respectively.

The contaminated soil is expected to have higher initial biodegradation rates than the uncontaminated soil due to prior bacterial acclimatization to naphthalene in the field. Figure 3.8 shows that naphthalene vapor concentrations are lower in the contaminated soil than in the uncontaminated. The source vapor concentrations fluctuated with each sampling period and between columns, which is attributed to small volume sampling inaccuracies. Concentrations throughout the column were therefore normalized to the source concentration measured at the bottom port. The normalization allows direct comparison of the column profiles on the same scale.

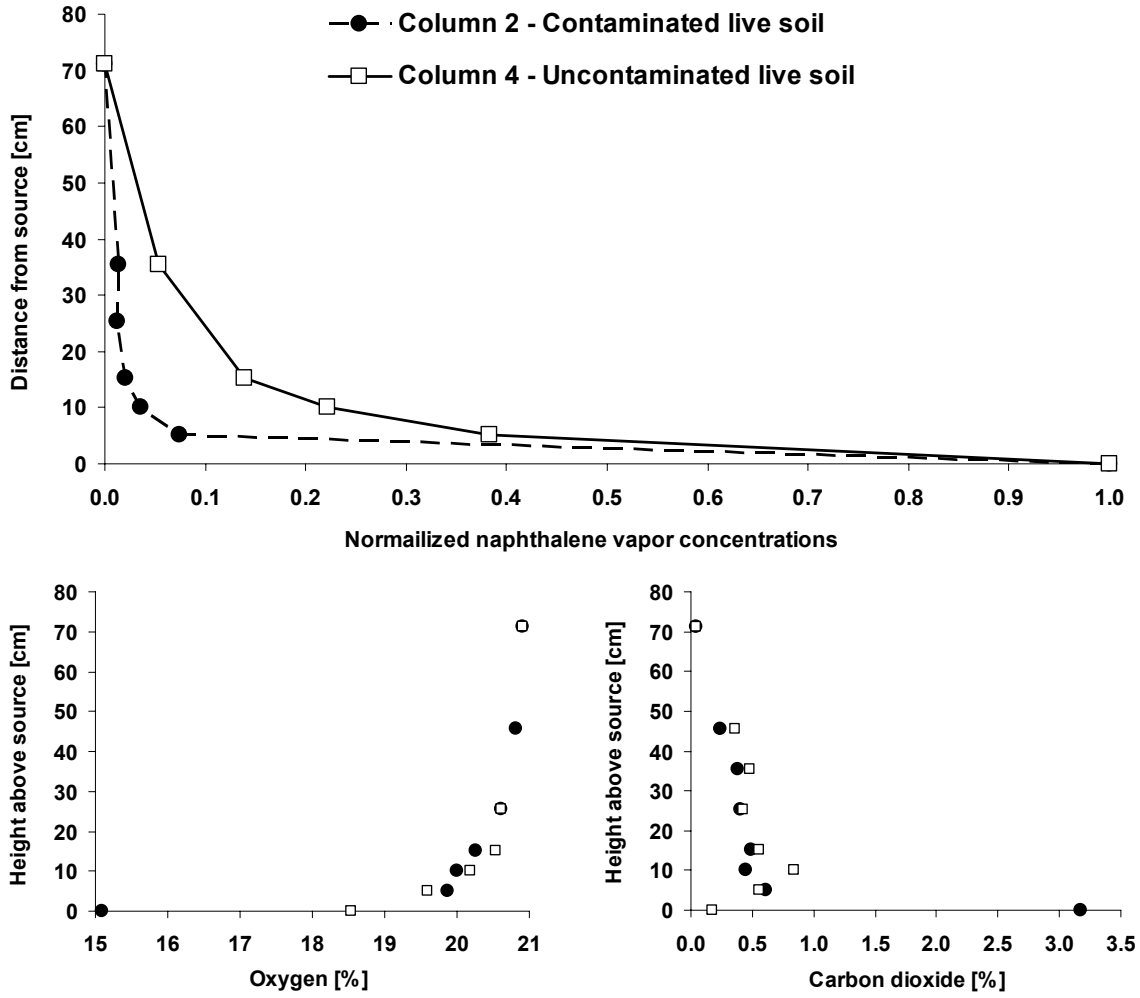


FIGURE 3.8 Comparison of normalized naphthalene vapor concentration, oxygen and carbon dioxide in live contaminated and live uncontaminated soil columns.

Oxygen and carbon dioxide profiles shown in the bottom of Figure 3.8 indicate a difference in bacterial activity. The live columns had decreasing oxygen levels with depth as shown on Figure 3.8, bottom left. Oxygen levels near the source of the live columns were 3.5% lower in the contaminated soil column compared to the uncontaminated soil. The live columns had decreasing carbon dioxide levels with depth and significant differences in carbon dioxide levels near the source as shown on Figure 3.8, bottom right. As seen in Figure 3.8, a significant amount of naphthalene is degraded within the first 5 cm of soil, and this explains the high carbon dioxide levels and depressed oxygen levels near the source. The difference between the two live soil columns is believed to be a result of previous acclimation of the microorganisms in the contaminated soil to naphthalene in the field.

The small amounts of naphthalene vapors released from this site do not possess a risk for human health or the environment at the observed rate. The column study shows rapid aerobic biodegradation within the first 5 cm of the vadose zone. This corresponds to very low flux out of the ground surface (Figure 3.3). The National Institute of Occupational Safety and Health recommended exposure limit (NIOSH REL) and the Occupational Safety and Health Administration permissible exposure limit (OSHA PEL) for naphthalene are both 50 mg m^{-3} (10 ppm) (20). Soil gas concentrations are generally below 1 mg/m^3 measured 1-1.5 meters below ground at the site (typical data shown on Figure 3.4).

Wilson (21) outlined an analytical solution that describes concentration as a function of distance from the source when diffusion and first-order biodegradation govern the fate and transport of a compound. The solution is one dimensional for steady-state conditions with boundary conditions of a source concentration at the bottom of the column and concentration of zero at the top of the column. From Fick's second law the naphthalene flux is derived by taking the derivative of the concentration profile (see Appendix B).
The effective diffusion coefficient in soil $D = \theta_a \tau_a D_a = (\theta_a^{3.33} / \eta_{tot}^2) D_a$, [m^2 day^{-1}],
where θ_a (m^3 air m^{-3} total) is the volumetric soil air content, tortuosity is a reduction factor (τ_a) for diffusion in porous media given by Millington and Quirk (22), η_{tot} (m^3 voids m^{-3} total) is the porosity and D_a [m^2 day^{-1}] is the molecular diffusion coefficient in air. The effective diffusion coefficient was calculated using a molecular diffusion coefficient (D_a) of 0.562 m^2 day^{-1} for naphthalene at 23°C as described by Cho et al. (23) The volumetric soil air content (θ_a) was calculated from averaged soil moisture contents in each column measured at the end of the experiment and the bulk density of 1.6 g/cm^3 for the soil. For both live columns θ_a was 0.2 m^3 air m^{-3} total; for the contaminated autoclaved column, θ_a was 0.25 m^3 air m^{-3} total; and for the uncontaminated autoclaved column, θ_a was 0.31 m^3 air m^{-3} total.

The biodegradation rates change slowly over time due to microbiological acclimation to naphthalene. From Fick's first law for diffusion and the initial water content of 0.11 g/g for the live columns it takes naphthalene takes 15 days to diffuse the length of the soil column. It was assumed that rate changes in the columns over time are slow compared to

the time it takes for a diffusion profile to reach semi-steady state. Wilson's solution was fitted with the concentration profiles to obtain first-order biodegradation rate coefficients (μ^1_c) for the column experiment. The flux rates throughout the columns were calculated based on the concentration profiles and fitted biodegradation rates using the solution outlined by Wilson (21), (Appendix B).

Figure 3.9 shows the model fit and resulting flux rates for the contaminated live column from a vertical concentration profile at day 207. The model fit yields a biodegradation rate coefficient of 13±1 day^{-1} and a similar model fit for the uncontaminated soil on day 207 yields a biodegradation rate coefficient of 12±1 day^{-1}.

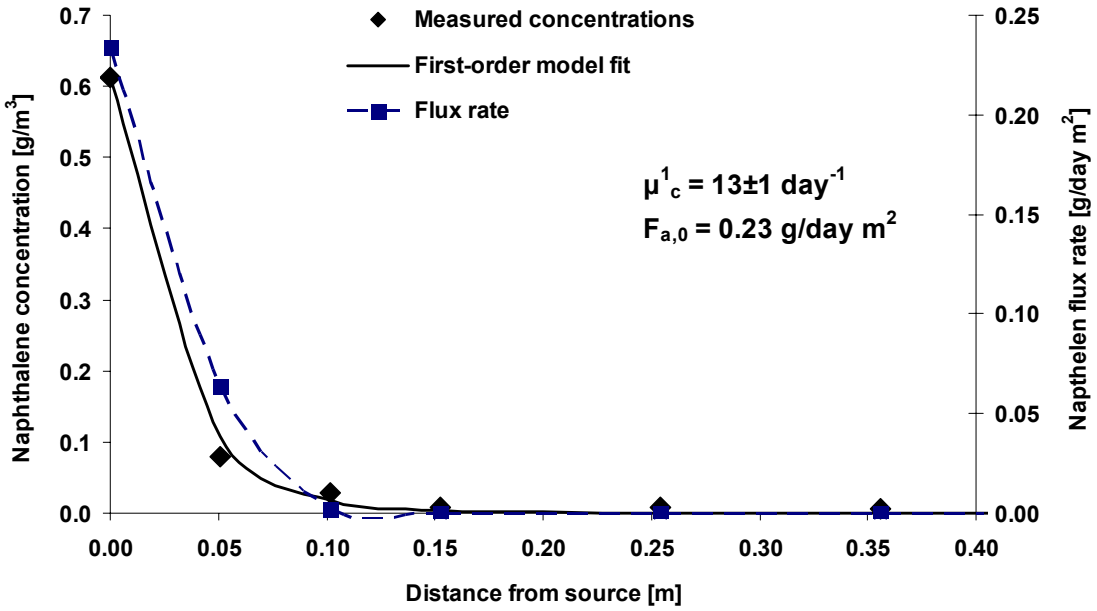


FIGURE 3.9. Naphthalene concentration and flux profiles for the contaminated live column along the column length after 207 days. Data for the bottom 40 cm shown.

The flux rates out of the source at day 207 were 0.23 g/day m^2 and 0.22 g/day m^2 for the contaminated and the uncontaminated soil, respectively. The contaminated and uncontaminated live columns both had an average soil moisture content of 0.125 g/g at the end of the experiment, which was a 13.6% increase from the initial moisture content of 0.11 g g^{-1}. The theoretical flux out of the source if there were no biodegradation is estimated to be 10 mg/ day m^2 at a maximum linear concentration gradient of 0.9 g m^{-3}/m and an effective diffusion coefficient of 0.0112 m^2/day for the live columns. Generally,

the flux without biodegradation would be 0.0158 m/day times the source vapor concentration in the columns in mg/m^3.

The main purpose of the autoclaved controls was to verify that approximately linear diffusion profiles exist when biodegradation is negligible. The autoclaved columns generally exhibited very low biodegradation rates and approximately liner concentration profiles. Biodegradation rate coefficients for the autoclaved columns were variable over time but remained below 1.8 day^{-1}, which is on the order of 10 times lower than the live columns. The autoclaved columns lost considerable moisture during the study, while the moisture was maintained in the live columns. A discussion of soil moisture, organic carbon and naphthalene sorption is provided in Appendix B. The effective diffusion coefficient in the autoclaved columns is therefore 4-8 times higher than in the live columns and the flux rates can not be directly compared.

Figure 3.10 shows the first-order biodegradation rate coefficients for the unsaturated and saturated live and autoclaved columns over time. By the end of the experiment, both the contaminated and uncontaminated live soil columns had reached approximately the same biodegradation rate of 13 and 12 day^{-1}, respectively. From Figure 3.10, it is evident that the contaminated soil column reached high degradation rates much faster than the uncontaminated soil column. It is evident that the large increases in biodegradation rates occurred well after the 15 days it takes naphthalene to diffuse through the column; hence the microbes in both live columns required an acclimitization period. Nevertheless the microbes in the contaminated soil adapted faster than the microbes in the uncontaminated soil.

Biodegradation rates in the contaminated live soil were initially around 5 day^{-1} but began to increase after two months of incubation reaching a maximum plateau of 25-26 day^{-1} after three months. The rates began to decline after four months and after 207 days at the termination of the experiment the rate was 13 day^{-1}. The inability to sustain the maximum biodegradation rates, despite a constant supply of the substrate naphthalene and oxygen levels maintained above 15%, indicates that some other factor than substrate or oxygen is limiting. Biodegradation rates in the uncontaminated live soil were initially around 2 day^{-1}, but began to increase after five months, a full three months after the contaminated live soil biodegradation rates increased steadily to 12 day^{-1} at the

termination of the experiment. The results show that microbes are initially degrading naphthalene more efficiently in the contaminated live soil column, which can be attributed to the prior acclimatization of the microbes to naphthalene in the field.

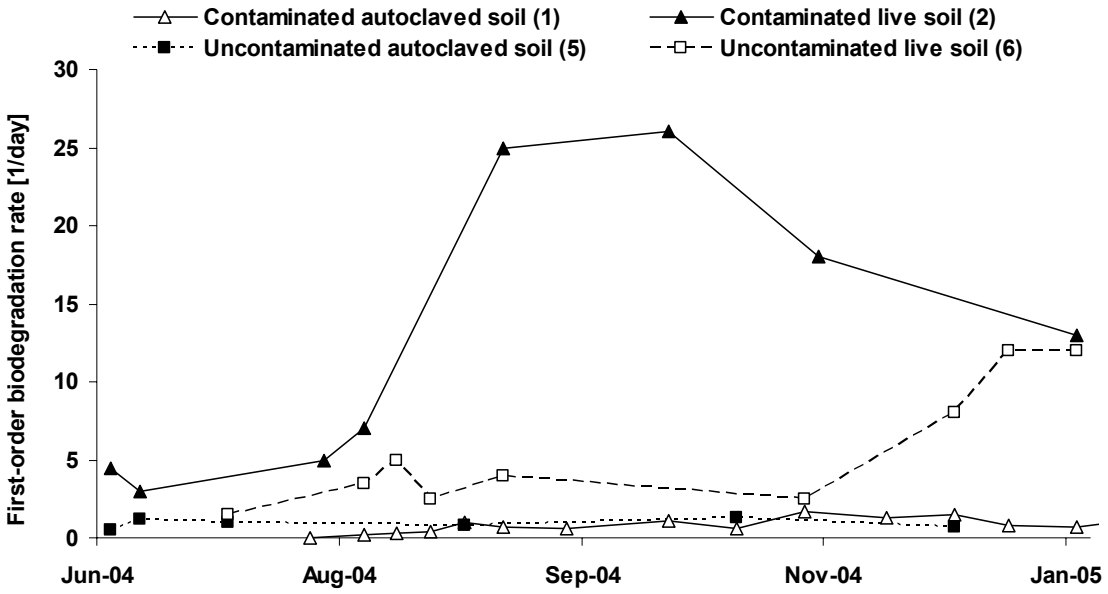


FIGURE 3.10 Aerobic first-order biodegradation rates of the uncontaminated and contaminated live and autoclaved laboratory soil columns over time.

The column experiments showed increasing aerobic biodegradation rates over time (Figure 3.10). The rate increases of 3-5 times shows great potential for enrichment of a naphthalene degrading microbial population in both soils as a result of long term exposure to naphthalene vapors.

Table 3.1 summarizes the biodegradation rates and flux rates out of the source for the columns at the beginning and end of the experiment as well as at the maximum. The theoretical flux out of the source for both the contaminated and the uncontaminated column is approximately 10 mg/m^2 day for the highest source concentration measured, or 0.016 mg/day relative to the source. The flux rates estimated from the data fits range from 0.2 to 5 mg/day relative to the source concentration. The overall effect of the biodegradation is therefore a 10 to 300 fold increase in the flux of naphthalene out of the source relative to the source concentration.

TABLE 3.1 First-order biodegradation rates of the uncontaminated and contaminated live and autoclaved columns over time. The flux rate numbers in ( ) are the rates relative to the source concentration.

	Contaminated soil (2)		Uncontaminated soil (6)	
Biodegradation rate [day^{-1}], day 60	7		3.5	
Biodegradation rate [day^{-1}], day 207	13		12	
Biodegradation rate [day^{-1}], max	26		12	
Flux rate [mg/m^2 day], day 60	48	(0.28)	18	(0.20)
Flux rate [mg/m^2 day], day 207	234	(0.38)	224	(0.37)
Flux rate [mg/m^2 day], max	754	(5.4)	224	(0.37)

The flux and biodegradation rates determined in the column experiment are believed to be high compared to field rates for the majority of the year. The rates were obtained from columns exposed directly to vapor from solid naphthalene. However field data obtained near the water table in August 2004, when the groundwater concentrations were highest, is in the same order of magnitude as concentrations measured in the columns (Figure 3.3). The biodegradation and flux rates in the columns are therefore believed to mimic the field rates when the water table is low. Biodegradation in the columns removes 90% of the naphthalene vapor flux within the first 5 cm of the vadose zone and 99% of the naphthalene is removed within the full column length of 71 cm. Typically the soil gas concentration declines by more than 90% from 2 m below ground to 1.5 m below ground (Figure 3.3). In August 2004 a few samples were obtained within the first 5-10 cm above the source and showed concentration drops from 13-30 mg/m^3 2 m below ground to 0.4-0.5 mg/m^3 1.4 m below ground, equal to a 97-98% removal (Figure 3.3). The field data agrees with the results of the column experiment and indicates that volatilization of naphthalene coupled with rapid biodegradation in the vadose zone is a significant removal mechanism at this site.

Water transpiration by the poplar trees reduces rainwater infiltration, removes moisture from the unsaturated zone and directly consumes groundwater. This results in increased gas saturation in the unsaturated zone and a lowering of the groundwater table. The mass

removal rate of naphthalene increased significantly after three growing seasons when the poplar tree roots reached the groundwater table (21). We believe that an effect of the transpiration from the phytoremediation system at this site is increased mass transfer of contaminants from the saturated zone to the unsaturated zone. The increased mass transfer is a result of a lower groundwater table, higher groundwater concentrations of contaminants, higher gas saturation in the unsaturated zone and a wider smear zone from amplified fluctuation of the contaminated groundwater.

Transpiration and phytovolatilization results indicate that a stand of fewer larger trees transpire more water overall, and a larger fraction directly from the groundwater, than a stand of smaller trees. The performance of the system might be enhanced by thinning of the tree stand to allow the remaining trees to grow bigger with deeper roots, leading to an overall increase in transpiration. This modification may benefit from supplementary planting of smaller plants or allowing native overgrowth of lower plants with a dense shallow root mass between the trees. This would facilitate water uptake from the unsaturated zone, increase permeability of the soil from root macro-pores and potentially enhance biodegradation in the rhizosphere of these plants in the unsaturated zone.

Acknowledgements

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CHAPTER 4

Uptake and phytovolatilization of naphthalene by poplar trees

Rikke G. Andersen, John T. Novak , Joel Burken, Mark A. Widdowson

The Charles E. Via Jr. Department of Civil and Environmental Engineering, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0105

Abstract

Plant uptake and phytovolatilization to the atmosphere is a potential pathway for the removal of volatile contaminants from soil and groundwater. The mechanism of phytovolatilization of naphthalene by poplar trees was quantified in the field at a creosote contaminated site. In situ measurements of naphthalene flux were measured directly on tree trunks in the field. A maximum flux rate of 249 $\mu g/m^2$ trunk surface area day was measured when soil gas and groundwater concentrations were high. The rates exhibited large seasonal and spatial variations. A laboratory uptake study and analysis of tree cores from the site provided supplementary evidence for naphthalene uptake by poplar trees. Naphthalene phytovolatilization rates and concentrations in plant tissue decrease with the height of the tree. Field and laboratory observations suggest that uptake occurs by transpiration as well as by passive diffusion from soil gas. Naphthalene phytovolatilization rates of 4.7 - 46 μg/day tree were determined based on the field measurements and tree sizes. The maximum estimated naphthalene uptake associated with the transpiration rates and groundwater concentrations was orders of magnitude larger. The difference between the estimated maximum uptake rates and measured phytovolatilization rates indicates that either the transpired water does not originate directly from groundwater or degradation in the roots and trees is substantial. Phytovolatilization appears to play a minor role in the loss of naphthalene from the site.

4.1 Introduction

Phytoremediation is increasingly being applied at contaminated sites for hydraulic control, plant uptake and enhanced attenuation of contaminants. Plants and the rhizosphere microbial ecosystem can reduce the contaminant mass, prevent exposure risk and decrease leaching and erosion (1). Other benefits of plants are improved soil quality and micro-climate, aesthetic value and restoration of ecosystems. Fast growing crops, such as poplar and willow, can also provide biofuel as well as paper, composite and solid wood products (2).

Subsurface contaminants can be taken up by plants via transpiration of contaminated groundwater or through partitioning into roots from soil gas or groundwater (3). Passive diffusion in and out of roots has also been observed for volatile contaminants (4). Plant uptake along with transpiration has been documented for several volatile organic contaminants (3, 5, 6, 7, 8). Transpiration is active when the stomata in the plant leaves are open during photosynthesis. Transpiration depends on weather (precipitation, temperature, wind, humidity, and sunlight), hydrogeology (depth to groundwater, water content in the unsaturated zone, soil permeability) and tree size (leaf area, root depth).

Field tree core data and laboratory uptake studies have shown a direct linear relationship between water concentrations in the root zone and uptake for chlorinated solvents and other organics (8, 9). Laboratory uptake studies with roots submerged in contaminated water have shown that the concentration of contaminants in the transpiration stream depends on the transpiration stream concentration factor (TSCF) of the compound and the water concentration (3, 5). The TSCF is contaminant specific and has been found to be correlated to the hydrophobicity represented by the octonal-water partitioning coefficient, K_{ow} (3). From uptake studies with different compounds, Burken et al. (3) proposed the relationship: $TSCF = 0.784*\exp[(-(\log K_{ow}-1.78)^2/2.44)]$. Contaminants with a log K_{ow} below 1 are too hydrophilic to pass through lipid root membranes and compounds with log K_{ow} above 3.5 tend to be bound in the roots rather than entering the xylem water (3). Naphthalene has a K_{ow} of 3.36 and a TSCF of 0.282 so it is expected to be taken up to some degree.

The concentration of contaminants in the xylem is dependent on how large a fraction of the total transpiration comes directly from groundwater and how much comes from

other sources including infiltration water and water in the vadose zone. Tree roots grow until they reach extractable water in the vadose zone and the transpiration water originates from this zone rather than from below the groundwater table. Furthermore the contaminant location affects the concentration in the transpiration water. Tree morphology, such as root depth and density, is therefore important in determining the final concentration of a contaminant in the transpiration stream. The tree morphology is related to local weather and the specific hydrogeology conditions such as depth to the groundwater table, soil permeability and water retention in the unsaturated zone.

Struckhoff et al (4) compared perchloroethylene (PCE) concentrations in tree cores to PCE concentrations in soil and groundwater at a field site. The study showed that the tree core concentrations had a stronger relationship with soil concentrations than with groundwater concentrations of PCE. The relationship between soil and tree core concentrations was hypothesized to be due to diffusion of PCE from soil gas into the roots.

Following plant uptake, contaminants may accumulate, phytodegrade or phytovolatilize (3). Incorporation into the plant tissue may occur directly or after transformation to a less toxic form. Naphthalene is readily biodegradable by microorganisms. Some plants may also be able to transform naphthalene, but phytodegradation has so far not been confirmed for naphthalene. Volatile contaminants in the plant xylem diffuse radially out of roots to lower concentration areas in the unsaturated zone (4, 10) or above ground to the atmosphere (10).

A concern related to plant uptake is the potential risk imposed by transferring the pollutants confined below ground to exposed biomass above ground. Plant accumulated contaminants may enter the food chain by ingestion or be deposited in the top soil as a result of plant litter. Volatile contaminants may be released to the air or accumulate in building structures and result in indoor exposure risk.

The objective of this study was to quantify the phytovolatilization of naphthalene by poplar trees in the field. Volatilization from tree trunks to the atmosphere was measured directly and total plant uptake was estimated based on transpiration rates and groundwater concentrations. In situ measurements of naphthalene flux out of tree trunks

were carried out throughout a year across the site to evaluate seasonal and spatial variations. Tree core analysis and a laboratory study provided supplementary proof of naphthalene uptake.

4.2 Materials and Methods

4.2.1 Site description

Phytoremediation with poplar trees was implemented at a creosote contaminated site in Oneida, Tennessee. The creosote was spilled during rail road tie treatment from the early 1950s to 1973. Creosote was discovered in a creek alongside the site in 1990. An interception trench and oil-water separator was installed to prevent off site contaminant migration. In 1997 a system of 1,146 hybrid poplar trees, *Populus deltoids x nigra DN34*, was planted on the site to provide hydraulic control and stimulate natural attenuation. Hybrid poplars are good candidates for hydraulic control and plant uptake of contaminated groundwater due to their fast growth, deep roots and relatively high transpiration rates. The site is located in a region with temperate climate and the majority of the 140 cm average yearly precipitation occurs during the winter and spring months. The aquifer underlying the site consists of sand and sandy clay to a depth of 3.0-3.5 m below ground with shale underneath. Creosote-based dense non-aqueous phase liquid (DNAPL) is pooled on the confining bedrock. The groundwater table within the unconfined aquifer remains relatively high in the winter from 0.6-1.2 m below ground, but decreases to 1.5-3.35 m below ground during the active growing season (11).

The contaminant concentrations and plume extent have been monitored, and the site has been the subject of research on mechanisms affecting the remediation process since installation of the system in 1997. The contaminant mass and plume extent has decreased over time (12). Increased removal rates occurred when tree roots reached the groundwater table after 3 growing seasons (12). Equilibrium between mass transfer into the groundwater from the DNAPL source and removal has stabilized the plume size and concentrations since 2001. The interception trench and the trees prevent contaminant migration off site.

4.2.2 Preliminary laboratory screening study

A three month hydroponic screening study for naphthalene uptake and phytovolatilization was conducted at the University of Missouri-Rolla in 2003. The method is described by Ma and Burken (10). The screening was run using triplicate poplar cuttings exposed to 10 mg/l naphthalene in Hoagland nutrient solution. One blank control cutting with Hoagland nutrient solution with no naphthalene was used as a control. The system is hydroponic, hence there is no soil present. The stems of the poplar seedlings were 0.7 cm in diameter and had a total length of 30.5 cm of which half was above the cap to the root zone. The flux chambers covered a 167 cm^2 surface section of stem from 2.5 to 10.1 cm above the cap to the root zone. A diffusion trap with a sorbent tube was placed on the stem from 2.5 to 10.1 cm above the tree root zone. The amount of naphthalene diffusing out of the strapped stem section was monitored by periodically replacing and extracting the attached sorbent tubes. The transpired water was recorded by weighing the system periodically and the transpired water was then replaced. At the termination of the experiment, the relative presence of naphthalene accumulated in tissue throughout the plant was determined by equilibrium between a tissue sample and a known headspace volume.

4.2.3 Field measurements

Flux chambers were mounted directly on tree trunks at the site in order to quantify the in situ naphthalene phytovolatilization flux rates. The flux measurements were carried out once a month from June to November 2004 at five locations shown in Figure 4.1. The locations are named after the closest multilevel groundwater sampling well (ML). Two sampling locations in the center of the plume were sampled from March 2004 to November 2004 (ML7 and ML22). The flux chambers were located 10-25 cm above ground. In July 2004 additional chambers were added to sample at vertical height up to 180 cm on tree trunks in the center of the plume (ML7).

The chambers consisted of 5 liter Tedlar bags wrapped around the tree trunks and sealed with plastic strips and weather resistant caulk against the bark. The chambers had outlet and inlet valves with Teflon tubes on opposite sides of the trunk. The naphthalene flux out of the tree trunks was trapped during 48 hour sampling events by pulling air from the chamber outlet Teflon tube and through XAD-2 sorbent tubes. The sorbent tubes were

wrapped in aluminum foil to avoid photodegradation. The cleaned air was recycled back into the chamber via the inlet valve in a closed system. A flow rate of 0.1 l/min minimized the risk of stripping naphthalene from the tree trunk at a higher rate than wind exposure. The continuous removal of naphthalene simulates exposure to clean atmospheric air. The sorbent tubes were capped, wrapped with aluminum foil, and stored at 4°C for no longer than 24 hours before analysis. Sorbent tubes from the field flux measurements were extracted with toluene following the NIOSH 5515 extraction procedure (13).

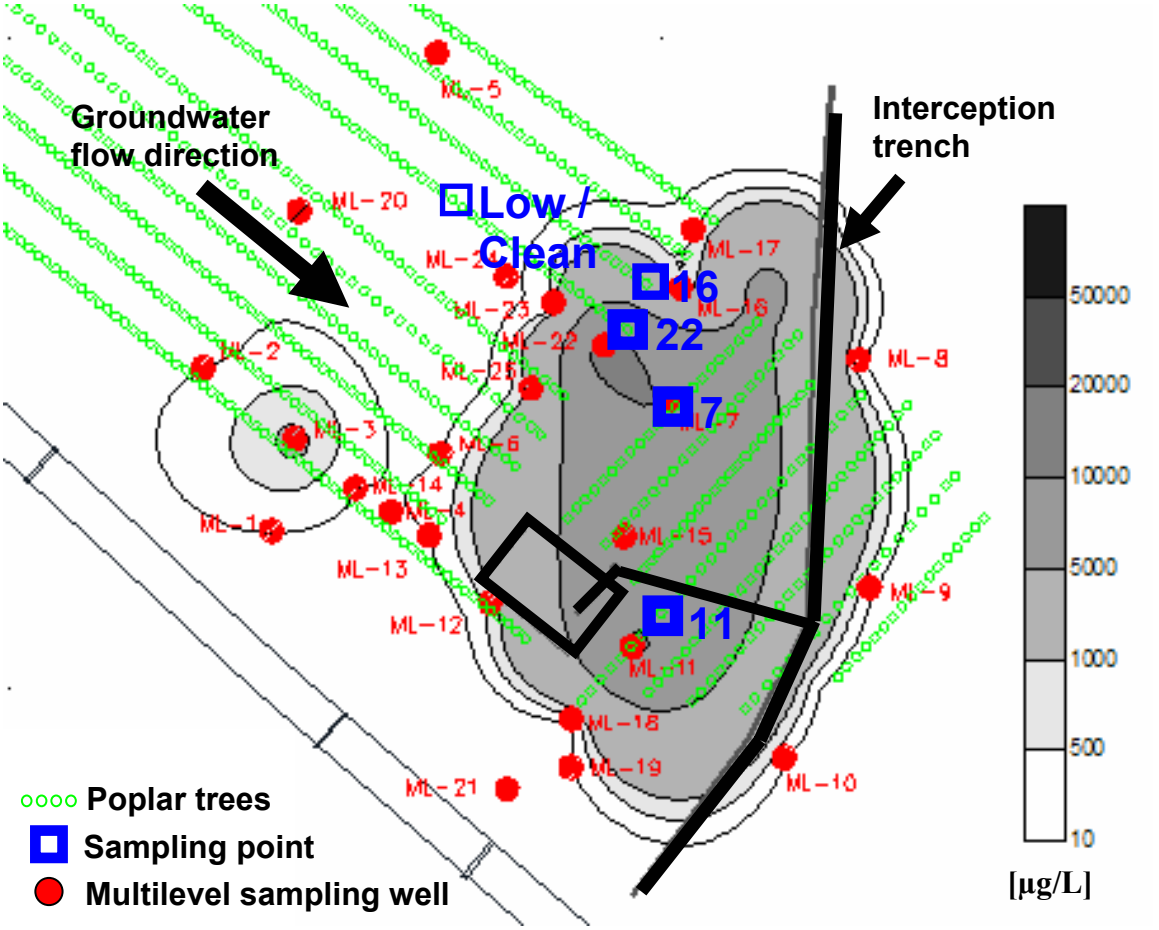

FIGURE 4.1 Deep (0-0.8 m above bedrock) naphthalene groundwater concentrations July 2004 (µg/L) and data sampling locations.

Core samples of tree tissue were collected as described by Ma and Burken, (9) from 21 trees across the site. The samples were transferred to 20 ml headspace vials and capped immediately. The samples were transported to the Engineering Research Center (ERC)

laboratories at the University of Missouri-Rolla for analysis by the method described by Ma and Burken (10). Phase distribution between gas in the headspace and water in the core tissue were calculated based on Henry's constant for naphthalene.

Groundwater elevations were measured once a month using a manual gauge in piezometers and recorded continuously with pressure transducers in three selected locations as described by Nelson (11). Groundwater level time series were used to evaluate the direct transpiration rates from the poplars (11). The transpiration rate was allocated to individual trees based on the square of tree diameter, which has been shown to be correlated to transpiration (14). Groundwater samples for PAH analysis were collected from multilevel sampling wells near the phytovolatilization sampling locations as described by Widdowson et al (12). Groundwater samples were extracted with methylene chloride and the extracts were analyzed on an automated Hewlett-Packard 5890 GC-FID and a DB-5 capillary column along with external liquid standards (12).

4.3 Results and Discussion

4.3.1 Naphthalene uptake laboratory results

The naphthalene flux rates out of the poplar seedling stems are summarized in Table 4.1. Transpiration rates for the seedlings were in the range of 28-66 mL/day. The total mass of naphthalene diffused out of the stem section was 0.42-0.74 mg/day. Tree 3 was sampled twice, after 4 weeks and after 6 weeks.

TABLE 4.1 Laboratory naphthalene uptake results, University of Missouri-Rolla.

		Naphthalene		Transpiration water
	Total time [days]	**Mass /day [µg/day]**	**Flux [µg/ m^2 day]**	**Volume/day [mL/day]**
Tree 1	14	679	**40611**	66.79
Tree 2	21	455	**27211**	62.24
Tree 3	28	420	**25150**	53.32
Tree 3	+ 14	744	**44528**	28.57

It is assumed that no diffusion loss occur in the root zone in the laboratory experiment since naphthalene vapors are confined and the concentration gradient between inside and outside of the roots therefore is zero (10). The naphthalene concentration in the uptake water predicted by the water concentration of 10 mg/L and the TSCF of 0.282 should be 2.82 mg/L. The naphthalene uptake rates based on the measured transpiration rates and the TSCF would be 80-188 µg/day if transpiration was the only uptake mechanism. This is five times lower than the measured stem flux. It is hypothesized that the reason for the higher naphthalene diffusion is that naphthalene diffusion from the gas above the water into the plants also contributes to the overall flux. The hydroponic system has no soil phase. This hypothesis could explain why the flux of naphthalene increases while the transpiration rate decreases from the first sampling period (week 0-4) to the second sampling period (week 4-6).

The presence of naphthalene in the plant tissue was assessed at the termination of the experiment. Figure 4.2 shows the naphthalene in the tissue represented as µg/L headspace. The data shows naphthalene accumulation decreases with the height of the sample along the transpiration pathway from roots to leaves. There was no naphthalene detected in the leaves and less naphthalene in the upper stem compared to the lower stem and in the stem in the root zone. The data show that the naphthalene diffuses out of the stem or degrades before it can reach the leaves.

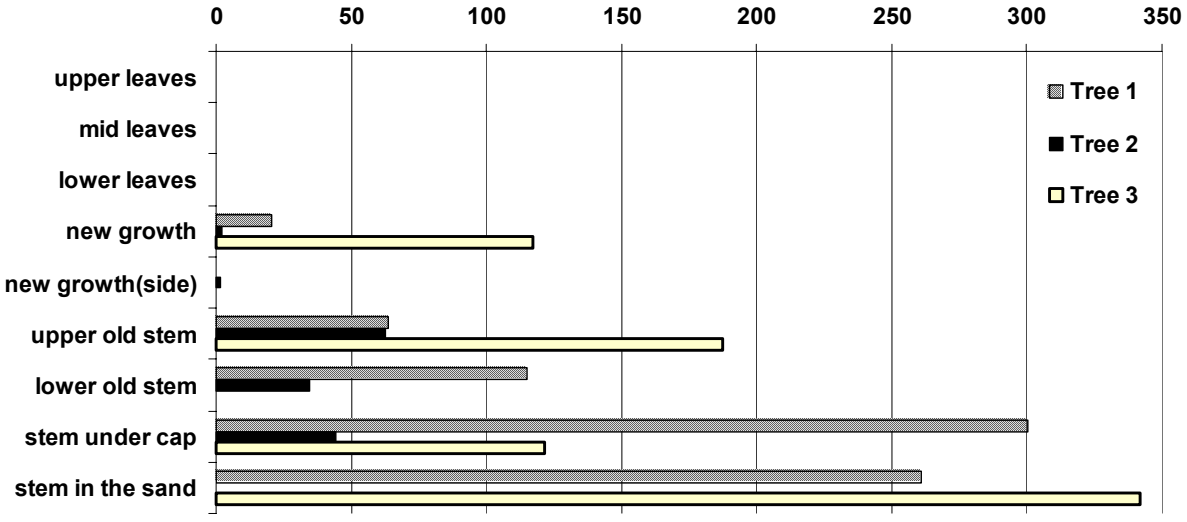

FIGURE 4.2 Relative presence of naphthalene in plant tissue [µg/L headspace].

4.3.2 Field transpiration rates and groundwater concentrations varies over time

Phytovolatilization via transpiration depends on the transpiration rate, groundwater concentration of the contaminant and the fraction of the total transpiration water which is drawn directly from the groundwater. A complication when interpreting field rates is that these variables change over time as well as between locations and trees. The transpiration rate and fraction of the transpiration that comes from the groundwater is dependent on weather, tree size and hydrogeology. The monthly total rainfall from March to October 2004 is shown in Figure 4.3a. The highest rain totals were recorded in May, late July, August and September. Figure 4.4b shows groundwater elevation along with estimated transpiration rates for a 232 m^2 area including the wells at ML7, ML16 and ML22 (b) and ML11 (c). The groundwater elevations are from P7 and from MW6, respectively. The well P7, used for transpiration rate at the area including ML7, ML16 and ML22, dried out from May to September. The transpiration data for this area in that period is therefore extrapolated from ML11 by multiplying by 1.5, which was the relationship factor between these two locations on May 4 and Sept. 19.

Figure 4.3 shows that transpiration rates increase in the spring, but as the groundwater elevation and rain decrease the direct transpiration rates decrease from May to early July. In August and September the transpiration rates increases, even though the groundwater table is still low. The transpiration increase is believed to be due to increased rain in July, August and September (Figure 4.3a). In October the transpiration rate decreases as the trees' active growing season is coming to an end.

The uptake of naphthalene is a function of the concentration in the groundwater as well as the transpiration rate. The groundwater concentrations of naphthalene increase as the groundwater table elevation declines in the summer at this site (Chapter 3).

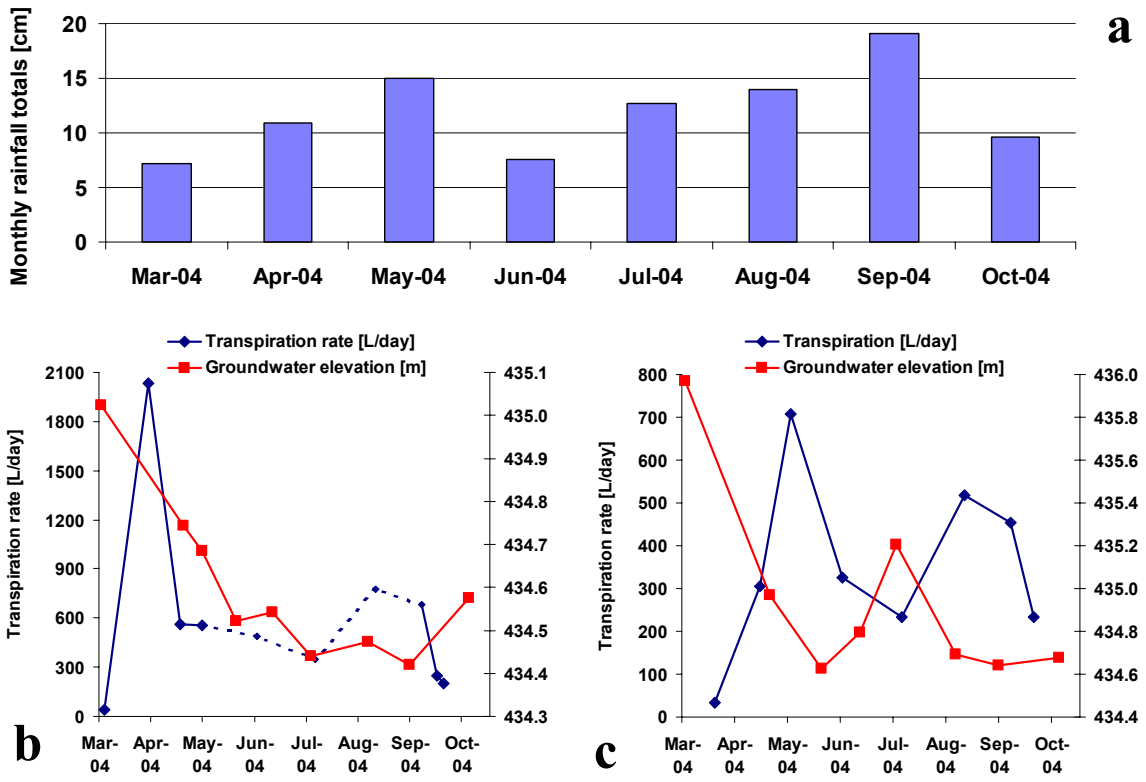

FIGURE 4.3 Total rainfall (4.3a) and transpiration groundwater elevations (4.3.b and 4.3.c) from March to October 2004. 4.3.b shows transpiration rates from 232 m^2 area including ML76, ML22 and ML16 and groundwater elevations from piezometer P7 inside this area. 4.3.c shows transpiration rates from 232 m^2 area including ML11 and groundwater elevations from MW6 inside this area.

4.3.3 Phytovolatilization measured in the field

The naphthalene phytovolatilization flux was measured directly on the trunks of five trees once a month. The study was used to determine the significance of naphthalene phytovolatilization at this site. Given the many variables in time and space described previously, five trees are insufficient for a statistical correlation of phytovolatilization with individual parameters. However, the data do provide an indication of the general rate of phytoremediation by phytovolatilization for naphthalene. Figure 4.4 shows phytovolatilization rates per m^2 of tree trunk area measured on the individual sampling trees at the five locations from April 2004 to October 2004. The rates shown on Figure 4.4 are all from chambers with dimensions of 15 cm height and covering the entire

circumference of the trunk at trunk heights from 10-25 cm above the ground. This results in that the chambers covers trunk surface areas of 0.04-0.11 m^2 depending on individual tree sizes.

The highest phytovolatilization rates occur from July to October 2004 (Figure 4.4) when the groundwater elevations are lowest (Figure 4.4) and the concentrations of naphthalene are highest (Chapter 3). It is evident from the data in Figure 4.4 that the phytovolatilization rates are highly variable over time and between locations.

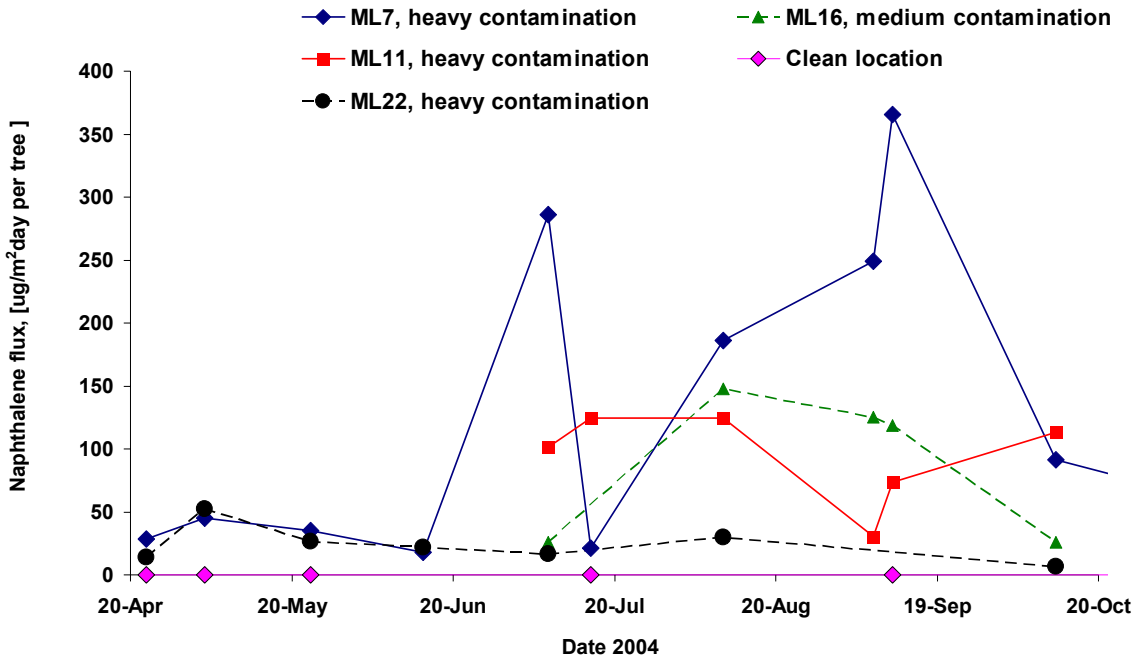

FIGURE 4.4 Phytovolatilization rates in µg/m^2 trunk surface area per day measured from 10 to 25 cm above ground on indiviudal trees at the five sampling locations April 2004 to October 2004.

The dramatic variation between the time series of phytovolatilization from the five trees is believed to be due to tree- and location specific variables. Table 4.2 lists local soil type, the average groundwater concentration in the closest well and tree size. As shown in Figure 4.4, some locations have small variability between sampling events (ML22) while others exhibit large variability (ML7). The naphthalene groundwater concentrations at ML22 are equal to or higher than the groundwater concentrations at ML7. The lower phytovolatilization rates for the tree at ML22 compared to the tree by ML7 indicates that either this tree has a smaller overall transpiration rate or that a larger fraction of the

transpired water is from the unsaturated zone and not from the groundwater. The tree by ML22 is smaller and therefore should transpire less water the tree by ML7. The smaller tree is expected to have fewer deep roots than the larger tree at ML7. Furthermore the location at ML22 has more clay and better moisture retention in the unsaturated zone than at ML7. The fraction of the transpiration water from the contaminated groundwater is therefore believed to be smaller at ML22 than at ML7.

TABLE 4.2 Characteristics of the sampling locations.

Location	Soil type	Groundwater naphthalene concentration, growing season, avg. top 0.5 m water [mg/L]	Tree diameter at trunk height of 137 cm [cm]
ML7	Sand	2977 - 8188	8.9
ML11	Clay / sand / silt	558 - 4736	9.7
ML16	Clay / sand / silt	170 - 475	19.4
ML22	Clay / sand / silt	3604 - 12470 *(and a NAPL spike of 49660)*	6.5
Clean	Clay / silt	0	9.7

The influence of tree size is most pronounced when comparing rates from the large tree at ML16 with the small tree at ML22 from July 2004 until October 2004 (Figure 4.4). The maximum rate at ML16 is 147 $\mu g/m^2$ day, compared to the maximum rate at ML22 of 30 $\mu g/m^2$ day. Transpiration rates have been found to be proportional to the tree diameter squared (14). The large tree located by ML16 is therefore expected to have approximately 9 times larger transpiration than the tree by ML22 (diameters listed in Table 4.2). The normalized flux rates by tree diameter squared results in a flux rate from ML22 that is only twice of the flux rate of ML16, even though the groundwater concentration at this site is 60 times higher. The reason the normalized flux rate is not 60 times higher at ML22 compared to ML16 is believed to be that a larger fraction of the total transpiration water is being drawn from the contaminated aquifer by the larger tree with deeper roots at ML16. The tree located by ML22 is smaller and has shallower roots and thus may consume a larger fraction of its transpiration water from the unsaturated zone in the summer months. The data show that larger trees have a higher volatilization rate per surface area when the difference in groundwater concentration is taken into account. Phytovolatilization can therefore be optimized by thinning out the tree stand to allow

fewer trees to grow larger and develop deeper roots. The effectiveness of the system can be further enhanced by including lower plants with dense root mass and water uptake in the unsaturated zone.

4.3.4 Phytovolatilization rate is influenced by weather

Transpiration and therefore phytovolatilization depend on weather conditions. The variability of the phytovolatilization over time is most pronounced for the tree located by ML7 (Figure 4.5, bottom). This location is characterized by sand from 1 m below ground to the bedrock, while the other locations have lenses of clay and silt (Table 4.2). The soil in the unsaturated zone at ML7 dries out to a greater extent here than in the other locations. It is believed that this tree takes up a larger fraction of the transpiration water from the contaminated groundwater during the summer than trees at ML11 and ML22.

During rain events there is a dramatic decrease in the phytovolatilization from the tree at ML7 (squares on Figure 4.5) compared to during dry sunny days (diamonds on Figure 4.5). This suggests that the tree transpire less during rain and/or retrieve a larger fraction of the overall transpiration water from the infiltrating rainwater as opposed to the deeper contaminated groundwater. Figure 4.5, top shows the atmospheric pressure and groundwater table corresponding to the sampling events. The phytovolatilization spikes May 4-5, July 7-9 and September 10-13 correspond to rising atmospheric pressure (clear skies and sun).

Data in Figure 4.5 show that phytovolatilization at ML7 also occurred in November 2004 and March 2005, which is outside the active growing season of the poplar trees. This indicates that some passive uptake mechanism is involved in addition to direct transpiration of contaminated groundwater. The laboratory uptake study also indicated transport by other means than transpiration. It is possible that the trees act as passive conduits for diffusion of gas phase naphthalene driven by a concentration gradient or a pressure gradient (4). The winter phytovolatilization rates are approximately 20% of the peak summer rate.

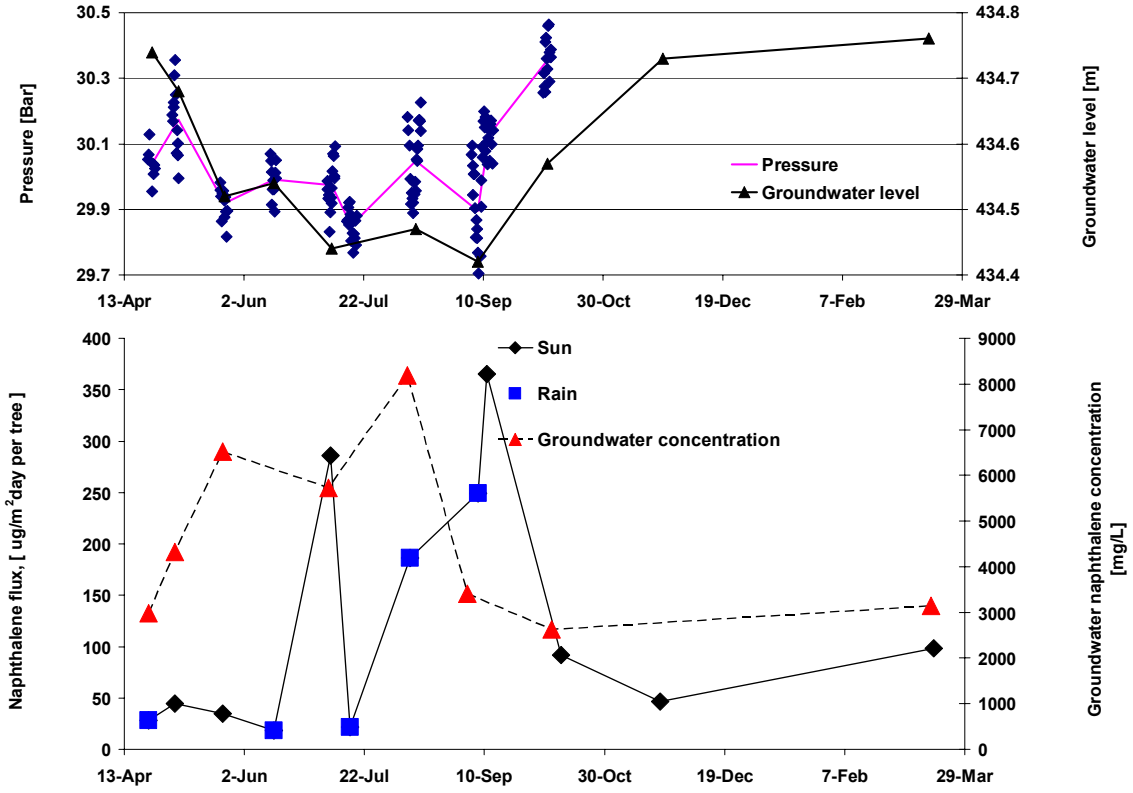

FIGURE 4.5 Phytovolatilization rates for individual tree and groundwater concentrations in contaminated area at ML7.

4.3.5 Naphthalene mass in tree cores

Tree core samples were collected from 21 trees in July 2004. The naphthalene concentrations in the cores ranged from 0.09-1.28 mg/L core water (Figure 4.6). The partition coefficient for naphthalene into wood is estimated from the K_{ow} to be around 56 L/kg (Communication with Burken, 2005). This results in estimated wood concentrations in the cores of 5 – 70 mg naphthalene/kg wood. Regulations for naphthalene concentrations in wood have not been established, but an oral reference dose (RfD) for chronic exposure to naphthalene of 0.02 mg/kg/day has been proposed by the EPA (15), which would be 1.4 mg/person day for a 70 kg person and this would be equivalent to a daily ingestion of 20-280 g wood with concentration of 5-70 mg naphthalene/kg wood. It is clear that the risk from the low levels of naphthalene in the wood is minimal. Risk associated with naphthalene is currently being re-evaluated (15).

Phytovolatilization rates, transpiration rates and groundwater concentrations were determined during the same sampling event during which the tree cores were sampled July 7-9 2004. The concentrations in the top 0-0.6 m of groundwater were 0-12000 mg/L at this time. Based on these groundwater concentrations and the TSCF of 0.282, the concentrations in tree water at the point of entry in the roots could theoretically be as high as 3380 mg/L.

Comparison of core concentrations and groundwater concentrations both sampled July 7-9 in 2004 shows no significant relationship (Figure 4.6). There are differences in the total transpiration based on tree size. The total transpiration is proportional to the tree diameter squared, but even after normalizing with tree diameter squared, there is no apparent relationship between the tree core concentrations and the groundwater concentrations. This could be due to variable losses of naphthalene before it arrives at the sampling location in the trunk. Geology and root depth also affect how much of the transpired water is contaminated groundwater. Differences in xylem flow on different sides of the same tree have been observed. Roots extending out in different radial directions from different sides of the tree can draw water with highly variable concentrations (10). Large trees also can have highly variable xylem flow patterns and the xylem flow can also be impacted by light direction, canopy closure and injuries to the trees (10). Given all these variables, tree cores did not appear to be useful for indicating plume naphthalene concentrations at his site. The core data does, however, provide a range of core concentrations as a second line of evidence for lower levels of naphthalene in above ground biomass than expected based on the TSCF and groundwater concentrations.

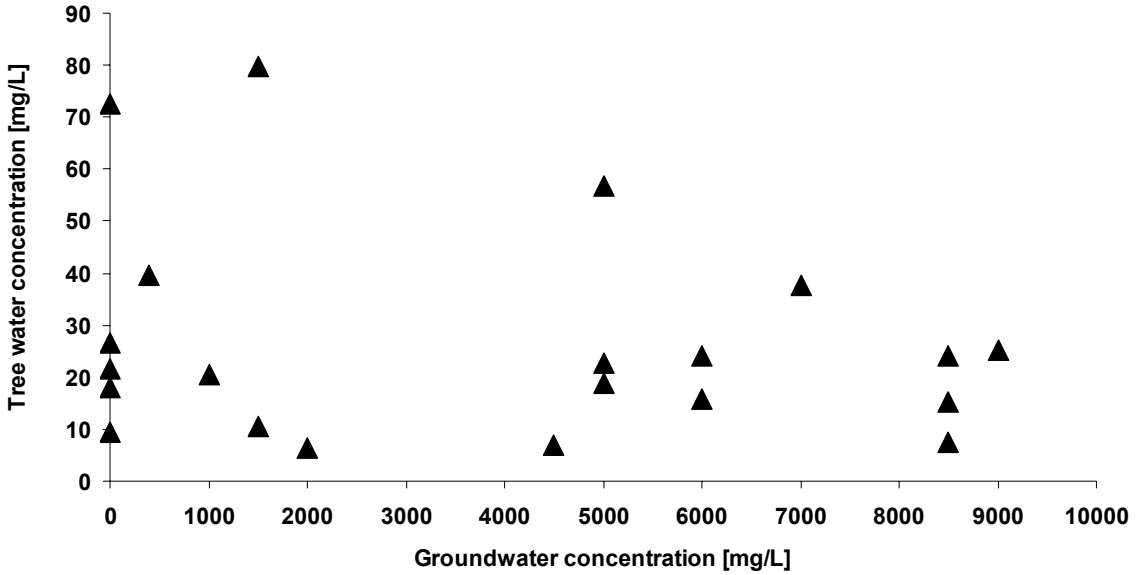

FIGURE 4.6 Naphthalene concentrations in tree core water normalized by tree diameter versus groundwater (spatially interpolated from measured values).

4.3.6 Phytovolatilization is lower than plant uptake estimated from transpiration

Vertical measurements of the phytovolatilization flux for naphthalene at this site were conducted at ML7 from July to October 2004 (Figure 4.7). The fluxes shown in Figure 4.7a were measured with the closed flux chambers generally used for the flux data presented in this study. The fluxes measured in heights up to 170 cm shown on Figure 4.9b were obtained with semi-closed chambers and had much lower fluxes (notice the scale for fluxes on the x-axis is 20 times smaller). The vertical measurements show that the flux declines with tree height. The fluxes measured with the closed flux chambers (Figure 4.7a) become negligible 0.5 m above ground surface.

The vertical measurements with permanent flux chambers at ML7 (Figure 4.7a) were used to derive a relationship for the flux with height at ML7 for the individual sampling events between July and October 2004. It is assumed that the decline with height is linear. For all other locations and earlier sampling events at ML7, the linear relationship is based on the flux measured at the base of the tree and the average decline rate from ML7 measurements. The total flux out of the tree trunks was estimated by integration of the linear declining flux over the trunk surface area. The surface area of this trunk is calculated from linear interpolation of the area from the diameters measured at the base of the trunk and at 1.37 m height.

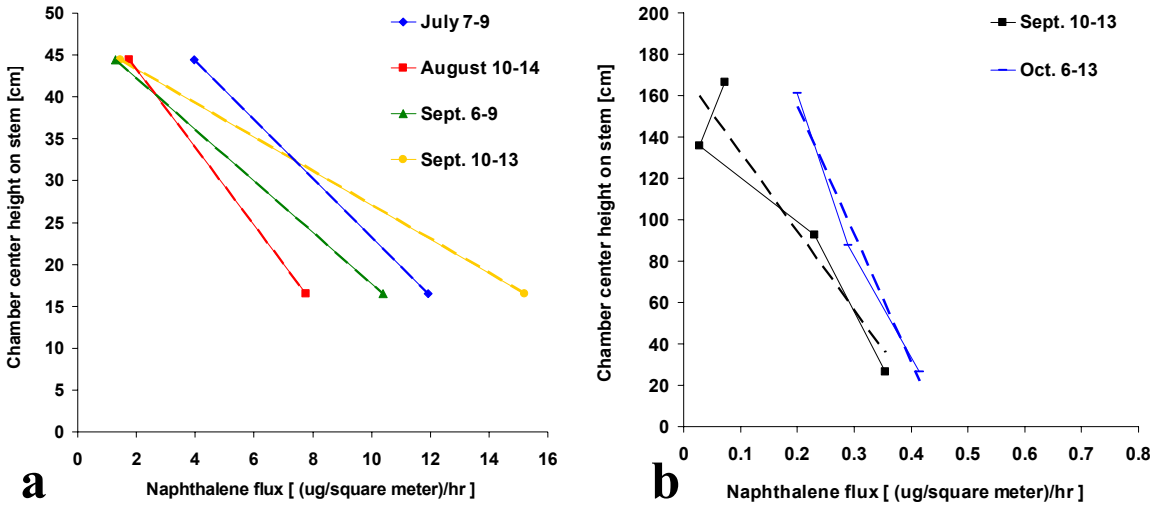

FIGURE 4.7 Phytovolatilization rates as a function of stem height. Plot a show results from closed flux chambers, while plot b shows results from semi-closed flux chambers

Table 4.3 and Figure 4.8a shows phytovolatilization rates for specific trees measured in July, August and September 2004 calculated from the flux chambers. The rates are between 4.7 and 46 µg naphthalene per day per tree. Table 4.4 and Figure 4.8b shows the theoretical maximum naphthalene uptake for specific trees with transpiration rates for July, August and September assuming that the transpired water is entirely from the contaminated groundwater. The maximum uptake rates were calculated to be between 1200 and 31200 µg/day per tree. The uptake of naphthalene with transpiration of contaminated groundwater was estimated as: Uptake = $C_{groundwater}$ * transpiration rate * TSCF, where TSCF for naphthalene is 0.282. The transpiration rates are calculated from groundwater table elevation fluctuations and precipitation, as described by Nelson, 2005 (11). The transpirations rates are allocated to individual trees by the trunk diameter squared at chest height (137 cm above ground) The data are derived assuming that all the transpiration water comes from the groundwater directly, but the actual fraction will be lower. The high maximum uptake rates estimated at ML7 and ML22 in August are the results of high groundwater naphthalene concentrations.

TABLE 4.3 Total trunk areas with detectable naphthalene flux, for individual trees average naphthalene flux rates for individual trees and total phytovolatilization of naphthalene from individual trees per day.

	Trunk area with flux [cm^2]			Flyx rate avg. over area with flux [$\mu g/cm^2$ day]			Total phytovolatilized [µg/day]		
	July	**Aug.**	**Sept.**	**July**	**Aug.**	**Sept.**	**July**	**Aug.**	**Sept.**
ML7	307.8	254.3	254.3	0.131	0.095	0.180	40.3	24.3	45.8
ML11	157.8	171.8	143.7	0.072	0.081	0.06	11.4	13.9	8.7
ML16	199.8	367.6	393.8	0.049	0.089	0.077	9.8	32.9	26.2
ML22	106.9	106.9		0.436	0.052		4.7	5.6	

TABLE 4.4 Transpiration rates, naphthalene concentrations in the top 0.6 m groundwater and total phytovolatilization of naphthalene from individual trees

	Transpiration rate [L/day per tree]			Naphthalene concentration in top 0.6 m groundwater [ug/L]			Total phytovolatilized [µg/day per tree]		
	July	**Aug.**	**Sept.**	**July**	**Aug.**	**Sept.**	**July**	**Aug.**	**Sept.**
ML7	6.10	13.49	11.83	5727	8188	3411	9852	31157	11379
ML11	4.07	9.00	7.89	4676	4736	3688	5362	12013	8202
ML16	24.40	53.99	47.33	170		226	1170		3020
ML22	2.74	6.06	5.31	12470	11613	49660	9631	19840	74379

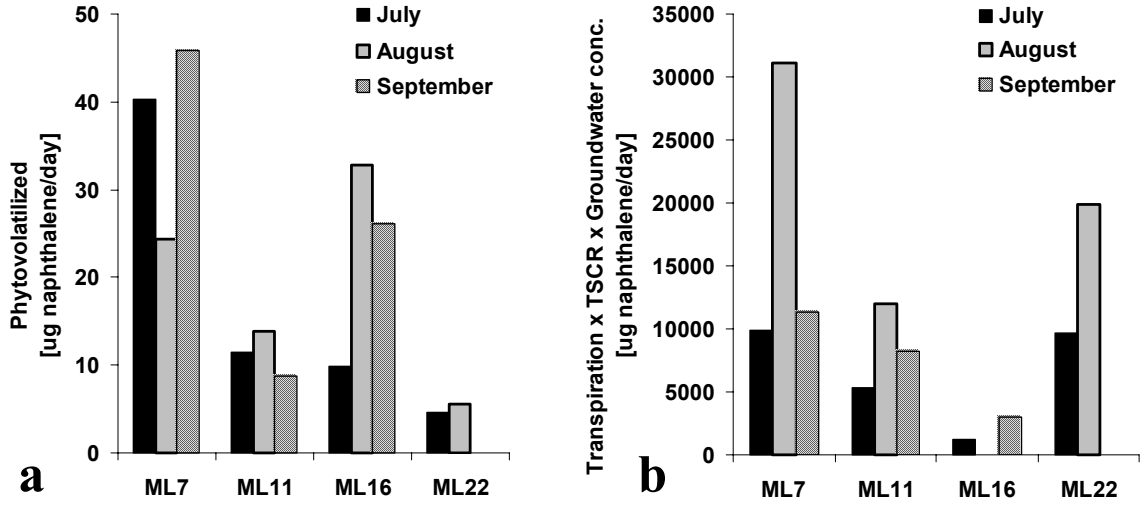

FIGURE 4.8 Rates of phytovolatilization measured for individual trees and theoretical uptake calculated for individual trees from transpiration x TSCF x groundwater concentration

The maximum possible uptake is approximately 100-4000 times higher than the measured phytovolatilization. The transpiration water is a mixture of contaminated groundwater, clean infiltration water and vadose zone water, so the actual concentrations in the xylem are expected to be lower. Tree roots will tend to grow until they reach the vadose zone immediately above the groundwater table and draw transpiration water from this zone rather than directly from the groundwater table below. The interface between the groundwater and the soil gas is a zone with rapid naphthalene biodegradation due to available oxygen and volatilized naphthalene (see Chapter 3). The concentration of naphthalene in the vadose zone water is therefore believed to be considerably lower than the concentration in the shallow groundwater.

The difference between the theoretical uptake of naphthalene from transpiration of groundwater and the much lower naphthalene mass phytovolatilized could also be due to loss mechanisms during the transport from the roots to the above ground biomass. Possible loss mechanisms are degradation in biofilm on the root surfaces, diffusion out of roots to the unsaturated zone and phytodegradation in the plant.

This study shows that naphthalene uptake and phytovolatilization occurs, but that phytovolatilization is much lower than predicted from groundwater concentrations and transpiration rates. The phytovolatilization rates are highly variable and change with weather and between locations. Phytovolatilization rates of naphthalene are higher from larger trees due to larger transpiration rates and deeper roots. There appears to be no direct relationship between naphthalene concentrations in the groundwater and naphthalene concentrations in the tree tissue. Phytovolatilization of naphthalene appears to play a minor role in the loss of naphthalene from the site. The indirect effects of transpiration for hydraulic containment and promotion of contaminant mass transfer to the vadose zone are thought to be of greater importance than the direct phytovolatilization.

Acknowledgements

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CHAPTER 5

Phytovolatilization and Bioremediation of Naphthalene at a Creosote-Contaminated Phytoremediation Site

Andersen, Rikke G.; Booth, Elizabeth C.; Nelson, Mike;
Marr, Linsey C.; Widdowson, Mark A.; Novak, John T; (Virginia Tech, VA, USA)

Preliminary comparison of removal mechanisms: Oral presentation and proceedings paper, The Eighth International In Situ and On Site Bioremediation Symposium, Battelle, Baltimore, Maryland, June 6-9, 2005.

Abstract

The role of vadose zone biodegradation relative to physical removal mechanisms induced by poplar trees at a creosote-contaminated site was investigated. Groundwater monitoring in a surficial aquifer for seven years demonstrated a reduction in polycyclic aromatic hydrocarbons (PAHs) with selective removal of naphthalene and three-ring compounds. Naphthalene mass loss mechanisms investigated at this site are plant uptake, phytovolatilization, volatilization and vadose zone biodegradation. Phytovolatilization was quantified in flux chambers mounted on trees and calculated from transpiration rates. Phytovolatilization was active during tree growth periods, but below detection during winter months. Vapor phase transport of naphthalene coupled with biodegradation in the unsaturated vadose zone was simulated using laboratory soil columns and confirmed using field monitoring. The results demonstrate significant volatilization of naphthalene followed by rapid biodegradation in the vadose zone within the first ~15 cm above the source of the vadose zone. Naphthalene flux measured in flux chambers at the land surface was up to five times higher in the summer than in the winter. Overall, the results show that vadose zone biodegradation is orders of magnitude higher than phytovolatilization and flux out of the ground surface.

5.1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are primary contaminants in soil and groundwater from Non-Aqueous Phase Liquids (NAPL) such as creosote, coal-tar and diesel fuel, which are widely applied or generated by industry for example in wood preservation or fossil fuel refinery. Phytoremediation, the use of plants to remediate contamination, has been shown to accelerate natural attenuation of PAHs in soil and groundwater (Banks et al, 1999), but the individual mechanisms involved are not completely understood. Phytoremediation can be an effective, low impact, aesthetically pleasing and economically favorable alternative to conventional remediation technologies. Plants create a favorable ecosystem in the rhizosphere for microbial communities and increased biodegradation of PAHs in rhizosphere soil compared to unplanted soil (Anderson et al, 1993). Immobilization of PAHs by humification can be enhanced in the rhizosphere (Banks, 1999) Transpiration lowers the groundwater table, thereby containing contaminated groundwater (Landmeyer, 2001), and at the same time increasing volatilization to the unsaturated zone (Davis, 2002). Direct plant uptake of contaminants followed by volatilization to the atmosphere or phytodegradation has been found to be important for different contaminants (Burken and Schnoor, 1998).

A creosote contaminated phytoremediation site in Oneida, Tennessee has been the subject of intense monitoring since a phytoremediation system comprised of over 1,000 hybrid poplar trees, *Populus deltoides x nigra DN34*, was installed in 1997 (Widdowson et al, 2005). A 0.3 m layer creosote layer on the bedrock 3 m below ground acts as a persistent source of PAH contamination in the surficial aquifer. Seven years of groundwater monitoring has demonstrated that the phytoremediation system along with an interception trench provide hydraulic control and preferential removal of naphthalene and 3-ring compounds. Increased removal rates were observed after three growing seasons as the tree roots reached the groundwater table. After a year of enhanced removal a new steady state was established at a lower contamination level as a result of equilibrium between dissolution of PAHs from creosote NAPL and removal mechanisms.

The overall goal for this research is to determine the governing phytoremediation mechanisms at this site and quantify the mass removal rates. Previous research has demonstrated that aerobic and anaerobic biodegradation of PAHs are enhanced in the

rhizosphere at this site (Robinson et al, 2003; Pitterle, 2004). Biodegradation in the saturated zone is significant throughout the year, but in situ biodegradation rates are highest in the summer and decrease by a factor of four during the winter months. (Pitterle, 2004). Naphthalene is the primary contaminant in soil gas and groundwater and at this site. Naphthalene mass loss mechanisms investigated in this study are plant uptake combined with phytovolatilization and volatilization combined with vadose zone biodegradation.

5.2 Materials and methods

Comprehensive field sampling was carried out to investigate the mechanisms of direct uptake combined with phytovolatilization, and vadose zone transport combined with biodegradation and volatilization at the land surface. Field sampling was conducted at six locations shown in Figure 5.1. Sampling took place from October 2003 to March 2005 during various weather conditions and tree transpiration rates.

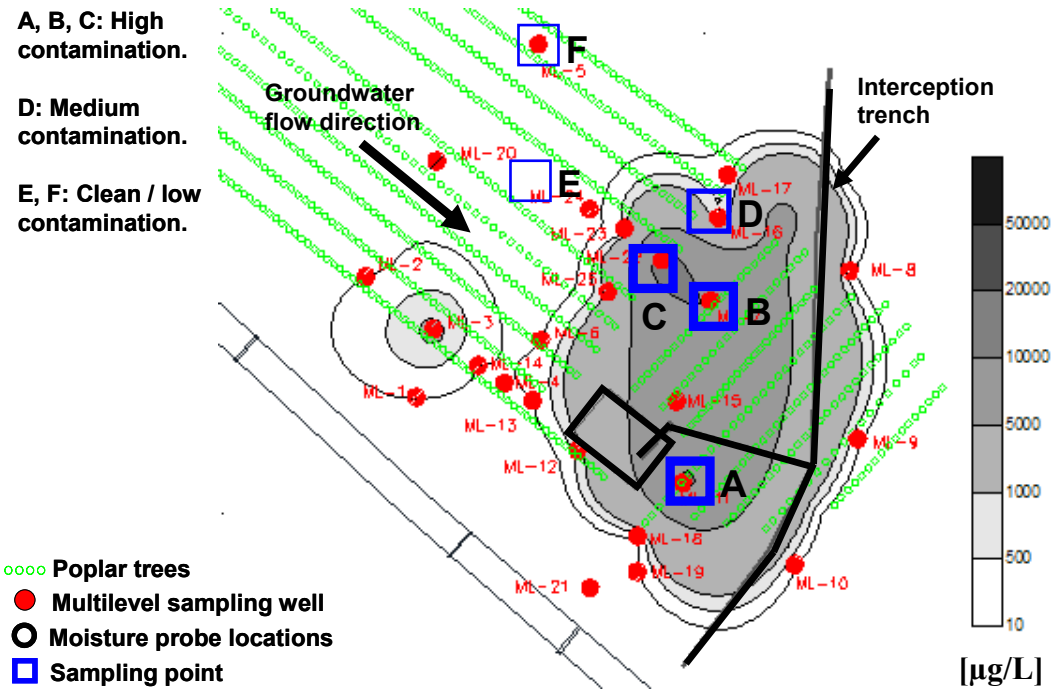


FIGURE 5.1 Deep (0-0.8 above bedrock) naphthalene groundwater concentrations (µg L^{-1}) in March 2004 and data sampling locations.

Phytovolatilization of naphthalene out of tree trunks was measured monthly from March to November 2004 and in March 2005. Sampling was conducted at several heights above ground on tree trunks. Naphthalene flux out of the soil surface was measured once a month from October 2003 to October 2004. Vertical subsurface profiles for naphthalene, carbon dioxide and oxygen in the soil gas were measured during the same period, but an optimal method for sampling of naphthalene in the soil gas was not finalized until July 2004. Weather data, moisture levels in the unsaturated zone, depth to the groundwater table and naphthalene concentrations in the groundwater were monitored during sampling events.

5.2.1 Phytovolatilization measurements

Flux chambers were mounted directly on tree trunks in order to quantify the phytovolatilization of naphthalene in situ. The chambers were Tedlar bags wrapped around the tree trunks and sealed with plastic strips and weather resistant caulk against the bark. The chambers had outlet and inlet valves on opposite sides of the trunk. Naphthalene fluxes out of the tree trunks were trapped during 48 hour sampling events by pulling air from the chamber outlet tubes and through XAD-2 sorbent tubes. The cleaned air was recycled back into the chamber via the inlet valve in a closed system. A flow rate of 0.1 l/min minimizes the risk of stripping naphthalene from the tree trunk at a higher rate than wind exposure. The continuous removal of naphthalene simulates exposure to clean atmospheric air.

5.2.2 Surface flux and soil gas measurements

Stainless steel chambers were built to measure the naphthalene vapors volatilizing from the ground surface. These chambers were installed in the ground surface and designed to trap naphthalene vapors escaping from the soil surface to the atmosphere. Similar to the tree chambers, naphthalene collected in the chamber was trapped on XAD-2 sorbent tubes and the clean air recycled back into the other end of the chamber. The method is further described by Booth (2005).

Permanent soil gas probes made from galvanized steel were installed by hand auger or fencepost hammer to enable assessment of vertical profiles of soil gas concentrations

of naphthalene, oxygen and carbon dioxide in the unsaturated zone. Oxygen and carbon dioxide concentrations in the soil gas were measured with a handheld GasTech monitor. One liter of soil gas was evacuated from each probe every 30 minutes, and naphthalene was trapped on XAD-2 sorbent tubes until 15 liters was collected. The slow flow rate of 2 L/hour allows time for the soil gas content of the semi-volatile naphthalene to recover by mass transfer from contaminated soil and groundwater.

5.2.3 Laboratory column experiment

Quantification of volatilization and biodegradation rates requires that these mechanisms be separated. Furthermore field soil gas measurements are influenced by dynamic fluctuations in moisture and groundwater level, and soil gas samples have not been recovered any closer than 15 cm above the groundwater table. To overcome these issues field observations are supplemented by a column study that was designed to quantify rates of volatilization and biodegradation of naphthalene in the unsaturated zone as well as to validate field observations. The column study is described in detail by Booth (2005). Detailed soil gas profiles for naphthalene, oxygen and carbon dioxide closer to the water/gas interface can be studied using soil columns and variables complicating field measurements can be controlled. The column experiment obtains steady state conditions for calculation of diffusion and biodegradation rates by similar means as in experiment conducted by Höhener et al (2003). The effects of volatilization versus biodegradation were separated by comparison of sterilized versus live columns, with sterilization achieved by repeated autoclaving of the soil over several weeks.

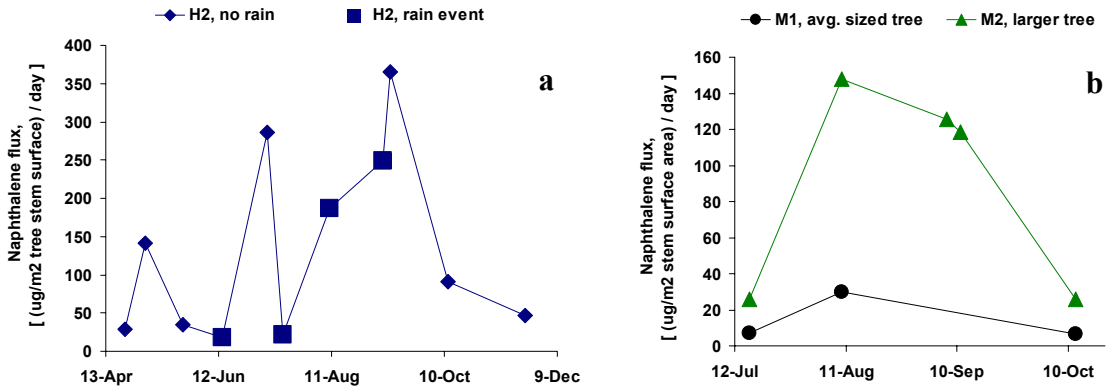
5.2.4 Analytical methods

The XAD-2 sorbent tubes from tree flux, surface flux, soil gas and columns were capped and stored in the dark at 4ºC until extracted with toluene using the NIOSH 5515 procedure. Groundwater samples were extracted with methylene chloride as described by Widdowson et al (2005). The extracts were analyzed along with external standards on a Hewlett Packard 5890 gas chromatograph with Flame Ionization Detection and DB5-MS fused silica capillary column for analysis of PAHs.

5.3 Results and discussion

5.3.1 Phytovolatilization of naphthalene

Phytovolatilization measured within the most contaminated area of the site from April to October 2004 is shown in Figure 5.2, left side. The larger square points were affected by rain events. The phytovolatilization starts in the Spring when the trees begin to transpire and peaks in the Fall when the groundwater table is lowest and groundwater concentrations of naphthalene are highest. The highest phytovolatilization rate was 365 $\mu g/m^2$ tree surface area per day in September 2004 in the most contaminated area.



Average tree, highly contaminated area

Large and average trees, medium contaminated area

FIGURE 5.2 In situ phytovolatilization per square meter of tree surface, 2004. H2, M1 and M2 refer to the locations shown in Figure 1.

Plant uptake of naphthalene is driven by transpiration and is proportional to the concentrations of the contaminant in the groundwater (Burken and Schnoor, 1998). Figures 5.2a and 5.2b show that phytovolatilization is higher from the contaminated area compared to the medium contaminated area, respectively. Maximum phytovolatilization rates from average sized trees in areas with high-, medium- and low naphthalene groundwater concentrations were 365, 30 and 7 $\mu g/m^2$ tree surface area per day respectively. During drier months, the groundwater table declines leading to decreased transpiration rates but also higher groundwater concentrations. Larger trees with higher transpiration rates and deeper roots can enhance the overall phytovolatilization rate by transpiration of larger amounts of water from the deeper contaminated groundwater. This

is demonstrated in the right side of Figure 5.2, which shows that phytovolatilization per trunk area is significantly higher from a larger tree compared to an average size tree.

Field data verify that the naphthalene flux decreases with the height above ground and becomes negligible at trunk heights above 60 cm. Assuming linear decline of the flux with tree height and using actual tree dimensions to calculate surface areas, the total phytovolatilization from 12 trees in a 21 m^2 section of the most contaminated area is estimated to be 445 µg/day during July 7-9. Estimated phytovolatilization rates from the same area and time based on actual transpiration rates are 100 times higher (Nelson, 2005). This indicates that naphthalene is being lost during or after plant uptake. Potential removal mechanisms are 1) diffusive loss during passage through the roots to the unsaturated zone where it may be sorbed to the soil, biodegraded or volatilize to the atmosphere (Struckhoff et al, 2005), 2) biodegradation in the rhizosphere during uptake, 3) phytodegradation within the plant or 4) rapid photodegradation in the flux chambers before naphthalene can be trapped on the sorbent tubes.

5.3.2 Vertical soil gas profiles

Generally the in situ soil gas concentrations of naphthalene are below 1 µg/l at elevations more than 0.5 m above the groundwater, and soil gas has not been sampled any closer than 15 cm above the groundwater table due to water rise induced by the pumping. Figure 5.3 shows soil gas content in a highly contaminated area measured 1.4 m below ground, approximately 0.5-1 m above the groundwater table. The volumetric soil gas content is the percentage of the porosity occupied by gas. From April to July 2004, the groundwater table declined from 2 to 2.3 m below ground (m b.g.), stayed low until September, and rose back to 2 m b.g. in November at the location shown in Figure 5.3. Accordingly the soil moisture content is at its lowest level from July to September and the highest volumetric gas contents are found in this period.

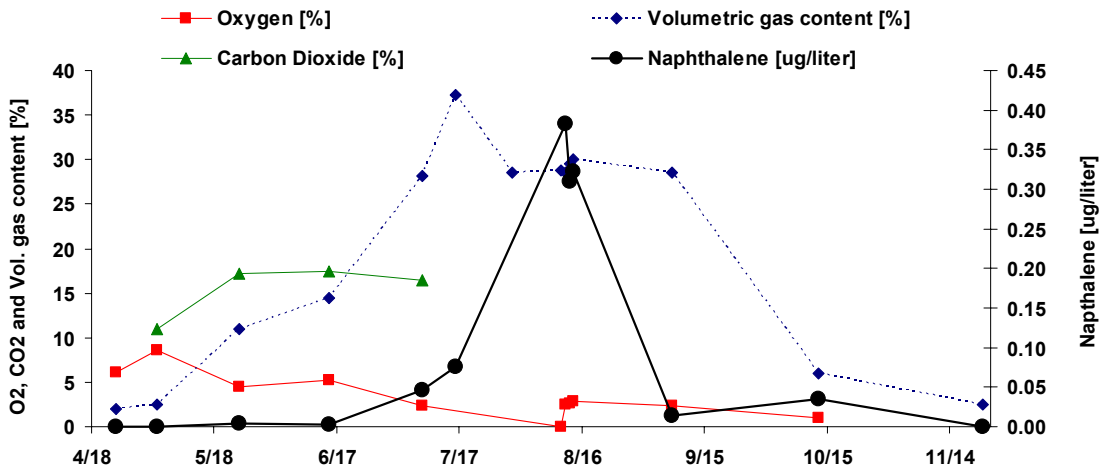


FIGURE 5.3 Soil gas concentrations 1.4 m b.g. in highly contaminated area, H2.

The highest soil gas concentrations of naphthalene were measured in August and September, when low groundwater levels allowed deeper soil gas sampling 2 m b.g (data not shown). Based on the actual groundwater concentrations of naphthalene and residual creosote exposed at low groundwater levels, soil gas concentrations are predicted to be up to 10 times higher from equilibrium relationships and diffusion. This indicates that biodegradation occurs in the vadose zone immediately above the groundwater.

Vertical profiles for naphthalene in columns with contaminated soil and in a contaminated area in-situ are shown in Figure 5.4. In situ soil gas concentrations measured in the most contaminated area in August 2004 are of the same magnitude as soil gas concentrations in the column study. The sterile control illustrates the profile if naphthalene was influenced by diffusion alone. The difference between the sterile and live columns shows that significant biodegradation occurs. The biodegradation occurs mainly in the first 5 cm above the naphthalene source in the columns.

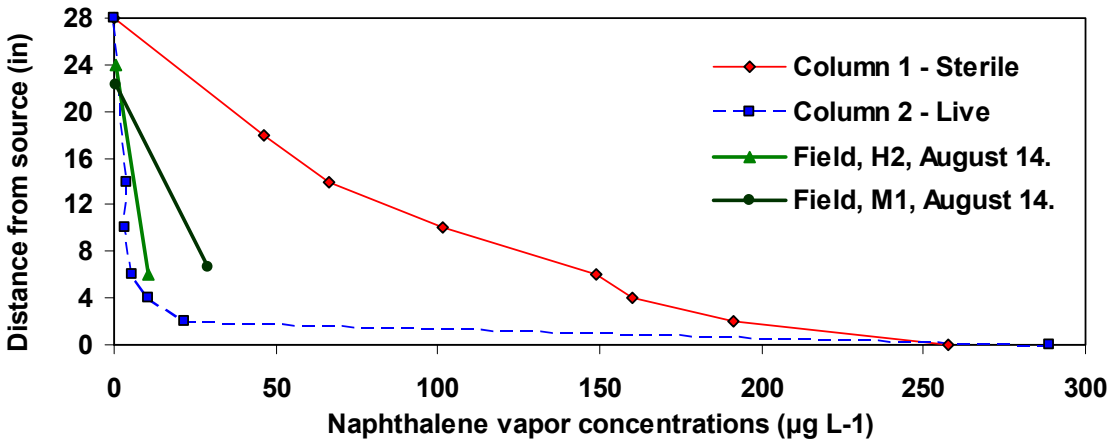
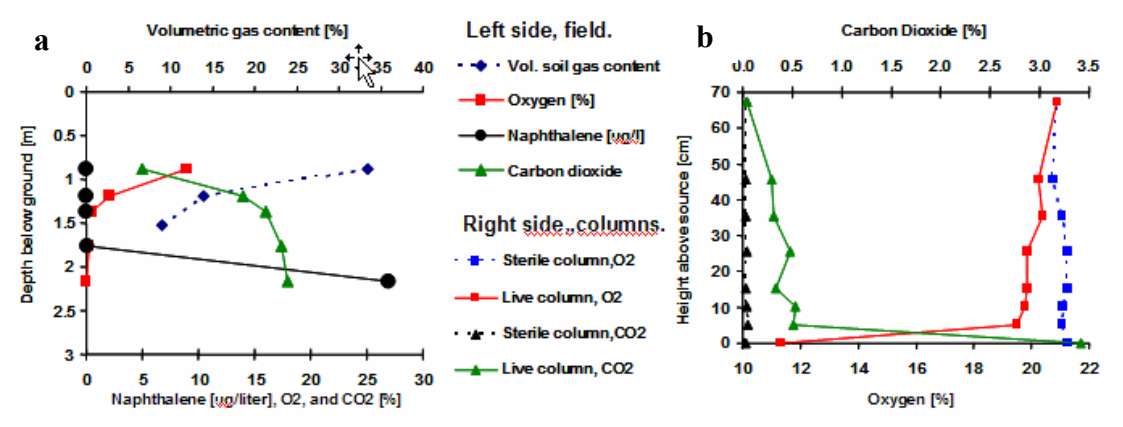


FIGURE 5.4 Naphthalene gas concentrations in soil columns and field. H2 and M2 refer to locations on Figure 5.1.

First-order aerobic biodegradation rates were fitted to soil column data for contaminated and uncontaminated soil. The maximum first-order rates were determined to be 25 and 12 day^{-1} for contaminated and uncontaminated soil, respectively. These rates where obtained after 3-5 months of acclimatization time with continuous supply of naphthalene and oxygen levels never falling below 5% right above the source.

Figures 5.5a and 5.5b show vertical profiles of oxygen and carbon dioxide content in soil gas for a contaminated area in the field and in columns with contaminated soil, respectively. Both the field and the live column profiles indicate aerobic biodegradation while the sterile column shows no indication of aerobic biodegradation. The field profiles are the result of oxygen depletion and carbon dioxide accumulation in the vadose zone as well as in the groundwater. Oxygen diffusion is limiting in the field profiles, as oxygen content is close to zero at depths below 1.5 m.

Aerobic biodegradation in a 15 cm zone above the groundwater with first order biodegradation rates 5-25 day^{-1} and soil gas naphthalene concentrations of 100-200 µg/l yields total mass removal of maximum 1400 to 9700 mg/day. These mass removal rates are under assumption of hourly replenishment of removed naphthalene by mass transfer from creosote and groundwater, which is suggested by field observations.



Profiles for O_2, CO_2, naphthalene and volumetric gas content in medium contaminated area, Sept 2004.

Profiles for O_2 and CO_2 in columns with contaminated soil, 4 months incubation.

FIGURE 5.5 Gas concentrations in soil gas probes (a) located in a contaminated area of the field site and laboratory columns containing contaminated soil (b).

5.3.3 Surface flux

Naphthalene fluxes out of the ground surface measured monthly from October 2003 to October 2004 are shown in Figure 5.6. The highest surface flux of 23 µg/m^2 per hour observed in August corresponds to a spike in naphthalene concentrations in the soil gas shown on Figure 5.3. This particular sampling event was characterized by a dramatic weather change in weather. The surface flux from a 21 m^2 section of the most contaminated area is estimated to be 790 µg/day from the rate of 1.57 µg/m^2 per hour on July 7-9, but higher at the peak in August. Generally the variations in fluxes are correlated to rainfall, pressure fluctuations, soil moisture content, temperature, humidity, and the groundwater table level.

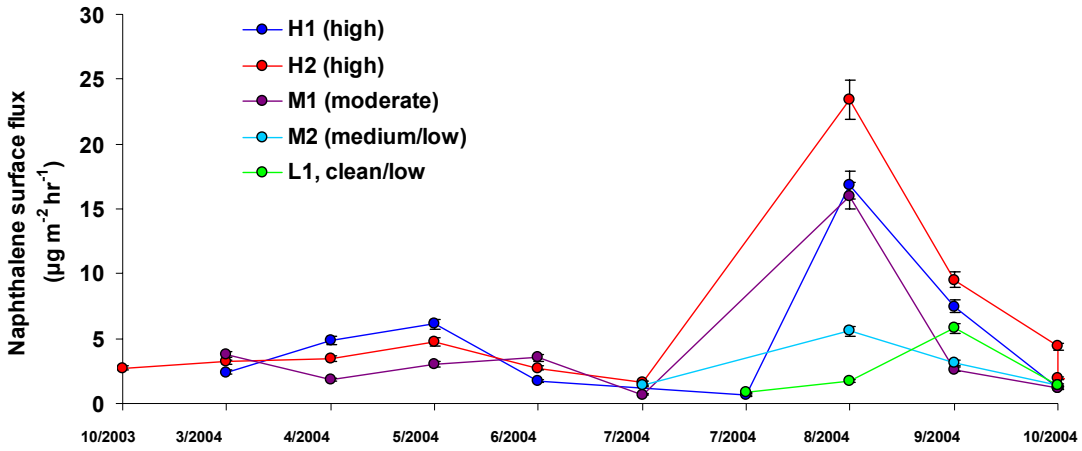


FIGURE 5.6 Naphthalene surface flux over time at various locations at the site.

5.4 Conclusions

Phytovolatilization and volatilization coupled with vadose zone biodegradation are most significant in summer and fall months when the soil moisture and groundwater table are lowest. The phytoremediation system enhances these mechanisms by transpiration of significant masses of water from the subsurface. Larger trees are beneficial for phytoremediation at this site due to higher transpiration rates and deeper roots. Rapid aerobic biodegradation immediately above the source was observed in a column study with soil gas exposed to solid naphthalene. The column results are supported by the field measurements of naphthalene in the soil gas and naphthalene surface flux.

The results indicate that direct losses of naphthalene to the atmosphere from surface flux and phytovolatilization are relatively low compared to biodegradation in the vadose zone. From the July 7-9 rates the total phytovolatilization is estimated to be 0.445 mg/day and the surface flux is estimated to be 0.79 mg/day from a 21 m^2 section of the most contaminated area. Aerobic biodegradation in the vadose zone in the same section yields a total mass removal of between 1400 to 9700 mg/day.

Biodegradation in the vadose zone and saturated zone is significant in summer and fall months. Smaller contributions to the mass removal comes from phytovolatilization and surface flux to the atmosphere especially in the late summer and early fall. Saturated zone biodegradation and groundwater flushing dominates the mass removal during winter

months. Rates for phytovolatilization, volatilization coupled with biodegradation in the unsaturated zone and biodegradation in the saturated zone will be utilized to estimate the overall remediation rates of the mechanisms combined over a year.

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CHAPTER 6

Quantification of naphthalene mass removal by individual phytoremediation mechanisms

Rikke G. Andersen, John T. Novak, Mark A. Widdowson, Linsey C. Marr

The Charles E. Via Jr. Department of Civil and Environmental Engineering,
Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0105.

Abstract

Naphthalene removal was quantified for different mechanisms at a creosote contaminated site using poplar trees for phytoremediation. Biodegradation of naphthalene in the saturated zone dominates by orders of magnitude over the removal by direct volatilization and phytovolatilization at this site. The total removal of naphthalene was estimated to require 50-100 years with phytoremediation, but more than twice as long without phytoremediation. The estimated removal of naphthalene was three times larger in the summer than in the winter due to slower biodegradation in the saturated zone and smaller rates of volatilization to the vadose zone in the winter. The removal rate estimates described in this paper are most sensitive to the assumptions for biodegradation rates and flux rates to the vadose zone. Concentration profiles in groundwater and soil gas control the estimation of flux rates. The research shows that phytoremediation enhances the overall naphthalene removal, mainly by stimulating faster biodegradation in the rhizosphere and promoting mass transfer of naphthalene to the vadose zone followed by rapid vadose zone biodegradation.

6.1 Introduction

Phytoremediation is an attractive remediation technology due to its relative low cost and maintenance requirement. Strategies for treatment of large diffuse contamination and plume remediation following source removal may benefit from phytoremediation, especially if an extended remediation time is acceptable. A limitation for widespread acceptance of phytoremediation applications is that the mechanisms are not yet completely understood nor have they been demonstrated in the field. Quantification of the contributions from each mechanism to the overall remediation rate is crucial for optimization of phytoremediation systems and prediction of the total remediation time.

Phytoremediation with poplar trees was implemented at a creosote contaminated site in Oneida, Tennessee. Research at this site for the past eight years has characterized and quantified many aspects of the phytoremediation process. The overall objective of the Oneida site research has been to investigate the active removal mechanisms and quantify the rates for each mechanism. Groundwater monitoring over the past seven years shows that the PAH mass and plume extent has decreased over time (1). Increased removal rates occurred when tree roots reached the groundwater table after 3 growing seasons (1). Equilibrium between mass transfer into the groundwater from the DNAPL source and removal has stabilized the plume size and concentrations since 2001. Naphthalene is the dominating PAH dissolved in the groundwater and the focus has therefore been on naphthalene removal. The removal mechanisms for naphthalene at this site are biodegradation in the saturated zone, direct volatilization, biodegradation in the vadose zone and phytovolatilization. The mechanisms are illustrated on figure 6.1 for summer and winter conditions.

Biodegradation is active year round. The biodegradation potential is enhanced in rhizosphere soil from the site and in situ respiration rates were 3-5 times higher in an area with trees compared to an area with no trees (2). Redox data, in situ respiration tests (2) and microcosm experiments (3) have shown that both aerobic and anaerobic biodegradation are active. Saturated zone biodegradation is primarily anaerobic at this site.

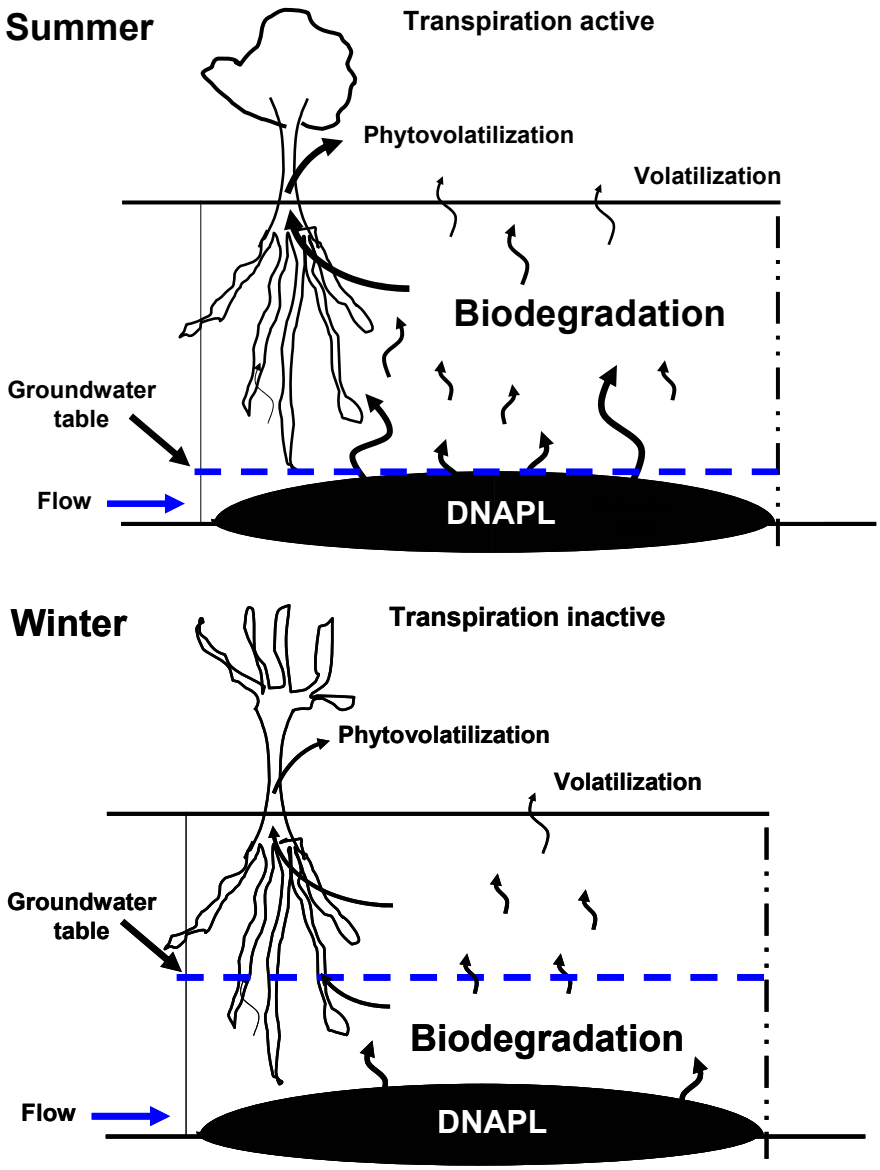

FIGURE 6.1 Phytoremediation mechanisms summer and winter

Volatilization of naphthalene to the unsaturated zone occurs year round, but is small in the winter. Field data shows increased volatilization of naphthalene to the vadose zone when the groundwater table is low and the groundwater concentrations are high in the summer and fall months (Chapter 3).

Rapid aerobic biodegradation of naphthalene is active in the vadose zone, based on field soil gas concentrations and laboratory column study (Chapter 3). Due to the low volatilization in the winter, biodegradation in the vadose zone is small. The canopy

interception of rain and the transpiration of water from the subsurface by the poplar trees contribute to lowering the water table and decreasing the moisture level in the unsaturated zone. Field data shows that these phytoremediation effects promote volatilization, coupled with rapid biodegradation in the aerated and rhizosphere-impacted vadose zone.

Phytovolatilization has been detected all year, but is highest in the summer and fall when the transpiration is active and groundwater concentrations are highest (Chapter 4). In addition, the moisture level in the unsaturated zone is lower in the summer and fall and therefore the fraction of transpiration water originating from the groundwater is higher (Chapter 4). The reason phytovolatilization has been detected in the winter is believed to be that the trees act as passive conduits for naphthalene in the vapor phase even when transpiration is not active (Chapter 4).

The objectives of this paper are:

1. Quantify the naphthalene mass removal by each mechanism for the entire site for summer and winter conditions and total for a year
2. Determine the impact of phytoremediation on the overall mass removal
3. Estimate the total remediation time based on the estimated removal rate and the estimated remaining naphthalene at the site
4. Identify knowledge gaps for quantification of important mechanisms

6.2 Materials and methods

6.2.1 Field measurements

Groundwater and soil concentrations of PAHs have been monitored for past 7 years since installation of the phytoremediation system in 1997. Groundwater samples for PAH analysis were collected from multilevel sampling wells as described by Widdowson et al (1). Groundwater samples were extracted with methylene chloride and the extracts were analyzed on an automated Hewlett-Packard 5890 GC-FID and a DB-5 capillary column along with external liquid standards (1). Groundwater elevations were measured once a month using a manual gauge in piezometers and recorded continuously with pressure transducers at three selected locations (4).

In addition to the background monitoring, a comprehensive field sampling scheme was conducted approximately once a month from March 2004 to March 2005 at the sampling locations shown on Figure 6.2. This sampling program was used for a detailed in situ quantification of naphthalene removal by the different mechanisms at the site.

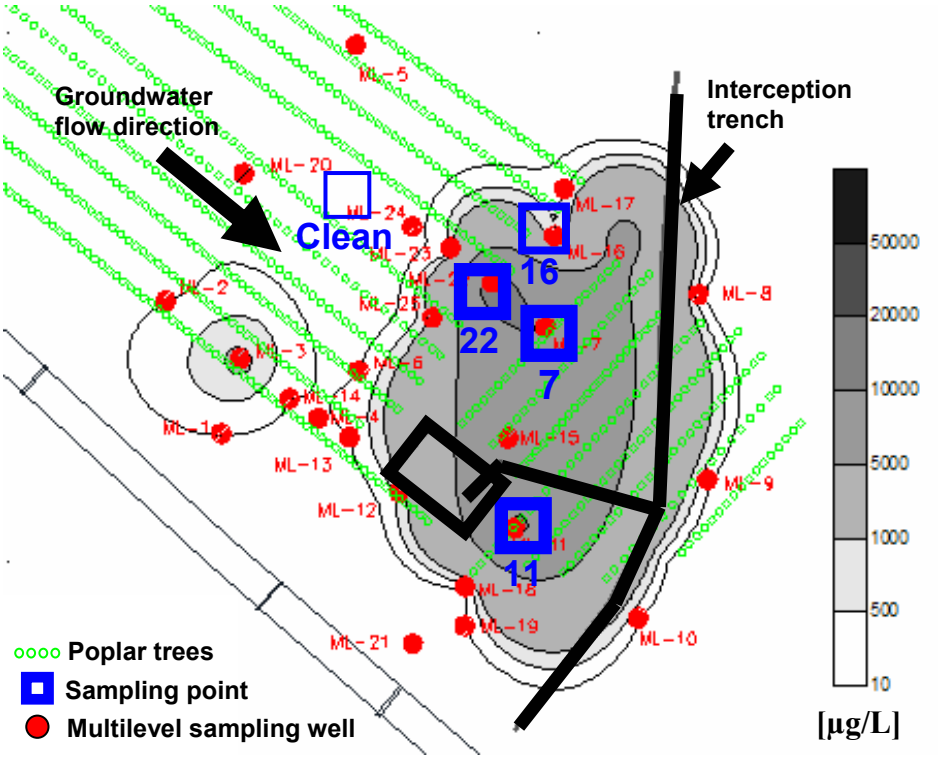

FIGURE 6.2 Site map with data sampling locations and naphthalene groundwater concentrations July 2004 (0-0.8 m above bedrock, [µg/L]).

Soil gas was recovered via soil gas probes installed across the site. Soil gas was slowly extracted over 8 hours and naphthalene was collected using sorbent tubes to determine the naphthalene concentrations in the soil gas. The oxygen and carbon dioxide contents in the soil gas were measured directly in the field and used as indicators of aerobic biodegradation. Moisture sensors installed in the unsaturated zone enabled parallel measurements of moisture levels in the unsaturated zone. The methods for sampling in the unsaturated zone are further described in Chapter 3.

The naphthalene flux out of the ground surface was measured by capturing naphthalene vapors collected in surface flux chambers onto sorbent tubes for sampling periods of 2 days. The method is described by Booth (5).

Phytovolatilization was measured directly by flux chambers with sorbent tubes mounted on tree trunks in the field at the same sampling locations throughout a year (Chapter 4).

6.2.2 Biodegradation rates

In situ respiration rates were obtained by push pull tests with injection of aerated water into the subsurface as described by Pitterle et al (2). The respiration rates were converted to aerobic naphthalene biodegradation rates by the stoichiometric relationship of 2.007 oxygen molecules consumed per naphthalene biodegraded. The tests were conducted in push pull wells installed in the contaminated area with trees and without trees as well as in an uncontaminated location. The tests were conducted during summer and winter seasons.

A seven month column study was conducted with soil from the site to imitate the diffusive flux and biodegradation of naphthalene vapors in the vadose zone as described in Chapter 3 and by Booth (5).

Aerobic and anaerobic microcosms were constructed with soil sampled by hand auger at the same locations and depths as the push pull tests were conducted. The soil samples were transferred directly from the hand auger to autoclaved glass jars with sealed lids. The deep samples used for the anaerobic setup were briefly exposed to air during sampling, but immediately transferred to a glove bag and purged with nitrogen before being sealed tightly. The samples remained stored in the glove bag until construction of the microcosms. All the soils samples were stored in a 4 ºC refrigerator until use.

Aerobic biodegradation as well as biodegradation by sulfate reduction and iron reduction have been observed at this site whereas methanogenesis is not significant (3). Oxygen was provided in the headspace air of the aerobic microcosms, but it is possible that oxygen was depleted or diffused slowly during the incubation period. Added water contained K_2SO_4 to obtain a concentration of sulfate of 150 mg/l, which is representative for sulfate concentrations measured in the groundwater at the site. The soil itself is a reservoir for Fe^{3+}. Other electron acceptors have not been shown to be consumed by biological processes at this site.

Everything used in the setup of the microcosms was autoclaved. The microcosm containers were 20 ml vials with rubber septa crimp caps. Each soil was thoroughly mixed prior to setup and a subset of the soil was taken aside for controls. The microcosm vials each received 3 grams of the relevant soil. The soil was spiked with 1 mL of a hexane solution with 250 mg/L naphthalene and 50 mg/L of acenapthylene, acenapthene, flourene, flouranthene, anthracene, pyrene and chrysene in hexane and the hexane was allowed to evaporate off in a fume hood. This method resulted in initial concentrations of naphthalene around 500-700 mg/kg and 20-50 mg/kg of the other PAHs. The reason for adding the seven other PAHs in lower amounts was to mimic the background concentration of other PAHs in the groundwater at the site.

Eight milliliters of autoclaved de-mineralized water was added to both the aerobic and anaerobic microcosms. The vials were crimped immediately upon addition of the water. The anaerobic microcosms were constructed the same way but inside an anaerobic glove-box purged with nitrogen. Autoclaved water used for the anaerobic microcosms was transferred to the glovebox immediately after autoclaving and allowed to cool inside the box in order to minimize dissolved oxygen in the water.

The control soils were autoclaved 15 times with three one week breaks to allow for germination of microbial spores. Three grams of the dry autoclaved soil was weighed out in the individual vials and then these were autoclaved again prior to the addition of PAHs. The anaerobic controls where spiked, water added and crimped inside the glovebox.

The microcosms were shaken on a vortex shaker and incubated in the dark on a slow agitating table to mimic the slow groundwater flowrate at the site at constant temperature of 20 ºC. The aerobic microcosms were shaken on a vortex mixer once a week to minimize diffusion limitations for oxygen into the water.

Microcosms were sacrificed in triplicate approximately every second week over a time period of 172-235 days for the anaerobic microcosms and approximately every week for 140 days for the aerobic microcosms. Ten milliliters of methylene chloride was added to the microcosms and the microcosms were agitated for 34-38 hours on a shaking table. The samples settled in the refrigerator for 24 hours to separate the soil, water and methylene chloride before methylene chloride was transferred and diluted if necessary

into 2 ml target vials for gas chromatograph analysis. Samples were analyzed along with external standards on a gas chromatograph with FID and DB5-MS fused silica capillary column for analysis of PAHs.

6.3 Results and Discussion

6.3.1 Naphthalene mass loss from the site

The naphthalene groundwater concentrations have been entered into a three dimensional grid and summarized for each sampling event from 1998-2004. The total naphthalene mass in the groundwater as a function of time is shown in Figure 6.3. The total mass is shown along with the mass in the lower 0.7 m of the groundwater. From 1997 to 1999, the mass in the groundwater was around 70 kg. From 1999 to 2000 the mass dropped down to a new low equilibrium around 50 kg total (Figure 6.3), where it remains. The concentration of naphthalene is expected to remain at this level until the source is nearly exhausted of naphthalene.

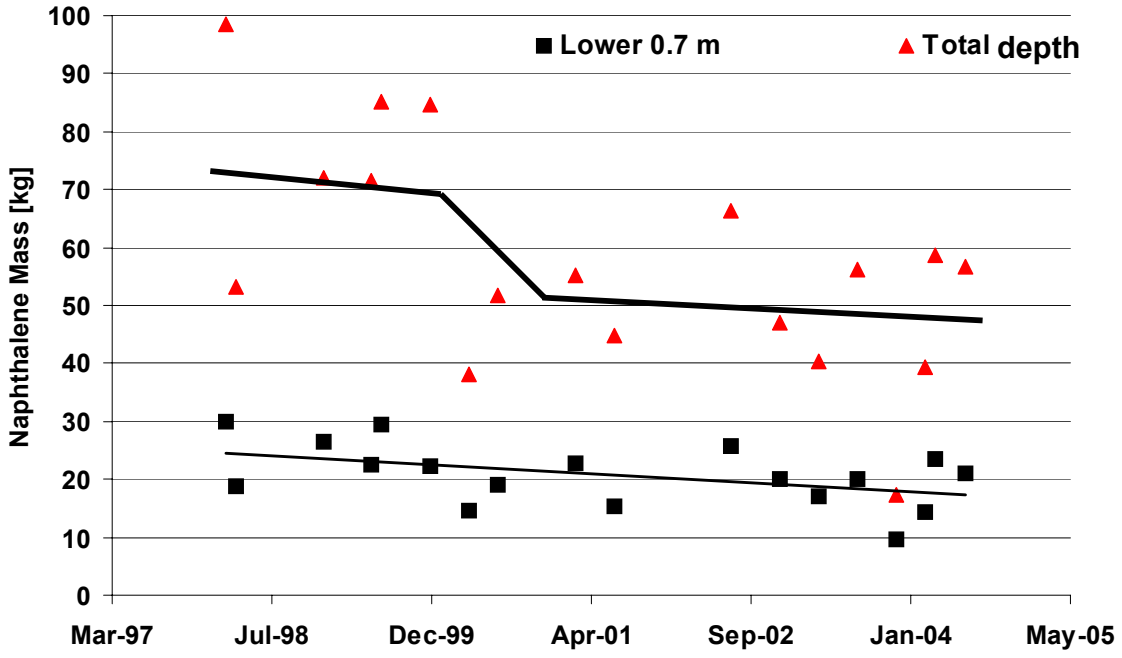

FIGURE 6.3 Change in naphthalene mass in the groundwater over time, exclusive of free phase creosote

In addition to the naphthalene dissolved in the groundwater, there is also residual naphthalene present in the soil and in the creosote DNAPL pooled on top of the bedrock. Based on observations during soil and groundwater sampling as well as groundwater naphthalene concentration contours, the areal extent of the remaining creosote DNAPL is around 200-400 m^2. The thickness is up to 30 cm deep, but is believed to average around 10 cm based on samples collected by hand augering. The porosity of soil is approximately 0.37. The volume of remaining creosote pooled on the bedrock is estimated to be 7-15 m^3. The density of creosote is 1066 kg/m^3 and the naphthalene content in different creosotes is 7-18 weight % (6). Some of the creosote at this site has been weathered for up to 50 years, so it is assumed that the naphthalene content is in the lower end of this range and is assumed to be approximately 10%. The total naphthalene remaining in the creosote is then estimated to be 750 – 1600 kg so the total naphthalene mass in the creosote plus in the groundwater is 800 – 1650 kg.

The observed total naphthalene mass removal is a result of biodegradation, volatilization and phytovolatilization. A description and quantification of each of the removal mechanism is given in the following sections.

6.3.2 Mass removal by saturated zone biodegradation

Respiration tests were conducted in the field throughout a year at locations with and without trees (Pitterle et al, 2005) and converted to aerobic naphthalene biodegradation rates by a stoichiometric factor 2.007 mg O_2 per mg naphthalene degraded. Aerobic and anaerobic microcosms were prepared with soils from the same locations and used to measure naphthalene loss rates. The biodegradation rates for contaminated soil from an area with trees compared to an area with no trees are shown on Figure 6.4 both from microcosm data and field data.

Both the microcosm and field respiration rates are higher in the presence of trees. The anaerobic microcosm rates in contaminated soil were 0.017 day^{-1} in soil from an area with trees and 0.011 day^{-1} in a soil from an area with no trees. The aerobic microcosm rates were 0.03 day^{-1} and 0.02 day^{-1} respectively. The respiration tests (push-pull) showed 3-5 times higher rates in the presence of trees. The reason for the plant enhanced biodegradation rates is assumed to be the higher microbial numbers and diversity in the

rhizosphere soil. This is due to rhizosphere effects such as increased organic matter and aeration. The oxygen consumption measured in the respiration tests may be a result of biodegradation of the extra organic matter in rhizosphere soil as well as naphthalene. The respiration tests therefore likely overestimate the rhizosphere effect on the biodegradation rates. Based on the microcosm results and respiration tests the rhizosphere effect is assumed to at least double the biodegradation rates and a factor of 2 is assumed for the rhizosphere effect.

The respirations tests indicated 2-4 times higher aerobic biodegradation rates in the summer than in the winter in the contaminated area with trees (2). The anaerobic biodegradation rates are assumed to be ~3 times higher in the summer as well.

The aerobic laboratory microcosms are believed to underestimate aerobic in situ biodegradation rates due to diffusion limitations in these relatively static systems. On the other hand, the rates of 1-15 day^{-1} deduced from the field respiration tests represent a situation with an unlimited oxygen supply. Biodegradation rates obtained in aerobic columns mimicking the aerated conditions in the vadose zone resulted in similar aerobic biodegradation rates of 3-26 day^{-1}. The top few cm of the groundwater may be aerobic but oxygen levels would be low. The upstream and side fringes of the plume are also expected to have some oxygen available to support rapid aerobic biodegradation. The total naphthalene mass in these border areas is estimated to be less than 100 grams, a small fraction of the total naphthalene mass. Even with rapid biodegradation rates of 1-26 $day^{-1,}$ the removal by aerobic biodegradation in the border areas is assumed to be low, due to the relatively slow diffusion of oxygen to these locations and the low mass of naphthalene. Aerobic biodegradation in the fringes of the plume in the saturated zone is assumed to be insignificant at this site.

The field respiration tests and laboratory vadose zone aerobic rates are not relevant for the biodegradation in the majority of the saturated zone depth. Redox data from groundwater sampling shows anaerobic conditions in the saturated zone in the contaminated area. The anaerobic microcosms showed biodegradation rates between 0.011 day^{-1} and 0.017 day-1. The microcosms were designed to ensure that there were no limitations in the anaerobic electron acceptors. The microcosm water contains 150 mg/L

sulfate and the soil contains ample amounts of iron. The anaerobic microcosm rates are therefore believed to mimic the in situ anaerobic biodegradation rates.

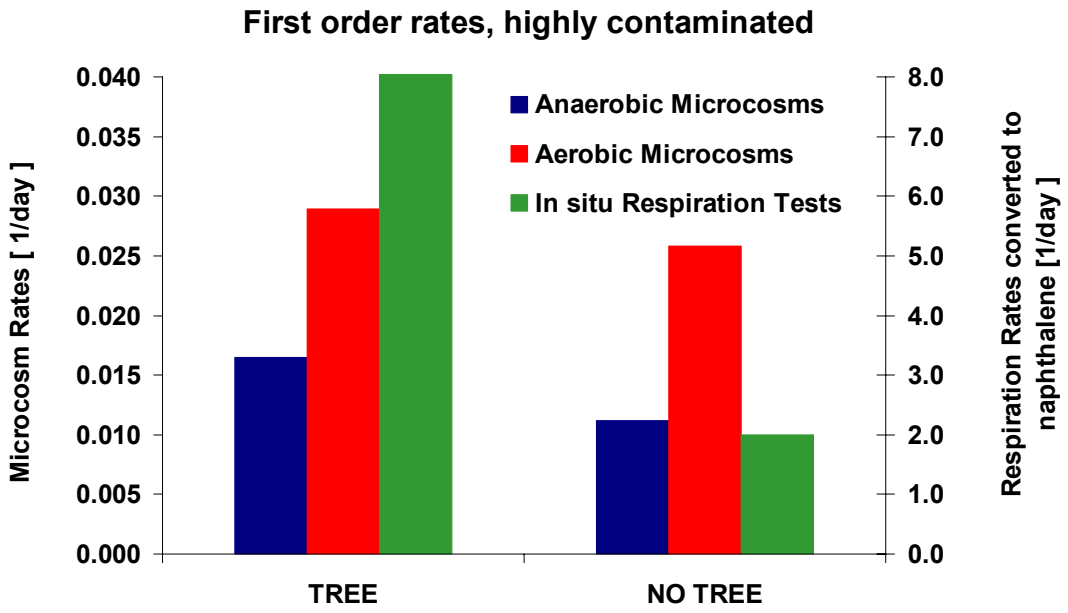

FIGURE 6.4 Biodegradation rates from aerobic field respiration tests and from aerobic and anaerobic laboratory microcosms

An alternate approach to estimating the biodegradation rate in the saturated zone is to investigate the mass removal based on the monitoring data. Figure 6.5 shows data from ML12 at the fringe of the plume and these data suggest a first order degradation rate of 0.0026 day^{-1} for saturated zone biodegradation if this was the only removal mechanism. In reality, volatilization coupled with vadose zone biodegradation as well as plant uptake contributes to mass removal from the saturated zone. The rate of 0.0026 day^{-1} may therefore slightly overestimate the rate, but it has been assumed for this estimation.

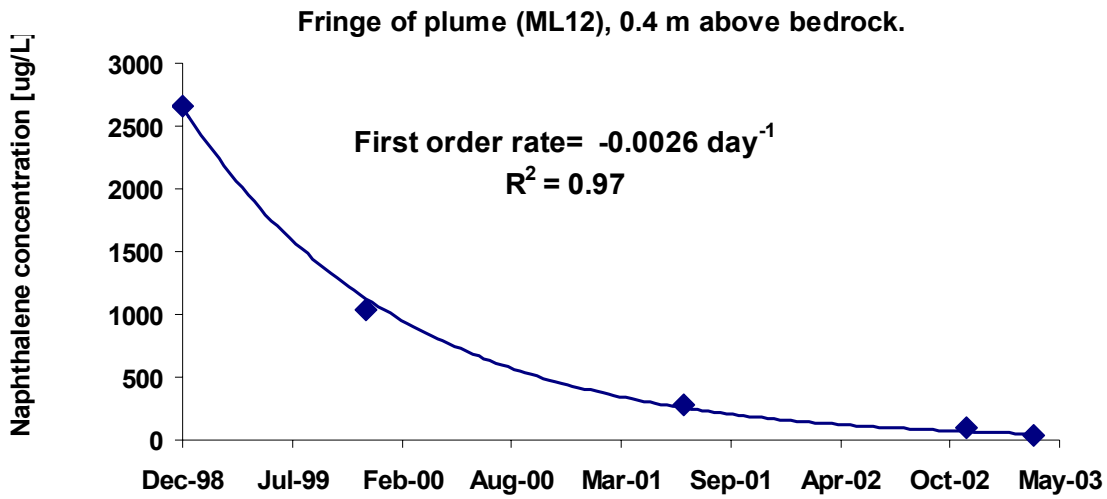

FIGURE 6.5 Naphthalene groundwater concentrations 0.4 m above bedrock in the fringe of the plume

Figure 6.6 shows a typical concentration profile at the center of the plume. The profile is approximately linear which indicates that diffusion governs the transport of naphthalene and that biodegradation is very low in the bottom 1 m of the aquifer. This is supported by the fact that the total mass is stable in the lower 70 cm of the aquifer above the DNAPL shown on Figure 6.6.

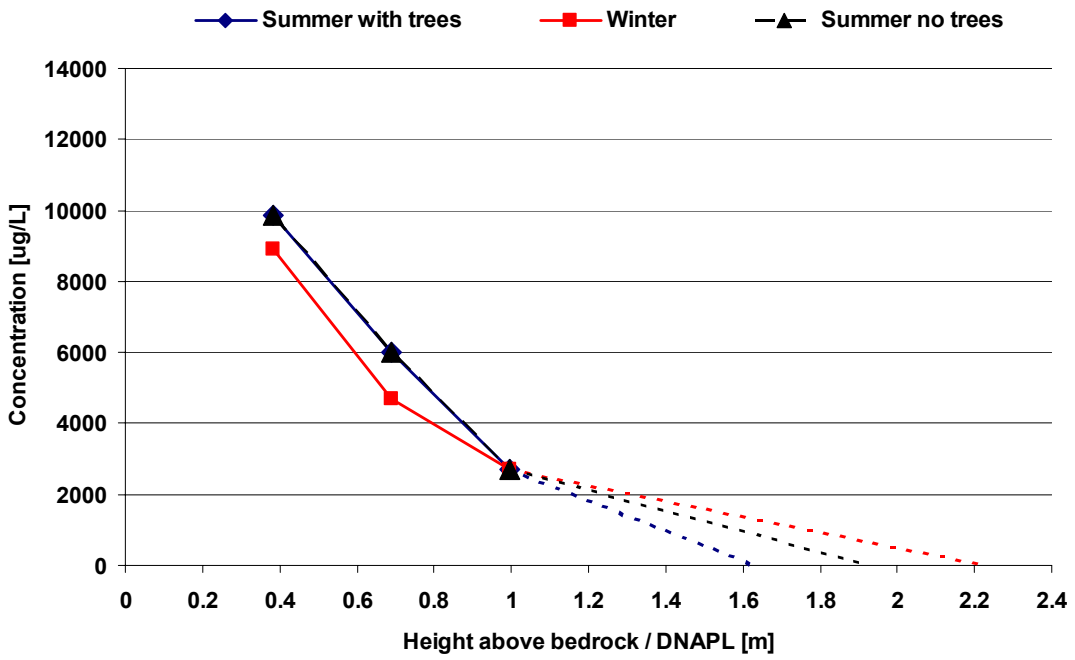

FIGURE 6.6 Naphthalene groundwater concentration profile in the plume center

Overall, the estimated biodegradation rate in the saturated zone is 0.0026 day-1 to 0.017 day-1 in the summer with phytoremediation. If the rates are believed to be ~3 times lower in the winter as suggested by the respiration test results, the winter rate would be 0.00087-0.0057 day-1. Assuming that the enhancing effect of the phytoremediation system is approximately a factor of 2, based on the microcosm (factor 1.5) and respiration test results (factor 3-5), the rate in the absence of phytoremediation is reduced to 0.00087-0.0057 day-1 in the summer and 0.00029-0.0019 in the winter without phytoremediation.

Table 6.1 summarizes the estimates for saturated zone biodegradation for the entire site in the summer and winter of 2004 with and without phytoremediation. The total naphthalene mass in the saturated zone above 1 m from the bedrock is around 25 kg in both summer and winter.

TABLE 6.1 Estimates of saturated zone biodegradation of naphthalene

	Phytoremediation		**No phytoremediation**	
	Rate range [day^{-1}]	**Mass removed [kg/year]**	**Rate range [day^{-1}]**	**Mass removed [kg/year]**
Summer	0.0026 - 0.017	12-77	0.0013 - 0.009	6-41
Winter	0.00087 - 0.0057	4-26	0.0004 - 0.003	2-14
Entire year		**16-103**		**8-55**

The mass removed by saturated zone biodegradation is estimated to be ~ 16-103 kg/year with the impact of the phytoremediation system but only around 8-55 kg/year without phytoremediation. The lower end of the range is believed to be more likely, since these rates were estimated from field groundwater data whereas the higher rates were obtained from microcosms.

6.3.3 Mass removal by volatilization to the vadose zone

The mass transfer of naphthalene from the saturated zone to the vadose zone can be estimated from concentration profiles and assumptions about diffusion and biodegradation through these subsurface zones. A complication is that groundwater concentrations and soil gas concentrations have not been measured in the field

immediately at the interface between the water and the gas. Groundwater concentrations are typically available from 0.1-0.3 m below the water table. The reason for this is that pumping of the groundwater wells creates a small cone of depression in the water table and this limits how close to the top of the water table it is possible to recover water samples. Soil gas concentrations are available from 5 cm above the water table in August and September, but closer sampling was not possible due to the inclusion of water when pumping air. For the remainder of the year, samples were not recovered any closer than 40 cm above the water table due to higher moisture levels in the vadose zone that reduced the gas permeability of the soil. The development of methods for measuring concentrations closer to the water-gas interface is highly desirable for future research.

In addition to the gap in naphthalene concentrations in the groundwater and soil gas close to the water table, the upper zone of the saturated zone and the lower zone of the vadose zone are suspected to be enriched for rapid aerobic biodegradation of naphthalene. This is based on observation of fast respiration rates in the saturated zone in situ (Pitterle, 2005) and rapid aerobic biodegradation in the vadose zone in laboratory columns (Chapter 3). The fluctuating water table enhances mass transfer of naphthalene and oxygen to this zone. The conditions created with moderate levels of naphthalene, oxygen and moisture are optimal for aerobic microorganisms degrading naphthalene. High rates of biodegradation would be expected in this region. On the other hand the steady state mass (Figure 6.3) and concentrations (Figure 6.6) in the lower 1 m of the saturated groundwater close to the groundwater, suggests that biodegradation is slow in the saturated zone. Volatilization to the vadose zone was investigated in the field by measuring naphthalene concentrations in the soil gas in the unsaturated zone across the site over one year (Chapter 3). Soil gas concentrations and ground surface flux rates indicated significant removal of naphthalene in the vadose zone. A laboratory column study confirmed that rapid aerobic biodegradation of volatilized naphthalene occurs in the vadose zone. The majority of the removal occurred in the first 5 cm above the source in the column study and within the first 0.5 m above the water table in the field (Chapter 3).

Hypothetically the concentration profile for naphthalene in the saturated zone and vadose zone is expected to be like that depicted in Figure 6.7, assuming no significant biodegradation in the lower 1 m of the saturated zone, anaerobic biodegradation in the

upper saturated zone, and rapid aerobic biodegradation 5-50 cm above the water table. Laboratory column results indicate that rapid aerobic biodegradation occurs in the first 5 cm above the water table as well, if the oxygen levels are sufficient. It is possible that aerobic biodegradation occurs at times of heavy rain a few cm below the groundwater table, but usually this region is anaerobic (7).

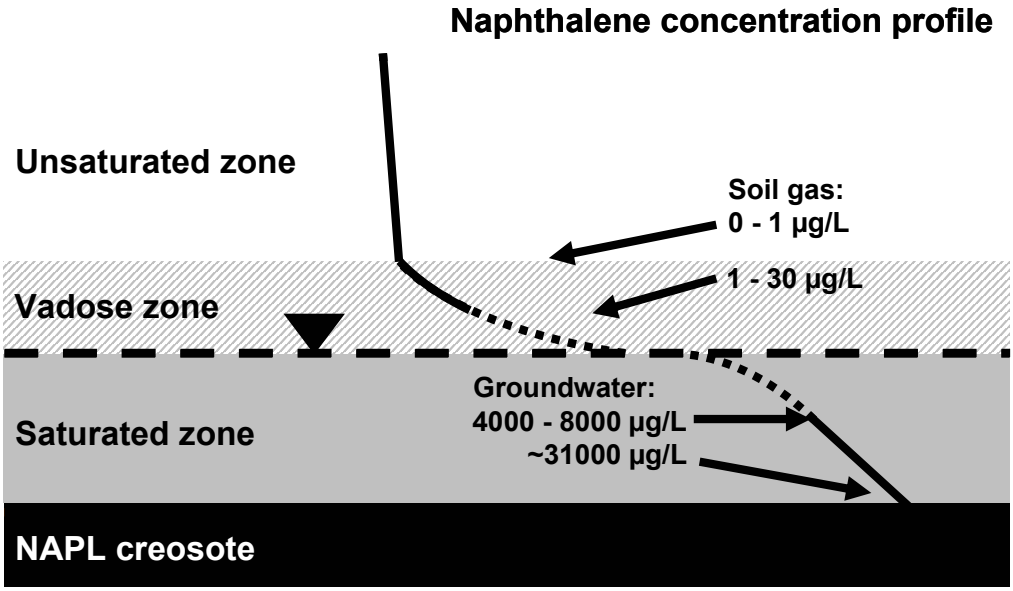

FIGURE 6.7 Hypothetical concentration profiles in the summer and winter

Biodegradation first order rates and flux are fitted to measured soil gas concentrations using the method outlined by Wilson (8). The boundary condition for the concentration profile is set at the deepest measured soil gas concentration. The input parameters and fitted values are listed in Table 6.9. The maximum soil gas concentrations were measured 5-8 cm above the groundwater table in the center of the plume in August 2004. Much lower concentrations were measured farther from the groundwater table. The soil gas concentrations were below detection limit throughout most of the year.

The flux out of the groundwater to the vadose zone must be at least equal to the flux measured in the soil gas. In reality it may be higher since biodegradation can occur in the region between the water table and the first soil gas sampling point. Field measurements of oxygen content in the soil gas suggest that 1-2 % oxygen is present close to the water table. Assuming that aerobic biodegradation right above the groundwater table occurs

with the fitted rates shown in Table 6.2 the concentration in the soil gas at the water table would be 10-60 μg/L in August and the flux rates would be 30-300 μg/m^2 hr.

TABLE 6.2 Estimate of naphthalene flux to the vadose zone based on soil gas data

August 12 and 14, ML7 From 8 cm above the water		**August 14, ML22 From 5 cm above the water**	
Height [m]	Concentration [μg/L]	Height [m]	Concentration [μg/L]
0	6-12	0	29
0.46	0.4-0.53	0.38	0.37
0.7	0.32-0.38	0.78	0.36
Total Length = 2.08 m		Total Length = 2.14 m	
1. order rate = 0.4-0.5 day^{-1}		1. order rate = 1.5 day^{-1}	
Flux = 16-38 μg/m^2 hr		**Flux = 155 μg/m^2 hr**	

The flux out of the groundwater to the vadose zone has also been estimated based on groundwater concentrations ignoring slow biodegradation in the groundwater and assuming Fick's second law for diffusion and an effective diffusion coefficient of 2.5×10^{-6} $cm^2\ s^{-1}$ = 9×10^{-7} $m^2\ hr^{-1}$, based on a tortuosity factor of 4. The groundwater concentration is believed to be 31 mg/L immediately above the DNAPL, which is at the solubility for naphthalene. The groundwater table was ~1.5 m above the DNAPL in the summer and ~2 m above the DNAPL in the winter of 2004. Based on this alone, the flux out of the groundwater by diffusion can be a maximum of 19 μg/ $m^2\ hr^{-1}$ in the summer and 14 μg/ $m^2\ hr^{-1}$ in the winter for an assumed concentration near 0 mg/L at the water table.

The groundwater concentration of naphthalene in the center of the plume (MLS7) is around 8 mg/L all year at 0.7 m above bedrock. This corresponds to 0.6 m above the DNAPL assuming that the DNAPL is 10 cm thick. Assuming that the concentration of naphthalene in the groundwater is 31 mg/L immediately above the DNAPL, this results in a flux out of the DNAPL of approximately 60 μg/hr m^2. The groundwater concentration is ~4 mg/L in the winter and ~7 mg/L in the summer in average in the sampling ports from 0.9-1.2 m above the DNAPL. The maximum flux occurs if the naphthalene

concentration at the water table is assumed to be ~0 mg/L. The maximum flux from 1.1 m above the DNAPL to the groundwater table is estimated to be ~15 ug/m^2 hr when the groundwater table is low in the summer and fall months and ~ 4 ug/m^2 hr when the groundwater table is high in the winter and spring months. The difference between the flux out of the DNAPL and the flux out of the groundwater to the vadose zone is believed to be due to biodegradation in the saturated zone.

Typical naphthalene concentration profiles in the center of the plume at this site are shown on Figure 6.2. Based on these groundwater profiles the flux is on average around 10 μg/m^2 hr in this location from 0.4-1 m above the DNAPL in both summer and winter assuming diffusion following Fick's second law and ignoring biodegradation.

The different approaches for estimating the flux of naphthalene from the groundwater to the vadose zone suggests flux rates of 4-14 μg/m^2 hr in the months with high groundwater table (November through April) and 15 - 300 μg/m^2 hr in the months with low groundwater table (May through October). Table 6.3 summarizes the estimates for naphthalene mass removal by volatilization.

TABLE 6.3 Estimate of naphthalene mass removal by volatilization to the vadose zone, with and without phytoremediation and during summer and winter conditions

	Phytoremediation		**No phytoremediation**	
	Rate range [μg/m^2 hr^{-1}]	**Mass removed [g/year]**	**Rate range [μg/m^2 hr^{-1}]**	**Mass removed [g/year]**
Summer	15-300	63-1252	4-14	17-58
Winter	4-14	17-58	4-14	17-58
Entire year		**~ 80-1300**		**~ 30 - 120**

The estimated flux is assumed to occur over a 950 m^2 area with groundwater concentrations of 1-20 mg/L (Figure 6.2). In this area the flux to the vadose zone is 17-58 g in the winter and spring and 63-1252 g in the summer and fall, which totals ~ 80-1300 g for the entire year.

The transpiration and canopy interception of rainwater from the trees is estimated to result in up to 0.3 m lower elevation of the groundwater table in the summer compared to

if there were no trees (4). The higher water table would result in lower volatilization rates throughout the year and the flux is estimated to be ~30-120 g for the entire year without the trees.

6.3.4 Mass removal by volatilization to the atmosphere

The flux of naphthalene out of the ground surface was measured at the same locations as the soil gas profiles throughout the year (5). The flux out of the ground surface to the atmosphere had a peak rate in August of 23.5 $\mu g/m^2$ hr when the elevation of the groundwater table is lowest, the groundwater naphthalene concentrations are highest and the unsaturated zone soil has lowest moisture. Three surface flux sampling locations were located within the 950 m^2 area with groundwater concentrations of 1-20 mg/L (Figure 6.2). The average flux rate for these three locations was 13 $\mu g/m^2$ hr in August and September, which is believed to represent July due to similar groundwater levels. From October through June the average was 3 $\mu g/m^2$ hr. The total removal by surface flux from the plume area is 1400 mg in the summer and fall (May through October) and 500 mg in the winter; total ~ 2000 mg in a year. If the trees were not present, the groundwater table would hypothetically be 0.3 m higher and this would result in winter surface flux rate for the entire year and a total removal by surface flux of only around 1000 mg.

6.3.5 Mass removal by biodegradation in the vadose zone

The mass removal in the vadose zone can be estimated from biodegradation rates deduced from field data and the laboratory column study, but a direct approach is to simply subtract the flux measured out of the ground's surface from the flux estimated coming out of the groundwater. Table 6.4 summarizes the mass removal by biodegradation in the vadose zone in the different scenarios calculated from the difference in flux out of the groundwater and flux out of the ground surface.

In addition to the physical effect of the lower groundwater table increasing volatilization, it is also assumed that the biodegradation rates are increased by a factor 2 in the vadose zone by the phytoremediation system (as discussed for the saturated zone). Since there is ~ 2 m of unsaturated zone for biodegradation and 90% has been shown to be removed in 0.5 m with the phytoremediation system in place, it is reasonable to

assume that 90% will be degraded within 1 m of unsaturated zone and therefore overall the flux out of the ground surface is not assumed to increase without the phytoremediation system.

TABLE 6.4 Estimate of naphthalene mass removal by biodegradation in the vadose zone, with and without phytoremediation, for summer and winter conditions

	Phytoremediation [g/year]			**No phytoremediation [g/year]**		
	Flux out of water	**Flux out of ground**	**Vadose zone biodegradation**	**Flux out of water**	**Flux out of ground**	**Vadose zone biodegradation**
Summer	63-1252	1.4	61-1251	17-58	0.5	16-58
Winter	17-58	0.5	16-58	17-58	0.5	16-58
Entire year	80-1310	~ 2	**78-1310 ~ 80-1300**	~ 34-116	1	**32-116 ~30-120**

Table 6.4 shows that the biodegradation in the vadose removes more than 90% of the naphthalene mass volatilized out of the water and only a very small mass escapes to the atmosphere.

6.3.6 Mass removal by phytovolatilization

Phytovolatilization was measured with flux chambers mounted directly on tree trunks at the site in order to quantify the in situ naphthalene flux rates throughout a year (Chapter 4). Phytovolatilization was detected within a 950 m^2 plume area with groundwater concentrations above 1 mg/L. Within this area there are 164 trees with an average diameter of 9 cm. The amount of phytovolatilization has been shown to vary with tree size due to the larger fraction of contaminated water taken up by larger trees with deeper roots. The sample trees next to ML7 and ML11 have diameters of 8.9 cm and 9.7 cm respectively, which is close to the average. These two trees have been used for the estimate of average phytovolatilization rates. The diameter squared has been found to be correlated to transpiration rates (9). The measured phytovolatilization rates were therefore normalized to an averaged sized tree by multiplying with the average diameter squared and dividing by the specific tree diameter squared. The factor is $(9\ cm)^2/(8.9cm)^2 = 1.023$

for the tree by ML7 and $(9\ cm)^2/(9.7\ cm)^2 = 0.8609$ for the tree by ML11. Table 6.5 shows the normalized phytovolatilization rates per tree surface area.

May through October have the highest phytovolatilization due to active transpiration, highest groundwater concentrations and the largest fraction of transpiration water originating from the groundwater.

TABLE 6.5 Estimate of naphthalene phytovolatilization rates throughout the year

Date	**Weather**	**Season**	**ML7, normalized [µg/m^2 day]**	**ML11, normalized [µg/m^2 day]**
April 23, 2004	cloudy / rain	spring / winter	**28.98**	
May 4, 2004	clear / sun	summer / fall	45.91	
May 24, 2004	clear / sun	summer / fall	35.87	
June 14, 2004	cloudy / rain	summer / fall	18.62	
July 8, 2004	clear / sun	summer / fall	292.76	87.43
July 16, 2004	cloudy / rain	summer / fall	21.88	107.37
Aug.,10, 2004	cloudy / rain	summer / fall	190.56	107.16
Sept., 7, 2004	cloudy / rain	summer / fall	**254.94**	26.34
Sept., 11, 2004	clear / sun	summer / fall	**373.65**	63.04
Oct.,12, 2004	clear / sun	summer / fall	93.65	97.65
Nov. 22, 2004	clear / sun	spring / winter	47.39	
March 16, 2005	clear / sun	spring / winter	**100.31**	
			cloudy / rain	clear / sun
		Average of all rates	[µg/m^2 day]	[µg/m^2 day]
		Summer	**104**	**136**
		Winter	**29**	**74**

Table 6.6 shows the average phytovolatilization rates per tree integrated up the height of the tree trunk as described in Chapter 4. The average summer and fall rate was 13.5 µg/day per tree on cloudy days and 17.7 µg/ day per tree on sunny days. April 2004 and November 2004 through March 2005 showed an average phytovolatilization of 3.8 µg/tree per day on cloudy days and 9.6 µg/m^2 day on sunny days. The number of cloudy days was 65 and the number of sunny days was 118 in the summer and fall months (4). The number of cloudy days was 92 and the number of sunny days was 90 in the winter and spring months (4). The total phytovolatilization from the 164 trees inside the plume

area is estimated to be 486 mg in the summer and fall and 199 mg in the spring and winter, totaling 685 mg for the entire year.

The maximum removal rates recorded and highlighted in bold in Table 6.5 yield total removal of 1293 mg in the summer and fall months and 249 mg in the winter and spring months, totaling 1542 mg in the entire year. Even with the maximum rates, the total phytovolatilization is small.

TABLE 6.6 Estimate of yearly naphthalene mass removal by phytovolatilization

	Summer and fall		**Winter and spring**	
	cloudy / rain	**clear / sun**	**cloudy / rain**	**clear / sun**
Rate [µg/day tree]	13.514	17.672	3.768	9.616
Rate [µg/day]	2216	2898	618	1577
Number of days	65	118	92	90
Total [µg]	144040	341964	56856	141930
Total season [mg]	**486**		**199**	
Total year [mg]	**685**			

6.3.7 Comparison of mass removal from different mechanisms summer and winter

The estimated ranges for removal contributions from the individual mechanisms in summer and winter conditions are summarized in Table 6.7 and shown for visual comparison in Figure 6.8. Table 6.7 shows that the summer removal is 3 times larger than the estimated winter removal. Biodegradation clearly dominates over volatilization and phytovolatilization both summer and winter, but the relative contributions from biodegradation in the saturated zone versus biodegradation in the vadose zone are uncertain.

The comparison in Figure 6.8 shows the lowest end of the estimated range for removal by saturated zone biodegradation and the highest end of the estimated range for removal by vadose zone biodegradation, since this is what we believe is closest to the actual removal. The vadose zone biodegradation is ~20 times smaller in the winter than in the summer, whereas the saturated zone biodegradation is 3 times smaller in the winter than in the summer.

TABLE 6.7 Summary of naphthalene mass removal by individual mechanisms during summer and winter conditions

	Summer [g]	Winter [g]	Total year 2004 [g]
Saturated zone biodegradation	12,000-77,000	4,000-26,000	**16,000-103,000**
Vadose zone biodegradation	61-1251	16-58	**77 - 1309**
Volatilization to atmosphere	1.4	0.5	**1.9**
Phytovolatilization	0.5	0.2	**0.7**
Total	**12,063-78,253**	**4,017-26,059**	**16,080-131,310**

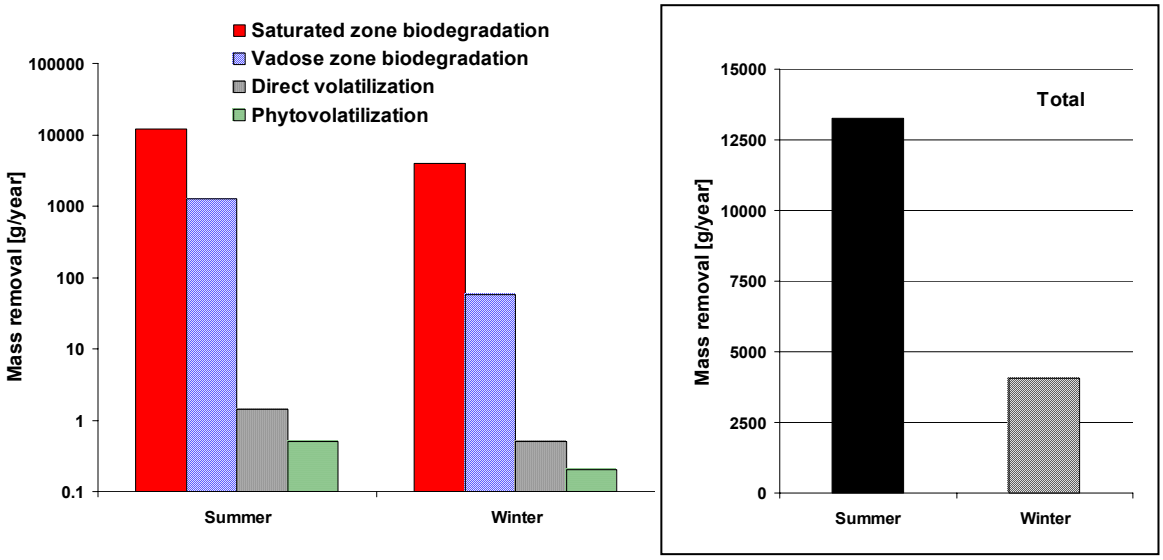

FIGURE 6.8 Comparison of naphthalene mass removal by individual mechanisms during winter and summer conditions

6.3.8 Comparison of mass removal with and without phytoremediation

The total year removal rates with and without the phytoremediation system are shown in Table 6.8 and Figure 6.9. The estimates shown on Figure 6.9 are the lower removal estimate for saturated zone biodegradation and the higher removal estimate for vadose zone biodegradation.

The estimates clearly show that phytoremediation enhances the removal rate for naphthalene. The estimated naphthalene mass in the residual creosote and groundwater is

850 - 1650 kg. The removal rate is 16-131 kg/year with phytoremediation and 6-55 kg/year without phytoremediation. The time it will take to remove all the naphthalene is therefore between 6 to 103 years with the phytoremediation system, and 15 to 275 years without the phytoremediation system.

TABLE 6.8 Summary of naphthalene mass removal by individual mechanisms with and without phytoremediation

	Phytoremediation [g/year]	**No phytoremediation [g/year]**
Saturated zone biodegradation	16,000-103,000	8,000-55,000
Vadose zone biodegradation	77 - 1309	32-116
Volatilization to atmosphere	1.9	1
Phytovolatilization	0.7	0
Total	**16,080-131,310**	**8,033-55,117**

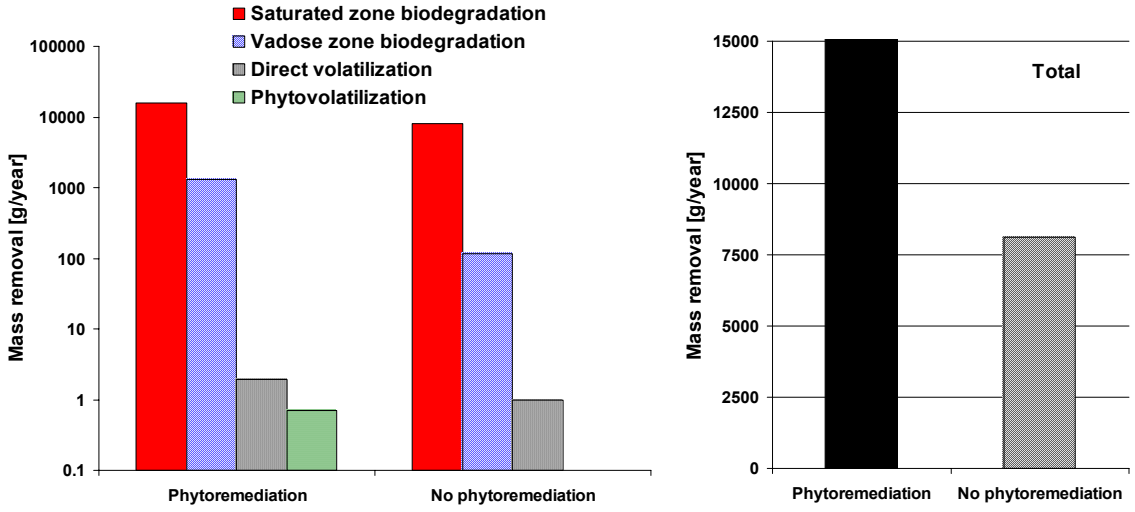

FIGURE 6.9 Comparison of naphthalene mass removal by individual mechanisms with and without phytoremediation

The true rate for biodegradation in the saturated zone is believed to be closer to the lower end of the range based on the groundwater data. The actual volatilization rate to the vadose zone is believed to be closer to the higher end of the estimated range based on soil gas data. The total removal rate would then be approximately 17 kg/year with the trees and 6 kg/year without the trees assuming the lower estimated rates for saturated zone

biodegradation and the higher estimated rates for vadose zone biodegradation. The remediation time for the estimated 850-1650 kg residual naphthalene would then hypothetically be 50-100 years with the phytoremediation system and 140-275 years without the system.

6.4 Conclusions and research recommendations

The biodegradation dominates over the removal by volatilization and phytovolatilization by orders of magnitude, even with the wide range of estimated removal rates presented here. The transfer of naphthalene to the atmosphere by volatilization and phytovolatilization is negligible at this site, which is a positive finding in regard to concern for exposure risk at similar sites.

The overall remediation time for naphthalene at this site is estimated to be reduced to half by the impact of the phytoremediation system. The removal rate is estimated to be significantly slower in the winter than in the summer.

The long estimated remediation time is not a problem at this specific site since there is no immediate exposure risk and the contamination is contained by the hydraulic action of the trees and the interception trench. Based on this research, we believe that phytoremediation with poplar trees can be a useful tool for remediation of dissolved naphthalene plumes after removal of the majority of the source, but phytoremediation is generally not appropriate as a stand alone remediation method for sites with a substantial residual creosote DNAPL.

The removal rate estimates described in this study are sensitive to the assumptions for biodegradation rates, groundwater elevation and concentration profiles in the groundwater and soil gas. Careful site assessment of these variables is therefore essential for the accuracy of the overall estimates of remediation time as well as site specific evaluation of the relative importance of biodegradation in the vadose zone and the saturated zone, respectively. Development of methods for in situ quantification of anaerobic biodegradation rates is desirable. In situ quantification of the rhizosphere effect on the biodegradation by other means than respiration test is also warranted, as oxygen consumption may be a result of enhanced biodegradation of naphthalene is well as the extra organic matter in rhizosphere soil. This may possibly be accomplished by

modification of the push pull test method discussed by Pitterle et al (2). A more direct determination of in situ flux rates to the vadose zone would greatly improve the confidence removal rate for vadose zone biodegradation. Ideally methods for direct flux or concentration measurements closer to the water table should be developed, but a more controlled approach could to apply the column model described in Chapter 3 with conditions closer to the actual in situ concentrations.

The observations at this site indicate that the zone just below and immediately above the water table has elevated biodegradation potential due to moderate contamination levels and higher redox levels. The effect of water interception and transpiration by a phytoremediation system can promote enhanced mass transfer of contaminants and oxygen induced by increased fluctuations of the water table. The effect of this is increased biodegradation. The results from the Oneida site research show that the phytoremediation system enhances the overall removal, mainly by stimulating faster biodegradation in the rhizosphere and promoting mass transfer of naphthalene to the vadose zone. Future research is needed to optimize and fully take advantage of this effect for similar contaminants and sites.

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