THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

THE ROLE OF VEGETATION IN THE PHYTOREMEDIATION AND ECOLOGICAL RECOVERY OF HAZARDOUS WASTE SITES

A Dissertation

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirement for the

degree of

DOCTOR OF PHILOSOPHY

By

PAUL EUGENE OLSON

Norman, Oklahoma

1999

UMI Number: 9957005

UMI®

UMI Microform 9957005

Bell & Howell Information and Learning Company
300 North Zeeb Road
P.O. Box 1346
Ann Arbor, MI 48106-1346

THE ROLE OF VEGETATION IN THE PHYTOREMEDIATION AND ECOLOGICAL RECOVERY OF HAZARDOUS WASTE SITES

A Dissertation APPROVED FOR THE
DEPARMENT OF BOTANY AND MICROBIOLOGY

BY

ACKNOWLEDGEMENTS

I have been truly privileged to be part of the Ph.D. program in the Department of Botany and Microbiology at the University of Oklahoma. The faculty, staff, and students at the University of Oklahoma have provided me with an unparalleled opportunity to explore the exciting realms of science, and in return develop my professional and career aspirations as a scientist. Many individuals at the University of Oklahoma and elsewhere have been instrumental in mentoring my progress that allowed for the successful completion of this dissertation.

I will forever be indebted to the mentoring of my doctoral advisor, John Fletcher. Dr. Fletcher has the unique ability to identify and conduct innovative research in many areas of endeavors. Through Dr. Fletcher's tireless patience and instruction, he took a naïve graduate student and molded myself into a motivated scientist. His shared experiences have provided me with a solid professional foundation from which to build from.

I would like to thank the members of my doctoral committee, Drs. Bruce Hoagland, Forrest Johnson, William Ortiz Leduc, Paul Philp, and John Skvarla, for providing advice and help during my research work. Without their patience and persistence, this dissertation would not have been possible.

A sincere gratitude goes to the Union Carbide Corporation. The progressive thinking of the workers at Union Carbide not only funded a great portion of my research, but allowed myself and fellow co-workers access to research sites on their facilities in Texas City, Texas to conduct my work for this dissertation. Without the timely help from Tom Wong, Richard Chapin, Bobby O'Byran at the Texas City plant, and Xiujin Qiu, Sunil Shah in West Virginia. this research project would not have been possible. Additional funding for this project was provided by the Graduate College at the University of Oklahoma.

Chemical analyses for this research were performed in the labs of Dr. Paul Philp in the Sarkey's Energy Center. The combined efforts of Dr. Philp and Jon Allen provided an easy atmosphere in which to complete the lengthy chemical analyses during this research. In addition. Joe Grego in the Department of Civil Engineering was always helpful in providing me with needed equipment. including Soxhlet glassware for soil extractions.

Many fellow students provided technical help and expertise that allowed me to complete this dissertation in a timely manner. In particular. I am indebted to Mary Beth Leigh. Burt Bluhm. Kevin McAllister. Jennifer Barker. Patty Smith. and Mike Kyle for help in sampling. data measurements. and/or analyses during this project.

And finally. I would like to dedicate this dissertation to my two sisters. Ann Lewis and Cindy Golbach. Their love. support. and constant reassurances gave me the strength to see this dissertation to completion. And because of my sisters and many others. my life holds a very bright future.

TABLE OF CONTENTS

List of Tables

Chapter 3.

Chapter 4.

List of Figures

Chapter 3.

Chapter 4.

Preface

Environmental and human health risks are potentially associated with the manufacture, use, and disposal of a variety of man-made chemicals. In 1976, the United States Congress enacted the Resource Conservation and Recovery Act (RCRA) to federally regulate the safe disposal of hazardous wastes and reclamation of areas previously contaminated with pollutants. The United States Environmental Protection Agency (USEPA) lists 129 chemicals and metals as priority pollutants subjected to federal RCRA regulations because of their inherent persistence in the environment, potential bioaccumulation, toxicity and/or carcinogenicity to living organisms. Organic chemicals represent 114 out of the 129 priority pollutants on the list and are divided into 12 classes, including the polycyclic aromatic hydrocarbons (PAHs).

Terrestrial sites, including unlined earthen settling ponds and disposal lagoons, currently contaminated with PAHs are generally in need of remediation as mandated by RCRA legislation. Remedial actions at PAH-contaminated sites in the U.S. typically involve invasive, engineered methods (i.e. excavation / incineration) that are usually very costly. Alternatively, less invasive, biological methods that stimulate microbial-degrading processes are gaining regulatory acceptance. One such approach for the clean-up of deeply contaminated hazardous waste sites is the economically and ecological-sound plant-based microbial remediation known as phytoremediation.

Although beneficial evidence exists regarding the use of phytoremediation for recalcitrant organic pollutants, the underlying mechanisms are not readily understood. Furthermore, the choice of plants suitable for the phytoremediation of organic pollutants has received little attention, as easy to grow grasses and agronomic plants are usually employed for remedial strategies. From an ecological context, the selection process should be directed toward plants that not only can tolerate industrial waste settings and the local climate, but have the potential to form rhizosphere-specific associations between plant roots and pollutant-degrading microflora. In addition, no phytoremediation studies have been completed in field conditions at a time frame consistent with the normal degradation of recalcitrant carbon compounds found naturally in the environment. As a result, virtually no attention has been paid to ecological and environmental factors that are likely to be the most important for the successful development of a phytoremediation technology of organic pollutants. This dissertation consists of four chapters that examine the ecological role of vegetation in the enhanced remediation and restoration of PAH-contaminated sites.

The first chapter examines the role of plant invasion and succession at a former sludge basin possessing high levels of organic contaminants in the phytoremediation of this site. Through natural events, pioneer plant species have modified the underlying sludge to allow for additional plants to colonize the site. Over time, tolerant plants growing at this former sludge basin have provided the means for the ecological recovery of this chemically distressed site. Chapter one has been written according to guidelines for the journal *Environmental Science and Pollution Research*.

The second chapter describes how plants provide the means for the natural attenuation and dissipation of recalcitrant PAHs at a former sludge basin. This investigation evaluated vegetative growth (5-15 years) of naturally occurring plants and the influence of rooting patterns and depth on pollutant concentrations in the basin. The results from this study, including contaminant fate, illustrate the dynamic, long-term ecological role of plants and associated microflora in the phytoremediation of recalcitrant organic pollutants. Chapter two has been written in a format suitable for the journal *Environmental Toxicology and Chemistry*.

The third chapter discusses the structure and depth of mature tree roots growing in heavily contaminated industrial sludge. The visual restoration of the sludge and decreases in contaminant levels associated with the growth of the tree roots provide useful information regarding future use of trees in the phytoremediation of sites contaminated with organic pollutants. Chapter three has been formatted in accordance to the guidelines of *Bioremediation Journal*.

The fourth chapter examines a fifteen-year range of mulberry trees naturally growing in an industrial waste basin. Above and belowground biomass measurements were used to estimate the spatial and temporal relationships of tree growth and contaminate fate. The results from this study provide comprehensive information regarding mulberry growth in contaminated soils that can be used to predict plant allometric growth and estimate the fate of soil contaminants at other sites employing trees for phytoremediation. Chapter four has been written for submission to the *Journal of Environmental Quality*.

Abstract

Examination of volunteer vegetation growing in industrial sludge contaminated with polycyclic aromatic hydrocarbons (PAHs) has been used as a means of identifying particular plant species whose roots foster soil restoration and the degradation of organic contaminants. Aerial photographs taken over the last 25 years have been used to characterize plant invasion and plant succession that has resulted in the development of a diverse and healthy community of plants (51 species from 22 families) currently growing at this contaminated site. Differences between the plant community composition at the contaminated sludge basin and a non-contaminated site suggested that a select group of plants have favorable characters to colonize, grow and modify polluted soils.

The growth of plant roots in contaminated soils is of paramount importance to the success of phytoremediation. Field observations, coupled with chemical analysis, indicated that the presence of plant roots growing in the contaminated sludge corresponded with a visual soil restoration and dissipation of recalcitrant PAHs. The distribution and depth of plant roots from tolerant vegetation growing at these former sludge basins were species and age dependent. In general, tree and shrub roots penetrated the sludge to a greater extent (~90 cm), compared to grasses (~50 cm) and forbs (~60 cm) at this site. However, PAH levels were significantly lower within the root zone (10-25 % the concentration present beneath the root zone) regardless of the vegetation compared to the non-rooted sludge. Thus, tolerant vegetation invaded and colonized a former sludge basin, and through extensive root growth, fostered the natural attenuation and ecological recovery.

Biomass measurements made on 31 mulberry trees of different ages and sizes were used to develop allometry relationships for both above and belowground components. These allometric relationships, in combination with the pollutant changes documented in this sludge basin, support the concept that an established plant community with associated microorganisms provides an ecologically stable, low cost, sustained bioremediation system for long-term treatment of sludge basins.

Chapter 1.

ECOLOGICAL RECOVERY OF VEGETATION AT A FORMER INDUSTRIAL SLUDGE BASIN AND ITS IMPLICATIONS TO PHYTOREMEDIATION

Abstract

Examination of a former industrial sludge basin containing organic pollutants showed that the basin had undergone substantial ecological recovery over the last 16 years through natural forces. Conventional phases of ecological recovery (plant invasion and succession) have occurred, but the structure of the diverse plant community (51 species and 22 families) was different from that at a recovering non-polluted disturbed site. Bermuda grass, mulberry, and sunflower, believed to be early invaders of the basin, still persist in large numbers indicating that these species are well suited to cope with normal environmental stresses at this area (i.e. seasonal drought and flood) as well as organic pollutants. There was evidence that early invaders of the site fostered disappearance of contaminants thereby creating more favorable conditions for a broader spectrum of plants to grow. Vegetation analyses of naturally vegetated hazardous waste sites hold promise as a screening device for identifying plant species and management practices worthy of further phytoremediation investigations.

1 Introduction

Ecological recovery of physically and chemically distressed land has been well documented by plant ecologists for abandoned agricultural fields (Bard, 1952; Bazzaz, 1968; Perino and Risser, 1972), mine spoils (Gibson, et al., 1985; Skousen, et al., 1990; Skousen, et al., 1995), and volcanic ash plumes (Frenzen, et al., 1988; Tsuyuzaki, 1995). In this context, ecological recovery has been defined as the return of a biological population or community to some aspect of its condition prior to a stressor's introduction (USEPA, 1998). In general, these studies have described the pattern and timing of ecological events whereby bare ground becomes inhabited by a changing community of plants with the eventual outcome being vegetation characteristic of a given geographical region. Ecological events that have been studied in detail at such sites include plant invasion, competition, succession, and community structure change (Pickett, 1982). Efforts to explain differences in disturbed sites have shown that the set of adverse growth conditions (plant stressors, i.e. low soil nutrients, high pH, etc.) prevailing at a given site influences both the pattern and rate of ecological recovery. For example, although seeds of many different kinds of plants are deposited by natural vectors (i.e., wind, birds, etc.), at a disturbed site only a few species grow and survive during the first few years of recovery because of selection pressures exerted by stress conditions.

Of great importance in understanding ecological recovery is knowledge that not only is the initial community of plant invaders (pioneer species) capable of withstanding severely adverse growth conditions, but that they are also capable of modifying the soil conditions and microclimate at a disturbed site (Tansley, 1935). These modifications produce conditions in which a broader spectrum of plant species can inhabit the site.

thereby facilitating plant succession. Plant species that follow the pioneer community often have more extensive root systems, increased foliage, and other features that are conducive to further improvement of the site for habitation by other life forms (animals, insects, worms, etc.). The invasion and succession of plants at disturbed sites is essential for ecosystem recovery because photosynthetic plants are the primary producers of food and shelter for all other living components of a healthy ecosystem.

From an ecological standpoint, hazardous waste sites are disturbed areas similar in most respects to other disturbed areas (abandoned agricultural fields, etc.) because they share common stress features. The distinguishing feature of hazardous waste sites is the presence of high levels of organic and/or metal contaminants. The presence of contaminants may be thought of as an additional selection pressure for determining what plants will colonize a site and how plant succession and ecological recovery will proceed. Common knowledge that abandoned hazardous waste sites are often covered with vegetation indicates that natural ecological events do occur, but to our knowledge no efforts have been made to study these events. Understanding which plant species colonize various types of hazardous waste sites and the pattern of plant succession could be useful in developing a more effective phytoremediation technology, whereby natural processes accelerated by the use of the most appropriate plants and management practices are tailored to cope with the chemicals and climate conditions at individual sites in different geographical regions, with the goal of establishing an ecologically-sound sustained phytoremediation systems.

The objective of this study was to determine how plant invasion and succession at a former sludge basin possessing high levels of organic contaminants compared to events occurring at a nearby disturbed but non-contaminated site. Examination of aerial photographs in combination with analysis of the vegetation growing at each site was used to make this comparison.

2 Experimental Section

2.1 Location Climate

The study was conducted at the Union Carbide chemical plant (Figure 1) located in Texas City, Texas (Galveston County). This installation is located on the eastern edge of the Texas Coastal Prairie (Correll and Johnston, 1979). The climax vegetation of the Texas Coastal Prairie is dominated by four tallgrass species: big bluestem (*Andropogon gerardii*), little bluestem (*Andropogon scoparius*), Indian grass (*Sorghastrum nutans*), and panic grasses (*Panicum* spp.). Over the last 20 years, large portions of the Texas Coastal Prairie has been transformed into woodland vegetation by trees and shrubs, with two aggressive introduced species, Chinese tallow (*Sapium sebiferum*) and eastern baccharis (*Baccharis halimifolia*), playing major roles in this transition (Bruce, et al., 1995).

The annual rainfall in Texas City is approximately 105 cm, and the growing season (frost-free days) exceeds 300 days [National Oceanic and Atmospheric Administration (NOAA): NOAA-National Climatic Data Center web page]. The average maximum temperature in midsummer (July) is 33.7°C, and the average minimum temperature reached in midwinter (Jan) is 8.6°C. The high summer temperatures are often accompanied by drought periods ranging from 8 to 12 weeks.

2.2 *Sludge Basin*

The sludge basin is a 0.45 ha rectangular area bordered on all sides by aboveground earthen embankments. The basin was used from the 1940s until the early 1980s to dispose of waste-water from the chemical plant. The water, taken originally from the Brazos River, contained a mixture of organic contaminants from the chemical plant and residual amounts of sediment (sand, silt, and clay) not removed during the filtration of the river water prior to its use in the plant. The mixture of organic contaminants discharged into the basin included a broad spectrum of small molecular weight benzene derivatives and higher molecular weight polycyclic aromatic hydrocarbons (PAHs) typically found in waste-water from petroleum chemical plants. Metals were not a part of the chemical processing at this plant and therefore were not disposed of in the basin. The waste-water was discharged from vacuum trucks into the basin at the southwest corner of the basin (Figure 2). Settling of the river sediment and associated chemicals during the basin's years of active use resulted in the accumulation of sludge heavily laden with an assortment of organic waste compounds. Discharging waste-water a truckload at a time from a fixed location had two important effects on filling the basin: (1) chemical contaminants were discharged in a heterogeneous manner both vertically and horizontally across the basin, because the chemical content of each truck load was different, and (2) the basin filled from the southwest corner to the northeast corner creating a pronounced topographical slope due to sediment deposition. Because of the uneven filling of the basin, the lower, eastern portion of the basin was submerged under water until 1982 when routine pumping was initiated to keep the basin drained.

2.3 Non-Contaminated Site

The non-contaminated site is a 2.4 ha parcel of land located approximately 800 m south of the sludge basin (Figure 1). In the mid 1980's, this land was overlain with soil taken from construction sites during expansion of the chemical plant onto undeveloped land. The overburden soil is mounded to a maximum height of 10 m, and an average side slope of approximately 30 degrees. This area was not used for disposal of chemical waste, and none of the marker contaminants analyzed for in this study were found at the site.

2.4 Railroad and Road Right-of-Ways

Mature, seed-bearing plants growing along the railroad and roads bordering the chemical plant (Figure 1) were surveyed since their close proximity to both the sludge basin and the non-contaminated area. These plants were the most likely donors of seed for volunteer vegetation that appeared during the 1980s on exposed sludge and bare ground at the sludge basin and non-contaminated site, respectively.

2.5 Aerial Photographs

Aerial photographs taken between 1958 and 1995 of the chemical plant and its surroundings were purchased from private vendors. Photographs ranging in scale from 1:4800 to 1:600 were available for the entire span of years studied. Photographs were studied to determine periods of physical and/or chemical alteration of the basin, and also to establish when bare ground was first invaded by vegetation. The general nature of the vegetation (grasses, forbs, or trees) from the first year of invasion up to 1995 was examined. The visual interpretation of photographs regarding the presence of certain kinds of vegetation (i.e. grasses versus trees) and the location of specific perennial plants (trees, shrubs) was compared to and verified by examining plants currently growing at the study sites.

2.6 Contaminant Distribution

The distribution of organic contaminants at the former sludge basin and the non-contaminated site was determined by quantifying the amounts of four representative marker-compounds [naphthalene (Nap), phenanthrene (Phe), pyrene (Pyr), and benzo(a)pyrene (BaP)] from cored material. These chemicals were chosen as indicator compounds because of their general presence in waste discharged from the Texas City plant and their persistence in the environment relative to lower molecular weight benzene derivatives. The water solubility, log K_{ow} (octanol-water partition coefficient), and solid phase vapor pressure for the 4 marker-compounds ranged from 31.0 to 3.8×10^{-3} (mg/L at 25° C), 3.37 to 6.04, and 10.4 to 7.0×10^{-7} (Pa at 25° C), respectively. These values provided a range of physical-chemical properties reflective of contaminants with moderate leaching and volatility tendencies (i.e. naphthalene) to those that are virtually immobile (i.e. benzo(a)pyrene) in the soil (MacKay, et al., 1992). In this study, the abundance of these representative marker-compounds was used as an indicator of the general distribution of the mixture of organic contaminants discharged into the basin.

Six cores, evenly distributed across the basin (Figure 3), were obtained and the 0-30 cm and 140-160 cm segments were removed for chemical analysis. Cored material was removed in a similar fashion from the non-contaminated site. Each sample was subjected to Soxhlet extraction and analysis according to U.S. EPA methods (USEPA. 1986a; USEPA. 1986b).

2.7 Soil Fertility

Soils removed from the sludge basin. the non-contaminated site. and right-of-ways were prepared according to standard methods used for soil fertility determination. Twelve random soil samples were cored from the first 30 cm from each of the three areas. These samples were transported to the laboratory. dried at room temperature. sieved (2 mm). and thoroughly mixed to represent a composite sample for each site. In addition. twelve samples were collected in the sludge basin below the root zone (> 150 cm) of dominant vegetation growing in the basin. and prepared in like fashion. The soil analyses were conducted by Soil Analytical Services. College Station. TX for the sludge basin material and by Ward Laboratories. Keany. NB for the non-contaminated site and right-of-ways. It was necessary to use two different analytical laboratories because of contaminants present in the sludge samples.

2.8 Vegetation Analysis

The vegetation currently growing at each of the study locations (sludge basin. non-contaminated. and right-of-ways) was surveyed and lists of dominant species and percent coverage estimates were made. The survey techniques used at each area were dependent on the size and configuration of the area. Tree ages were determined by counting annual rings from trunk core borings.

2.8a Sludge Basin

The entire basin was sectioned into twenty, 15 m x 15 m subplots. Within each subplot, vegetational coverage (abundance) was determined from smaller 1.5 m x 1.5 m quadrates. The entire basin was surveyed and encountered species were tallied. Recorded plant species were also categorized into the following vegetational groups: a. trees and shrubs; b. forbs; and c. grasses. The most prominent plant species influencing the community structure, in terms of ground coverage, were plotted on a grid map identifying their exact location in the basin.

2.8b Non-Contaminated Site

Six belt transects measuring 75 m long and 30 cm wide were evenly positioned over the 2.4 ha area to determine principal plant species and relative coverage of vegetation growing at this disturbed location. Principal plant species and relative coverage was recorded for vegetation found growing within the 30 cm wide transect.

2.8c Right-of-Ways

The dominant plant species and relative percent cover of vegetation growing along approximately 4 km of rail and road right-of-ways on the perimeter of the Union Carbide property (Figure 1) were determined. This was accomplished by walking the linear distance of these vegetated areas and recording relative coverage of dominant plant species on appropriate map grids.

3 Results

3.1 Land Use

3.1a Sludge Basin

Analysis of photographs (Figure 2) indicated that materials were discharged into the basin from the southwest corner as evidenced by the continual expansion of a peninsula of discharged material into standing water at the eastern end of the basin. This practice appeared to end in the early 1980s since there was no change in the topography of the basin after that time with the exception of a drainage ditch dredged on the south side of the basin. At present, high ground in the southwestern corner slopes to low ground along the eastern edge of the basin. There is also a modest ridge running in a north-south direction in the middle of the basin. These topographical features observed in the aerial photographs taken since the 1970s still persist today.

3.1b Non-Contaminated Site

Photographs show that the non-contaminated site was an unpaved parking lot when the sludge basin was in use during the 1960s and 1970s. Analysis of later photographs showed that piles of soil deposited at this location in the mid 1980s have not been physically moved or altered since its deposition.

3.2 Chemical Characterization

A comparison of the fertility features at the three locations studied showed that the levels of N, P, and K were highest in the sludge basin (Table 1). Analysis of nutrients at two different depths in the sludge basin showed approximately the same levels of N but less P and K in the upper 30 cm (Table 1). The pHs were similar at the three locations except for the high pH of 9.3 observed in the sludge material at the 150 cm depth in the basin.

None of the four marker-compounds (naphthalene, phenanthrene, pyrene, and benzo(a)pyrene) were detectable at the non-contaminated site. In contrast, these compounds were widely distributed both horizontally and vertically in the former sludge basin (Figure 3). Three of the contaminants (Nap, Phe, Pyr) were present in every sample analyzed at both depths. The concentration of the contaminants in the upper 30 cm of the basin, where plant roots were plentiful, was always lower than the amounts present in the sludge material at the 150 cm depth where no roots were present. Contaminants at the shallow depth averaged less than 10% of the values present in the underlying sludge. The absence of high molecular weight PAHs from the root zone in this forensic field study suggests that the phytoremediation processes described in the laboratory work of Reilley, et al. (1996) have occurred *in-situ* over the past 16 years at this field site. Of special interest in this regard are pyrene and benzo(a)pyrene because of their minimal tendency to leach (Knox, et al., 1993) and/or volatilize (Park, et al., 1990).

3.3 Plant Invasion and Succession

3.3a Sludge Basin

Aerial photographs (Table 2) showed that the first herbaceous vegetation, composed of grasses and forbs (weedy annuals), colonized the basin in the early 1980s when efforts were started to keep the basin dry through pumping (see Land Use, above). Woody vegetation invaded the basin over the next 5 years. Perennial shrubs (*Baccharis* *halimifolia*) invaded the basin in 1982, and trees (*Morus* spp. and *Celtis laevigata*) appeared in 1985. Mixed taxonomic characters (i.e. leaf morphology) suggest that the mulberry growing in the basin and adjacent areas is a hybrid of *Morus alba* and *Morus rubra*, a common occurrence among mulberry growing in the wild (Wunderlin, 1997). By 1987, herbaceous plants (grasses and forbs) and woody vegetation (trees and shrubs) accounted for approximately 65% and 10% of the site coverage, respectively. From 1990 to 1995, the vegetational patterns in the basin changed successionally with herbaceous vegetation and woody species representing 55% and 40% respectively of the total coverage on the basin by 1995. Herbaceous cover on the basin increased from 10% to 75% between 1980 and 1989 and than decreased to 55% by 1995. Woody coverage on the basin has continued to increase from 5% in 1985 to 40% in 1995.

3.3b Non-Contaminated Site

Plant invasion had occurred at the non-contaminated area by 1989 as shown in aerial photographs (Table 2). Thus, invasion of this area was approximately 5 years later than invasion at the former sludge basin. The early pioneer species at the non-contaminated area appeared to be grasses and forbs (Table 2). By 1995, herbaceous vegetation and woody plants accounted for 60% and 35% relative coverage of the site, respectively.

3.4 Plant Community Structure

3.4a Right-of-Ways

The rail and road right-of-ways on the perimeter of the Carbide property (Figure

1) were the closest and largest communities of mature seed-bearing plants during the 1980s when exposed sludge and bare ground prevailed at the sludge basin and the non-contaminated site, respectively. Therefore, the vegetation in the right-of-ways was an important source of seeds during both plant invasion and succession at both of the areas studied. Aerial photographs showed that the right-of-ways had been vegetated continuously from 1958 to present without any major disturbance (Table 2). Inventory of species presently growing along right-of-ways showed Chinese tallow and eastern baccharis were equally dominant in coverage and abundance and accounted for nearly 52% of the coverage (Table 3). Ragweed and Bermuda grass were the next most prominent plants accounting for the majority of the coverage by forbs and grasses (4.7% and 7.4%), respectively.

3.4b Sludge Basin

At present the entire sludge basin is covered by dense, robust vegetation with deep green, well-formed foliage. The exception to this observation is the largely unvegetated drainage ditch along the south side, and a former truck-pad in the southwest corner of the basin (Figures 2 and 4). Inventory of the plants growing in the basin (Table 4) identified 51 species from 47 genera and 22 families. In general terms, the vegetation is best described as a healthy, diverse community of plants, whose structure is typical of a dynamic, evolving community growing at a formerly disturbed area [i.e. abandon agricultural field (Bard, 1952; Bazzaz, 1968; Perino and Risser, 1972)]

The composition of the dominant vegetation at the basin according to vegetation type (trees/shrubs, forbs, grasses) was 41%, 22%, and 27%, respectively (Table 3). The three most prominent species based on area of ground covered (a reflection of abundance) were mulberry tree (36%). Bermuda grass (19%). and sunflower (9%). accounting for 64% of the total cover on the basin. Based on aerial photographs. the largest and oldest distinguishable plants were mulberry (Figure 4). Twenty-eight mulberry trees in the basin had trunk diameters greater than 20 cm and were between 8 to 14 yr old based on counting annular tree rings. The older mulberry trees presumably grew from seed deposited into the basin from an outside source (Figure 1) since they are too old for an early mulberry invader into the basin to have reached maturity and served as a seed source. The seeds from which the oldest trees grew. germinated in sludge material within 2 to 4 years after the basin was drained in 1982. There are a few large sugarberry trees that have reached the fruit-bearing stage and numerous baccharis. most of which have died beneath the canopy of the taller mulberry (Figure 4).

The mulberries are distributed across the basin in a random. scattered pattern with the exception of two areas where Bermuda grass and sunflower are in pure stands. The exclusion of woody plants from these areas is attributed to seasonal flooding because of the topography of the basin as described earlier in the results.

The composition of the plant community in the basin is quite different from that of the right-of-ways and the non-contaminated site (Table 3). Noteworthy differences are the abundance of Chinese tallow in the right-of-way areas in contrast to no tallow trees in the basin except for a few saplings that grew in 1997 from naturally dispersed seed. several of which have since died. The community composition of the basin versus the non-contaminated site is also drastically different. Four woody species (willow. rattlebush. salt cedar. and Chinese tallow. each accounting for over 1% of the cover at the non-contaminated site) are not present in the basin except for a few 1 year-old Chinese tallows (Table 3). In like fashion, several prominent grasses at the non-contaminated site (bushy bluestem, silver bluestem, fingergrass, and vasey grass) were minor components in the basin whereas Bermuda grass was abundant in the basin and almost nonexistent at the non-contaminated site.

3.4c Non-Contaminated Site

This area is equally dominated by the shrub (*Baccharis halimifolia*), forbs *Solidago* spp. and *Heterotheca subaxillaris*), and grasses (*Andropogon saccharoides* and *Chloris canterai*). Woody species, forbs, and grasses account for 32%, 33%, and 33% coverage (abundance), respectively. Young trees, such as Chinese tallow and willow, are starting to bear seed at the time of this study.

4 Discussion

Examination of aerial photographs showed that the former sludge basin studied in this investigation was used as a disposal area up until the early 1980s. Comparison of the basin's topography at that time with its present profile established that it has remained virtually unchanged with the exception of a runoff-sump dredged on the south side of the basin to facilitate the drainage and removal of excess rainwater following severe storms. Thus, there is no visual evidence that waste material has been removed from the basin during the last 20 years, or that it has been covered up with non-contaminated soil. Further evidence that the latter has not happened is the presence of the marker-compounds in the upper 30 cm soil samples taken from across the basin (Figure 3).

Ecological recovery through plant invasion and succession is in progress at the former sludge basin examined in this investigation. These processes appeared to start almost immediately when the basin was taken out of service since aerial photographs taken in 1982, the first year that the basin was drained, showed 50% of the exposed sludge covered with vegetation. Examination of aerial photographs taken between 1982, and 1995 showed a gradual replacement of the initial herbaceous pioneer species with perennial woody vegetation. This general pattern of ecological recovery (plant invasion followed by succession) typifies events reported in classical ecological studies of non-polluted, disturbed areas (Bard, 1952; Bazzaz, 1968; Perino and Risser, 1972; Pickett, 1982) and also parallels the ecological changes observed at the non-contaminated site in this study. At present the basin is completely covered by a dense, diverse community of 51 species representing 47 genera and 22 families, evidence that the presence of organic contaminants has not precluded the start of ecological recovery at this contaminated former sludge basin.

Although the general pattern of ecological recovery at both the sludge basin and the non-contaminated site was the same, the composition of the plant communities at the sites were different even though the two sites are only 800 m apart and shared the same natural sources of seed for pioneer species that invaded both sites in the 1980s. At present, approximately half of the ground at the sludge basin is covered by mulberry (36%) and Bermuda grass (19%); whereas at the non-contaminated site the predominant cover species are baccharis (24%), goldenrod (15%), fingergrass (13 %) and silver bluestem grass (10%) [Table 3].

There are many differing factors between the non-contaminated site and the sludge basin including soil fertility, drainage pattern, and organic pollutants that potentially contributed to the community differences at the two sites. Other random events, such as seed dispersion and herbivory, were instrumental in governing plant invasion and succession. We believe that the most important factor accounting for the more advanced stage of succession at the sludge basin is the longer time for recovery (5 additional yrs) and the more favorable growth conditions that prevail there (higher soil fertility and better water penetration) in comparison to the non-contaminated site. These features would give a competitive advantage to fast growing, large species with early and prolific reproduction potential. Evidence that this has happened in the basin is the presence of numerous large (10-15 cm trunk diameter), dead baccharis trees present beneath the dense canopies of over-towering 4 m tall mulberry trees (Figure 4).

There is evidence that some of the organic contaminants in the basin may have exerted a selection pressure as phytotoxins at certain times during the early stages of ecological recovery at the basin. This contention is based on examination of woody plants growing at the two sites. Chinese tallow, willow, rattlebush, and salt cedar (Table 3) were four woody species that grew at the non-contaminated site, but were not present in the basin. The best example of apparent exclusion of a species from the basin over the years by a potentially phytotoxic compound was the absence of Chinese tallow trees. This species is a fast growing, early reproducing, invasive tree (Bruce, et al., 1995), whose seed is dispersed by birds (Jubinsky and Anderson, 1996) similar in these respects to mulberry. Mature seed-bearing Chinese tallow trees have existed in the vicinity of the basin for at least 20 years (Table 2) and are presumed to have supplied the seed that has germinated and grown into mature seed-bearing trees at the non-contaminated site.

During the summer of 1997, Chinese tallow saplings grew in the basin but several developed etiolated leaves, typical of herbicide damage, and died during the later part of the summer when the upper soil was dry. Based on this observation, it is reasonable to assume that Chinese tallow seeds have been dispersed into the basin over the past 15 years, and that they have germinated but failed to survive. The death of the seedlings during the dry part of the summer suggests that the proposed phytotoxic effects to certain plant species (i.e. Chinese tallow) at this stage of the basin's ecological recovery are restricted to lower depths of the basin. It can be speculated that certain chemical and/or physical stressors affect some plant species when water is drawn from deeper regions of the contaminated basin. On the other hand, mulberry trees appear to withstand the unknown stressors. If chemical stressors were the problem, then it follows that the influence of these proposed phytotoxic compounds would only occur during drought periods when moisture is no longer available in shallow soil. The status of the recovering ecosystem may continue to improve (become less phytotoxic) over time as more of the proposed chemical stressors are degraded and/or mobilized through phytoremediation processes mediated by plants growing in the basin: mulberry (Donnelly, et al., 1994; Hedge and Fletcher, 1996; Leigh, 1997), sunflowers (Boyle and Shann, 1998), and grasses (Aprill and Sims, 1990; Ferro, et al., 1994; Gunther, et al., 1996). Examination of vegetation growing at hazardous waste sites serves as a means of identifying plant species that can cope with hazardous waste compounds in combination with other naturally occurring stressors such as seasonal drought and flooding. Using vegetation analysis at hazardous waste sites as a first level of screening for a desirable phytoremediation plant, it should be followed by controlled experimentation, preferably under field conditions, to fully evaluate a species' phytoremediation capabilities. Field testing conducted over a broad range of chemical and environmental conditions is needed to ascertain the general utility of a particular plant species for phytoremediation.

5 Conclusions and Future Outlook

The progress of early ecological recovery documented in this study provides useful ideas for future development of more effective phytoremediation technology. Phytoremediation to date has been patterned after agriculture and forestry practices where commercially important species are grown as monocultures (Schnoor, et al., 1995; Cunningham, et al., 1996). There have been very few screening studies comparing the phytoremediation performance of a broad spectrum of different species either in the laboratory or in the field. An expedient way to close this information gap is to capitalize on contaminated sites as a first level of screening to identify species with the potential to remediate pollutants over time under adverse growth conditions (i.e. drought). When more attention has been given to screening and field testing, it is unlikely that neither the same species will be found equally useful under all climatic and soil conditions, nor that a single species grown as a monoculture is the most effective phytoremediation system since it contradicts natural ecological recovery involving multiple species in an evolving plant community (Clements, 1928; Tansley, 1935) as observed in this investigation.

This study, to our knowledge, is the first effort to document plant colonization and community structure change at a hazardous waste site over an extended period (20 yrs). The observations and interpretations resulting from this forensic study (looking backwards from 1998 to 1978) provide strong incentive for conducting future long-term

(20 yrs) research at selected hazardous waste sites. Field studies designed with test and control plots. large enough to evaluate ecological processes (plant. animal. and microbial). could be used simultaneously to evaluate different phytoremediation strategies and their influence on ecological recovery. an important but not well-understood aspect of ecological risk assessment (Norton. et al.. 1992; USEPA. 1998).

6 References

Aprill. W.: Sims. R.C. (1990): Evaluation of the use of prairie grasses for stimulating polycyclic aromatic hydrocarbon treatment in soil. Chemosphere **20**. 253-265

Bard. G.E. (1952): Secondary succession on the Piedmont of New Jersey. Ecol. Monogr. **22**. 195-214

Bazzaz. F.A. (1968): Succession on abandoned fields in the Shawnee Hills southern Illinois. Am. Midl. Nat. **84**. 90-104

Boyle. J.J.: Shann. J.R. (1998): The influence of planting and soil characteristics on mineralization of 2.4.5-T in rhizosphere soil. J. Environ. Qual. **27**. 704-709

Bruce. K.A.: Cameron. G.N.: Harcombe. P.A. (1995): Initiation of a new woodland type on the Texas Coastal Prairie by the Chinese tallow tree (*Sapium sebiferum* (L.) Roxb. Bull. Torrey Bot. Club **122**. 215-225

Clements. F.E. (1928): Plant Succession and Indicators. H.W. Wilson Company. New York City. 453 pgs.

Correll. D.S.: Johnston. M.C. (1979): Manual of the Vascular Plants of Texas. The University of Texas at Dallas. Second Printing. 1881 pgs.

Cunningham. S.D.: Anderson. T.A.: Schwab. A.P.: Hsu. F.C. (1996): Phytoremediation of soils contaminated with organic pollutants. Adv. Agronomy **56**. 55-114

Donnelly. P.K.: Hegde. R.S.: Fletcher. J.S. (1994): Growth of PCB-degrading bacteria on compounds from photosynthetic plants. Chemosphere **28**. 981-988

Ferro. A.M.: Sims. R.C.: Bugbee. B. (1994): Hycrest crested wheatgrass accelerates the degradation of pentachlorophenol in soil. J. Environ. Qual. **23**. 272-279

Frenzen. P.M.: Kransny. M.E.: Rigney. L.P. (1988): Thirty-three years of plant succession on the Kautz Creek mudflow. Mount Rainier National Park. Washington. Can. J. Bot. **66**. 130-137

Gibson. D.J.: Johnson. F.L.: Risser. P.G. (1985): Revegetation of unreclaimed coal strip mines in Oklahoma: II. Plant communities. Reclam. Reveg. Res. **4**. 31-47

Gunther. T.: Dornberger. U.: Fritsche. W. (1996): Effect of ryegrass on biodegradation of hydrocarbons in soil. Chemosphere **33**. 203-215

Hegde. R.S.: Fletcher. J.S. (1996): Influence of plant growth stage on the release of root phenolics by mulberry as related to development of phytoremediation technology. Chemosphere **32**. 2471-2479

Knox. R.C.: Sabatini. D.A.: Canter. L.W. (1993): Subsurface Transport and Fate Processess. Lewis Publishers. Boca Raton. FL.

Leigh. M.B. (1997): The Release of Phenolic Compounds into the Soil by Fine Root Turnover of Mulberry (*Morus rubra* L.). MS Thesis. University of Oklahoma. Norman. OK.

Jubinsky. G.: Anderson. L.C. (1996): The invasive potential of Chinese Tallow-tree (*Sapium sebiferum* Roxb.) in the Southeast. Castanea **61**. 226-231

MacKay. D.: Shiu. W.Y.: Ma. K.C. (1992): Illustrated Handbook of Physical-Chemical Properties and Environmental Fate for Organic Chemicals. Volume II: Polynuclear Aromatic Hydrocarbons. Polychlorinated Dioxins. and Dibenzofurans. Lewis Publishers. Chelsea. Michigan. 597 pgs.

Norton. S.B.: Rodier. D.J.: Gentile. J.H.: van der Schalie. W.H.: Wood. W.P.: Slimak. M.W. (1992): A framework for ecological risk assessment at the EPA. Environ. Toxicol. Chem. **11**. 1663-1672

Park. K.S.: Sims. R.C.: Dupont. R.R.: Doucette. W.J.: Matthews. J.E. (1990): Fate of polycyclic aromatic hydrocarbon compounds in two soil types: Influence of volatilization. abiotic loss and biological activity. Environ. Toxicol. Chem. **9**. 187-195

Perino. J.V.: Risser. P.G. (1972): Some aspects of structure and function in Oklahoma old-field succession. Bull. Torrey Bot. Club **99**. 233-239

Pickett. S.T.A. (1982): Population patterns through twenty years of old field succession. Vegetatio **49**. 45-59

Reilley. K.A.: Banks. M.K.: Schwab. A.P. (1996): Dissipation of polycyclic aromatic hydrocarbons in the rhizosphere. J. Environ. Qual. **25**. 212-219

Schnoor. J.L.: Licht. L.A.: McCutcheon. S.C.: Wolfe. N.L.: and Carreira. L.H. (1995): Phytoremediation of contaminated soils and sediments. Environ. Sci. Tech. **29**. 318-323

Skousen. J.G.: Call. C.A.: Knight. R.W. (1990): Natural revegetation of an unreclaimed lignite surface mine in east-central Texas. Southwest. Nat. **35**. 434-440

Skousen. J.G.: Johnson. C.D.: Garbutt. K. (1995): Natural revegetation of 15 abandoned mine land sites in West Virginia. J. Environ. Qual. **23**. 1224-1230

Tansley. A.G. (1935): The use and abuse of vegetational concepts and terms. Ecology **16**. 284-307

Tsuyuzaki. S. (1995): Vegetation recovery patterns in early volcanic succession. J. Plant Res. **108**. 241-248

USEPA. (1986a): Method 3540. Soxhlet Extraction. SW-846: Test Methods for Evaluating Solid Waste (Physical/Chemical Methods). United States Environmental Protection Agency: Office of Solid Waste. Washington. D.C.

USEPA. (1986b): Method 8100. Polynuclear Aromatic Hydrocarbons. SW-846: Test Methods for Evaluating Solid Waste (Physical/Chemical Methods). United States Environmental Protection Agency: Office of Solid Waste. Washington. D.C.

USEPA. (1998): Guidelines for Ecological Risk Assessment. Federal Registry **63**. 26845-26924

Wunderlin. R.P. (1997): "Moraceae (Link): Mulberry Family." In: Flora of North America Editorial Committee (Eds.). *Flora of North America North of Mexico*. Oxford University Press. New York; Oxford. pp. 388-399

Table 1. Comparison of soil characteristics at the three locations analyzed for vegetation cover.

	Location[a]			
	Rail and Road Right-Of-Ways	Former Sludge Basin		Non-Contaminated Site
	Depth (cm)			
Soil Characteristics	30	30	150	30
PH	7.6	6.9	9.3	7.9
Nitrogen (mg/Kg)[b]	758	1161	816	176
Phosphorus (mg/Kg)[c]	28	1150	1780	6
Potassium (mg/Kg)[c]	53	191	361	120
Texture %				
Sand	48.5	50.5	50.5	54.5
Silt	38.5	16.0	16.0	24.5
Clay	13.0	33.5	33.5	21.0

[a]Locations are marked on topographical map shown in Figure 1.
[b]Total
[c]Available

Table 2. Changes in the percent ground cover between 1958 and 1995 at three different locations in and adjacent to the Union Carbide chemical plant in Texas City, Texas as estimated from aerial photographs.

	Location[a]								
	Rail and Road Right-Of-Ways			Former Sludge Basin			Non-Contaminated Site		
Year	Bare	Grass/ Forb	Trees/ Shrub	Bare	Grass/ Forb	Trees/ Shrub	Bare	Grass/ Forb	Trees/ Shrub
1958	5%	50%	45%	100%	0%	0%	10%	80%	10%
1967	5%	45%	50%	100%	0%	0%	100%	0%	0%
1970	n/a[b]			100%	0%	0%	n/a		
1973	n/a			100%	0%	0%	n/a		
1975	n/a			100%	0%	0%	n/a		
1977	5%	40%	55%	100%	0%	0%	100%	0%	0%
1978	n/a			100%	0%	0%	n/a		
1980	5%	35%	60%	90%	10%	0%	100%	0%	0%
1982	n/a			50%	50%	0%	n/a		
1985	n/a			35%	60%	5%	n/a		
1987	n/a			25%	65%	10%	n/a		
1989	5%	30%	65%	5%	75%	20%	30%	45%	25%
1990	n/a			5%	70%	25%	n/a		
1993	n/a			5%	65%	30%	n/a		
1995	5%	25%	70%	5%	55%	40%	5%	60%	35%

[a]Locations are marked on topographical map shown in Figure 1.
[b]Aerial photograph for the specific site was not available.

Table 3. The percent ground covered by different plant species at three locations in and adjacent to the Union Carbide chemical plant in 1997.

Species[a]	Family	Location[b]: Rail and Road Right-Of-Ways	Former Sludge Basin	Non-Contaminated Site
Trees/Shrubs				
Baccharis halimifolia[c] (Eastern Baccharis)	Asteraceae	25.2%	1.9%	24.1%
Celtis laevigata[c] (Sugarberry)	Ulmaceae	7.4%	2.8%	0.0%
Morus spp.[c] (Mulberry)	Moraceae	5.1%	36.2%	0.1%
Salix nigra[c] (Black Willow)	Salicaceae	2.3%	0.0%	2.3%
Sambucus canadensis[c] (Elder-Berry)	Caprifoliaceae	2.1%	0.3%	0.2%
Sapium sebiferum[c] (Chinese Tallow)	Euphorbiaceae	26.5%	0.0%	2.7%
Sesbania drummondii[c] (Rattlebush)	Fabaceae	0.0%	0.0%	1.3%
Tamarix parviflora[c] (Salt Cedar)	Tamaricaceae	0.0%	0.0%	1.2%
Trees/Shrubs Total		68.6%	41.2%	31.9%
Forbs				
Ambrosia trifida[d] (Giant Ragweed)	Asteraceae	4.7%	1.1%	0.8%
Aster subulatus[d] (Saltmarsh Aster)	Asteraceae	1.7%	0.3%	3.7%
Conyza canadensis[d] (Horseweed)	Asteraceae	2.4%	0.9%	3.5%
Eupatorium serotinum[c] (Boneset)	Asteraceae	0.0%	1.5%	0.0%
Helianthus annuus[d] (Common Sunflower)	Asteraceae	2.2%	9.4%	0.3%
Heterotheca subaxillaris[d] (Camphor Weed)	Asteraceae	1.1%	0.0%	8.3%
Pluchea purpurascens[d] (Canela)	Asteraceae	0.0%	1.9%	0.0%
Solidago spp.[c] (Goldenrod)	Asteraceae	3.5%	3.4%	15.4%
Typha latifolia[c] (Common Cattail)	Typhaceae	1.3%	0.1%	1.1%
Verbena litoralis[c] (Seashore Vervain)	Verbenaceae	0.0%	3.5%	0.2%
Forbs Total		16.9%	22.1%	33.3%
Graminoids				
Andropogon glomeratus[c] (Bushy Bluestem)	Poaceae	0.2%	0.6%	3.7%
Andropogon saccharoides[c] (Silver Bluestem)	Poaceae	0.1%	0.1%	10.2%
Chloris canterai[c] (Fingergrass)	Poaceae	1.8%	0.1%	13.5%
Cynodon dactylon[c] (Bermuda Grass)	Poaceae	7.4%	18.9%	0.4%
Cyperus spp.[c] (Umbrella Sedge)	Cyperaceae	1.4%	3.9%	0.4%
Digitaria adscendens[d] (Southern Crabgrass)	Poaceae	0.0%	1.3%	0.0%
Paspalum urvillei[c] (Vasey Grass)	Poaceae	0.6%	2.5%	4.8%
Sorghum halepense[c] (Johnsongrass)	Poaceae	2.5%	0.0%	0.0%
Graminoids Total		14.0%	27.4%	33.0%

[a]Common name is shown in parenthesis.
[b]Locations are marked on topographical map shown in Figure 1.
[c]Perennial Vegetation
[d]Annual Vegetation

Table 4. Inventory of vegetation growing in the former sludge basin during 1996-1998.

Family	Species Name	Common Name
Amaranthaceae	*Amaranthus viridis*	Pigweed
Asteraceae	*Ambrosia trifida*	Giant Ragweed
	Aster praealtus	Willowleaf Aster
	Aster subulatus	Saltmarsh Aster
	Baccharis halimifolia	Eastern Baccharis
	Conyza canadensis	Horseweed
	Coreopsis tinctoria	Plains Coreopsis
	Eupatorium serotinum	Boneset
	Gnaphalium purpureum	Cudweed
	Helianthus annuus	Common Sunflower
	Lactuca serriola	Prickly Lettuce
	Pluchea purpurascens	Canela
	Pyrrhopappus multicaulis	False Dandelion
	Solidago altissima	Tall Goldenrod
	Solidago gigantea	Late Goldenrod
	Sonchus asper	Sow Thistle
Brassicaceae	*Lepidium virginicum*	Peppergrass
Campanulaceae	*Tridanis perfoliata*	Venus' Looking Glass
Caprifoliaceae	*Sambucus canadensis*	Elder-Berry
Chenopodiaceae	*Chenopodium ambrosioides*	Mexican Tea
Cucurbitaceae	*Cucumis melo* var. Dudaim	Muskmelon
Cyperaceae	*Cyperus* spp.	Umbrella Sedge
Euphorbiaceae	*Sapium sebiferum*	Chinese Tallow
Fabaceae	*Melilotus indicus*	Sour Clover
	Vicia augustifolia	Narrow-Leafed Vetch
Geraniaceae	*Geranium texanum*	Wild Geranium
Moraceae	*Morus* spp.	Mulberry
Phytolaccaceae	*Phytolacca americana*	Pokeweed
Poaceae	*Andropogon glomeratus*	Bushy Bluestem
	Andropogon saccharoides	Silver Bluestem
	Chloris canterai	Fingergrass
	Cynodon dactylon	Bermuda Grass
	Dactyloctenium aegyptium	Crowfoot
	Digitaria adscendens	Southern Crabgrass
	Echinochloa crusgalli	Barnyard Grass
	Echinochloa cruspavonis	Japanese Millet
	Elymus canadensis	Canada Wild-Rye
	Leptochloa filiformis	Sprangletop
	Paspalum urvillei	Vasey Grass
	Polypogon monspeliensis	Rabbitfoot Grass
	Setaria geniculata	Foxtail
Ranunculaceae	*Ranunculus sardous*	Buttercup
Rubiaceae	*Galium aparine*	Catchweed Bedstraw
Solanaceae	*Solanum americanum*	Black Nightshade
Typhaceae	*Typha latifolia*	Common Cattail
Ulmaceae	*Celtis laevigata*	Sugarberry
Urticaceae	*Boehmeria cylindrica*	False Nettle
Verbenaceae	*Lantana horrida*	Texas Lantana
	Verbena bipinnatifida	Common Vervain
	Verbena litoralis	Seashore Vervain
Vitaceae	*Ampelopsis arborea*	Pepper-Vine

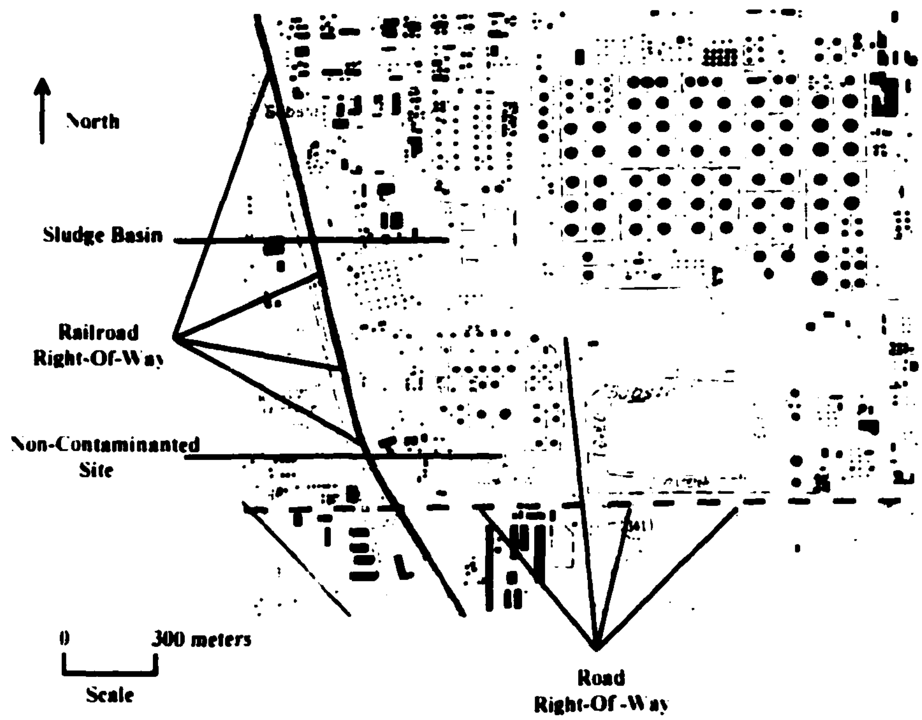

Figure 1. Topographic map showing the research location in Texas City, Texas. Vegetation was surveyed growing at four locations inside and adjacent to the chemical plant in 1997. USGS Virginia Point Quadrangle (Texas-Galveston CO.) 7.5 Minute Series.

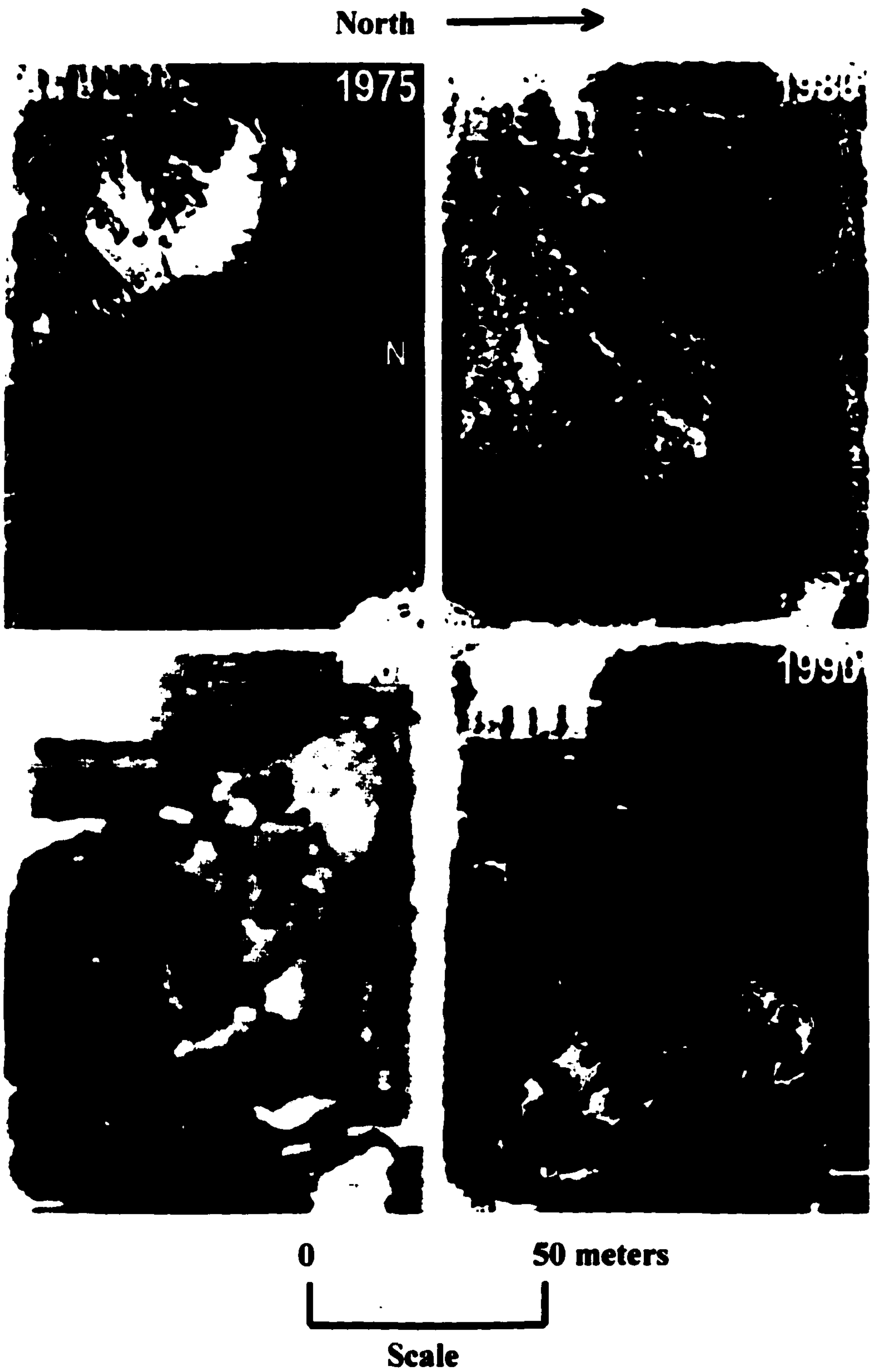

Figure 2. Representative aerial photographs showing vegetation changes at the sludge basin between 1975 and 1990.

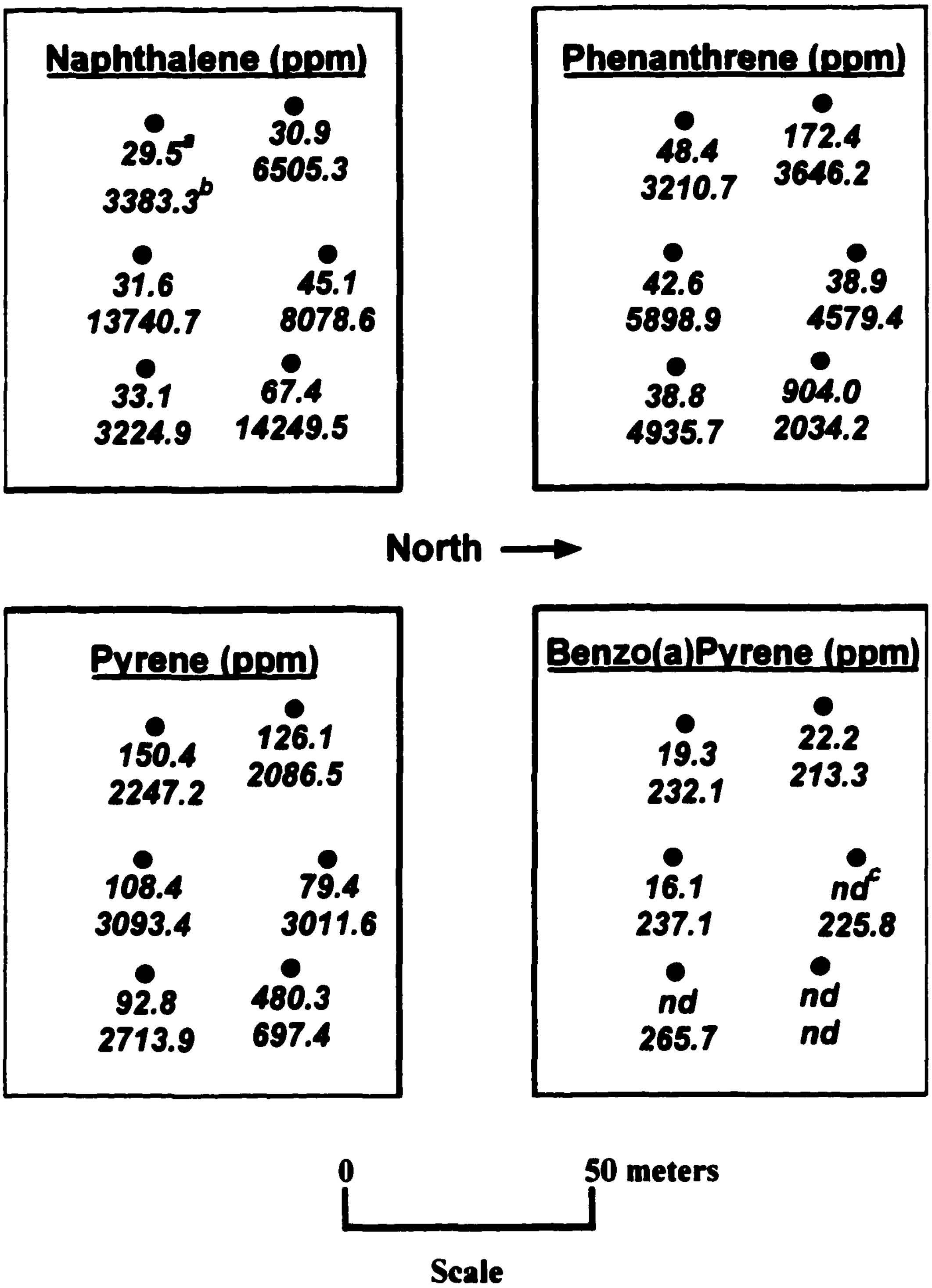

Figure 3. Selected PAH concentrations (ppm) from two depths across the sludge basin in 1998.

[a]0-30 cm

[a]Non-rooted parent sludge

[a]nd = non-detect (detection limit < 2 ppm)

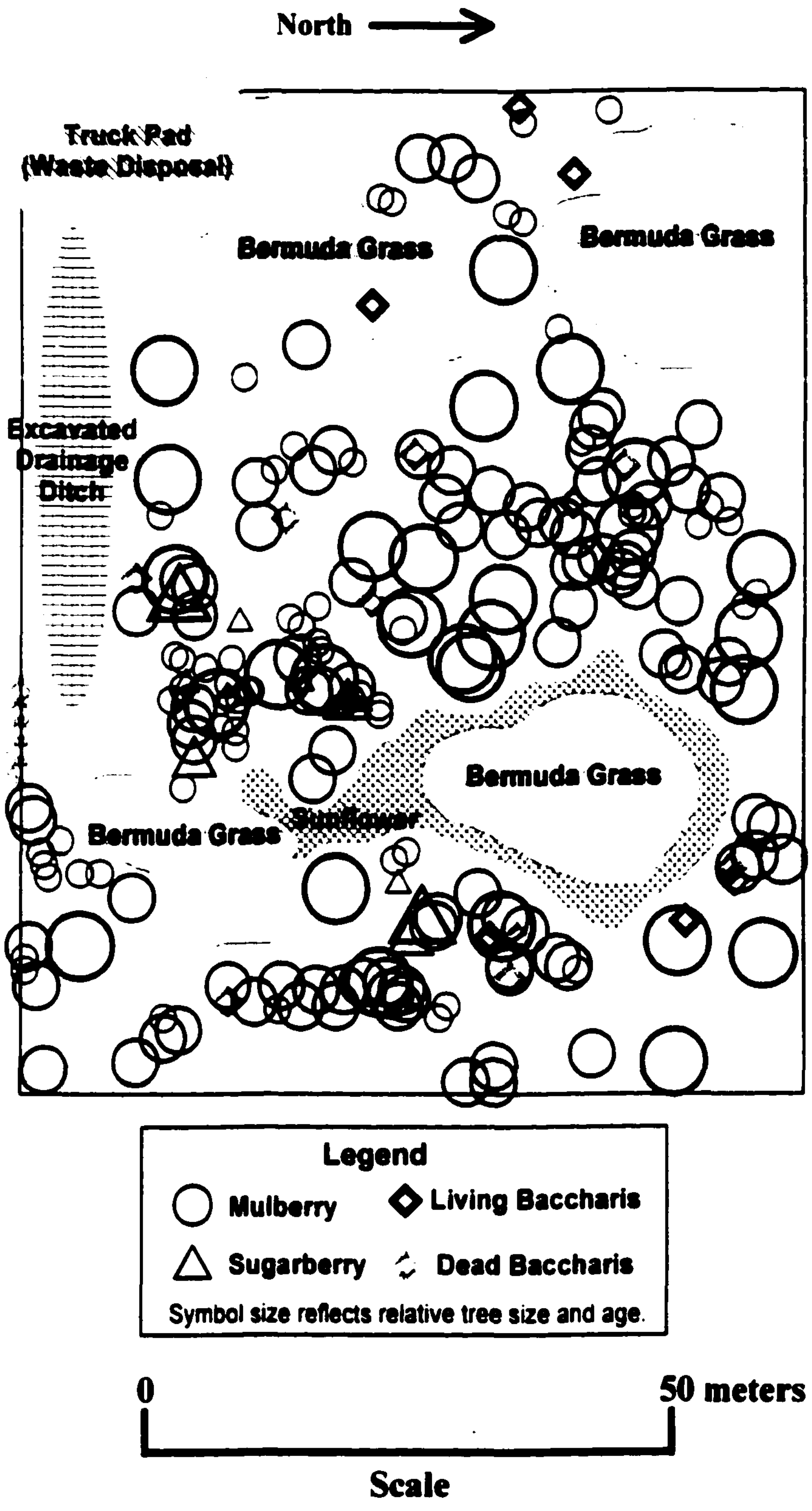

Figure 4. Distribution of dominant vegetation and relative size of tree species in sludge basin in 1998.

Chapter 2.

NATURAL ATTENUATION/PHYTOREMEDIATION IN THE VADOSE ZONE OF A FORMER INDUSTRIAL SLUDGE BASIN

ABSTRACT

The natural attenuation of organic pollutants in the vadose zone of a former industrial sludge basin was assessed in reference to naturally-occurring vegetation that has been growing at the site over the last 16 years. Prior to the invasion and colonization by vegetation at this site, the concentrations of polycyclic aromatic hydrocarbons (PAHs) in the basin were presumably similar to the concentration currently present in the non-rooted parent sludge (> 16,000 ppm). Examination of this basin showed that the PAH concentrations decreased significantly within the root zone of tolerant vegetation growing at the site compared to the non-rooted sludge. The upper rooted sludge (0-60 cm in depth) averaged less than 25 % of the original concentrations of PAHs found at this site. The average rooting depth of vegetation growing in the sludge basin was greater for trees (90 cm) compared to forbs (60 cm) and grasses (50 cm). At the bottom of the root zone of these tolerant plant species, the PAH concentrations averaged approximately half of the concentration of the non-rooted sludge. The natural attenuation of the vadose zone at this sludge basin demonstrates that over time the combined influence of dynamic plant growth and changes in the community of root-associated microorganisms are

instrumental in improving contaminated ecosystems. while providing direction for the development of a gradual. sustained phytoremediation technology for polluted terrestrial sites.

INTRODUCTION

It has become well established that contaminated groundwater environments often undergo natural attenuation (also known as intrinsic bioremediation and natural restoration) whereby the concentration and toxicity of pollutants decrease over time (Wiedemeier. et. al.. 1995; Wiedemeier. et. al.. 1998). Reduction of the presence and influence of toxicants through natural attenuation is attributed to the *in situ* action of a combination of physical and biological processes including dilution. dispersion. sorption. volatilization. and biodegradation. To date. natural attenuation has been well described and documented in numerous lotic systems with contaminated groundwater plumes (Leeson and Alleman. 1999). but comparable. detailed description and analysis of natural attenuation and associated processes in the vadose zone of polluted terrestrial sites has not been documented even though these sites are biologically active as evidenced by the natural invasion and robust growth of plants at hazardous waste sites.

A recent. forensic examination of vegetation at a former sludge basin established that ecological recovery of the plant community at the site (over a 16 year-period) had undergone the same sequence of ecological events that are characteristic of vegetation recovery in other stressed habitats (Olson and Fletcher. 1999). Through natural seed dispersal. germination. and seedling establishment. pioneer species successfully invaded and grew in the former disposal basin within 2 years after the drainage of surface waters that had accumulated as a result of precipitation. Although some of the original plants (i.e. 16-year-old mulberry trees) are still present at the site, other plant species (i.e. eastern baccharis) have been replaced as a result of plant succession. In 1998, the basin supported the robust growth of a diverse community of 51 species from 22 different plant families, evidence that the above ground portion of this polluted ecosystem was undergoing ecological recovery (Olson and Fletcher, 1999).

Since plant ecologists have recognized over the last century that pioneer species and early members of a newly established, changing plant community are instrumental in modifying the soil conditions, chemistry, and microclimate of a recovering soil ecosystem (Tansley, 1935), it is reasonable to propose that volunteer plants growing in contaminated soil at hazardous waste sites foster the natural attenuation of soil pollutants. This hypothesis was examined in the present study by quantifying both the lateral and horizontal distribution of polycyclic aromatic hydrocarbons (PAHs) in a revegetated former industrial sludge basin with reference to the distribution of plant species and depth of plant roots.

METHODS

Assessment of Natural Attenuation

To establish the occurrence of natural attenuation, it would be desirable to compare the level of present contaminants to levels determined at several earlier sample times distributed over 20 years with cross reference to major physical and biological events (i.e. surface water drainage, plant invasion etc.). Unfortunately, at older waste-disposal areas, there is a general lack of quantitative records with regard to the makeup of disposed wastes and the resulting chemical contents of sludge basins during their operation or upon closure. Therefore, neither baseline information regarding the contaminant levels prior to plant invasion nor periodic sample data thereafter are available for the sludge basin examined in this study or for sludge basins in general. In the absence of these data, an alternative method was used in this study.

Natural attenuation in the vadose zone at this site was judged by comparing the concentration of contaminants in the parent sludge collected below the root zone of plants (~160 cm deep) with contaminants present at 3 shallower depths (0-30 cm, 30-60 cm, and the bottom of the root zone). The depth of the bottom of the root zone ranged from 30 to 120 cm depending on the plant species, plant age, and location in the basin. The rationale for using this calculation as an estimate of attenuation is the assumption that the chemical makeup of the original waste disposed of throughout the basin (both vertically and horizontally) during its active use prior to 1980 would have most closely resembled that of sludge collected beneath the root zone in this study, since it is generally accepted that the physical and biological conditions favoring degradation of organic chemicals decreases with increasing soil depth. Thus, examination of the deeper sludge in 1998 provides a reference and/or internal standard for evaluating natural attenuation events that have transpired in the upper strata over the last 16 years since the basin was drained of surface waters. In this study, the material collected below the rooting zone will be referred to as "parent sludge".

In 1982, when the lagoon was drained of surface waters, it is believed that the contaminants were randomly distributed in the basin; whereby, in general, the kinds and variable amounts of contaminants were relatively uniform in concentration in the top and bottom layers of the lagoon. This contention is consistent with the method of disposal used to fill this basin prior to 1980. Individual truck loads of variable amounts of contaminants were discharged into the basin, a practice conducive to the random distribution of high and low concentrations of chemicals at all depths within the basin. Of equal importance is the knowledge that examination of aerial photographs taken from 1960 to 1989 have shown that no human activities interfered or altered the natural events that have transpired at this former sludge lagoon since its drainage of surface waters in the early 1980's (Olson and Fletcher, 1999). In this investigation, the importance of biological processes in natural attenuation focused on PAH analysis since their physical properties are, in general, not conducive to either leaching and/or volatilization (MacKay, et. al., 1992).

Site Description

The research was conducted at a former waste disposal basin (0.45 ha) in the center of an organic chemical manufacturing plant in S.E. Texas. This basin was used previously to examine plant invasion and succession (Olson and Fletcher, 1999). From the 1940s to early 1980s, wastewater containing a mixture of organic contaminants (PAHs and benzene-derivatives) and sediments (sand, silt, and clay) from primary clarifier river water was discharged into the basin from trucks. The resulting sludge accumulated in this basin to an average depth of 4 m.

In the early 1980s, routine pumping was initiated to keep the basin drained of surface waters, and subsequently was naturally colonized by vegetation tolerant of the site conditions (Olson and Fletcher, 1999). The site is currently revegetated (Figure 1) with a healthy, diverse community of plants (51 species and 22 families) and is dominated in terms of relative abundance and coverage by mulberry trees (*Morus* spp.), Bermuda grass (*Cynodon dactylon*), and common sunflowers (*Helianthus annuus*).

Coring and Root Measurements

Sediment/sludge samples were taken from 60 locations in the basin (Figure 1). The locations of the individual coring-sites were selected to provide samples from all regions of the basin in order to evaluate the influence of both plant species and age on industrial sludge. A post-hole digger was used to remove material from the upper 60 cm of the basin where roots were plentiful. Deeper material was removed from beneath the post-holes with a coring device made from 5 cm diameter PVC pipe. The length of intact cores removed with the PVC pipe averaged 2 m. At each sampling location in the basin, 500g composite samples were taken from the 0-30 cm layer, 30-60 cm layer, bottom of root zone, and non-rooted parent sludge (Figure 2). The samples were placed into amber jars with Teflon-lined caps, and transported to the University of Oklahoma for PAH analysis. The rooting patterns and depth of dominant vegetation growing at the site were determined by visually examining all cored material for roots, along with the roots of 8 trees, grasses, and forbs each whose root systems were partially excavated by shovel.

Chemical Analysis

Organic compounds in the cored material were extracted (USEPA, 1986a) and subsequently analyzed for PAH concentrations (USEPA, 1986b). Ten g of cored material was placed in a cellulose thimble and Soxhlet extracted (1:1 hexane/acetone) for 24 hours. The resulting extract was condensed to 10 ml with a Kuderna-Danish apparatus. Samples were diluted with acetone when necessary and analyzed for the 16 EPA Priority Pollutant PAHs [naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, dibenzo(ah)anthracene, benzo(ghi)perylene] by gas chromatography using internal standardization [deuterated phenanthrene (Phe d10)] as a response factor and external standards to quantify concentrations.

A gas chromatograph (Varian® 3300 GC) equipped with a flame-ionizing detector (GC-FID) was used to determine PAH concentrations in extracted samples. A DB-5ms capillary column (30 m x 0.32 mm i.d.) at a 1.0 µm film thickness was used for separation and identification of individual PAHs. The programmable injector was cooled to an initial temperature of 40°C, followed by an immediate temperature increase of 180° C/min. to a final temperature of 310° C, and held at this temperature for 95 minutes. The initial oven temperature was 40°C and held for 1.5 minutes, followed by an increase in temperature of 4°C/min. to a final temperature of 310°C and held for 30 minutes at this final temperature. The detector temperature was maintained at a constant 310°C. Helium was used as the carrier gas and maintained for maximum separation at a flow-rate of 2.4 mL/min. Concentrations of PAHs were recorded as mg/Kg dry weight sludge or parts-per-million (ppm). The reproducibility of extraction and analytical methods were > 90% as demonstrated with duplicate analyses of selected samples.

Statistical Analysis

Summary statistics and differences among the individual 16 priority PAHs and total PAHs in relation to depth in the basin were performed using SAS System for Windows, release 6.12 (SAS Institute Inc., Cary, NC, USA). Spatial variability existed among PAHs in both a horizontal and vertical direction within the basin. Because of spatial variability, it is a common practice to transform environmental data prior to statistical analyses. As a result, the underlying data structure for both original PAH values and log-transformed data were examined for basic statistical assumptions and based on those assumptions the most appropriate analysis was performed.

In general, all PAH concentrations (ppm) were determined to be normally distributed with occasional outliers using the original, non-transformed values. In the upper rooted zone, PAHs were much more highly variable, however transformed data were ultimately not used nor were outliers removed. Analysis of residual errors indicated that the variance was not necessarily decreasing with an increasing sample size, suggesting a heterosedastic variance inherent in this data set. This variability reflects the random nature of PAHs discharged into the basin over time, and the uneven physical and biological weathering. As a result, a variance-weighted ANOVA (Welch's ANOVA) was employed and least significant differences (Fisher's LSDs) were preformed to determine statistical differences between group means of non-transformed data for individual PAHs and total PAHs as a function of depth into the basin.

The horizontal distribution of PAH contaminants by depth in the basin and relative rooting depth were visually depicted with isopleths. Horizontal spatial correlation and variogram modeling of total PAH depth, and rooting depth in the basin was determined using geostatistical software (GS+ Professional Version. 1.2a; Gamma Design. Inc. Plainwell. MI. USA). Best-fit analysis estimated an isotropic linear model with sill and nugget variance for both total PAH depth and maximum rooting depth at each core location. This information was processed with general kriging procedures to develop contour maps interpolating the horizontal distribution of total PAH concentrations and rooting depth in the basin using surface mapping software (Surfer. version 6.04; Golden Software. Inc.. Golden. CO. USA).

RESULTS

All but 3 of the 240 samples collected at 4 depths from 60 widely distributed locations across the basin (Figure 1) possessed PAH compounds. This three dimensional distribution of PAH contaminants throughout the basin provides convincing evidence that during its active use the basin was filled from bottom to top with industrial waste containing PAH contaminants and that no clean soil had been introduced to cover the waste or promote plant growth.

The average total concentration of PAHs in the parent sludge present beneath the root zone (as defined in the method section) was 16.854 ppm (Table 1). The highest total PAH concentration for a single sludge sample (61.218 ppm) came from a core removed in quadrant G-5 (Figure 2) and the lowest concentration (382 ppm) for parent sludge was from quadrant I-5. In addition to the wide range of total concentrations present in the sludge. there was also a substantial variation in the ratio of individual PAH compounds (Table 2). The high variability in both total amounts and ratios of individual PAH compounds present in the parent sludge beneath the root zone is consistent with the contention expressed in the methods section that contaminants were originally discharged in a heterogeneous fashion into the basin because of differences in individual truck loads of waste material disposed of in the basin.

Material removed from the bottom of the root zone (Figure 3) contained PAHs with a total average concentration of 9190 ppm (Table 1), which was only 54% of that present in the parent sludge (16,854 ppm). Although there was substantial variability in individual samples collected at this depth across the basin, the average value was statistically different from that of the parent sludge (Table 1). Similar analysis of data collected on individual PAHs also showed high sample variability but statistical differences were observed for the 15 detected PAHs in comparison to the parent sludge except for acenaphthene and chrysene. It is especially noteworthy that these statistical differences prevailed for physically inert compounds such as pyrene and benzo(a)pyrene that have water solubilities of 0.132 and 0.0038 g/L, log K_{ow} of 5.18 and 6.04, and Henry's Law constants of 0.92 and 0.046 respectively. The disappearance of these recalcitrant compounds over time suggests the involvement of biological processes since the prospect of these compounds leaving the basin via volatilization and/or leaching is minimal.

Comparison of PAH concentrations in the root zone showed that the total average concentration in the 0-30 cm stratum (1121 ppm) was 50% of that in the 30-60 cm stratum, but due to the contaminant variability between some samples the difference was not statistically significant (Table 1). Likewise the average concentration of all individual PAHs examined was lower in the 0-30 cm stratum than in the 30-60 cm stratum, but none of the values were statistically different from each other. However, there was a statistical difference between the concentration of PAHs in both the 0-30 and 30-60 cm strata in comparison to PAH concentrations in material collected from the bottom of the root zone and also the parent sludge from beneath the root zone (Table 1). These statistical differences prevailed for all individual PAHs examined except for indeno(1,2,3-*cd*)pyrene, dibenzo(*ah*)anthracene, and benzo(*ghi*)perylene. Thus, there was a statistically significant decrease in the average and individual concentration of PAHs within the basin in the vertical direction starting with the highest in the non-rooted parent sludge, 50% less at the bottom of the root zone and 90% less in the rooted zones.

The generality of the vertical distribution of PAHs across the basin was examined by considering the horizontal distribution of contaminants with reference to plant distribution, plant age, and root depth. Through the use of the kriging method, PAH concentration data collected at each coring-site was used to prepare contour maps estimating the horizontal concentration of PAHs in four strata across the basin corresponding to the four depths reported in Table 1. Examination of the parent sludge map (Figure 2) shows that there was a higher concentration of PAHs in the portion of the basin lying southeast of a diagonal line drawn from H-1 to A-5. Two locations (Figure 2, quadrants C-7 and G-5) had concentrations in excess of 35,000 ppm, twice the average concentration for parent sludge in the basin (Table 1).

The contour map of PAH concentrations at the bottom of the root zone showed that the majority of the basin had a reduced level of PAHs (<5,000 ppm) in comparison to the parent sludge stratum where approximately half the basin had PAH levels in excess of 15,000 ppm. The two quadrants noted earlier to have PAHs in excess of 35,000 ppm in the non-rooted parent sludge also had elevated levels in the stratum at the bottom of the root zone. In one quadrant (D-6), the PAH concentration at the bottom of the root zone was higher than that observed in the parent sludge beneath the root zone.

Examination of the contour maps for the two upper strata showed that both of these strata had PAH concentrations of <2000 ppm for most of the basin. The higher average PAH concentration reported for the 30 to 60 cm depth in comparison to the 0 to 30 cm depth (Table 1) was primarily due to higher levels of PAHs centered around quadrants B-7 and D-6 (Figure 2). The higher levels of contamination in the upper strata in these two quadrants appear to be at the top of columns of contamination of increasing concentration that extend to the parent sludge. The vertical distributions of PAHs in these quadrants are of special interest with regard to plant distribution and rooting depths (as discussed later).

The plants now growing in the basin are a result of natural plant invasion and succession previously documented through the analysis of aerial photographs taken between 1960 and 1989 (Olson and Fletcher, 1999). Within two years after the basin had been drained of surface waters, >75% of the area was covered by rapidly growing grasses and forbs. Over time, the majority of the original pioneer herbaceous vegetation was replaced by trees. At present, two dominant plant species (mulberry trees and Bermuda grass) are distributed in large patches of relatively pure stands of either trees or grass (Figure 1). Although phytotoxic compounds present in the upper strata of the basin in 1982 may have influenced early plant invasion and succession, this is believed to have been a temporary condition (Olson and Fletcher, 1999), and the slow invasion of the remaining grass areas by mulberry and other tree species does not appear to be due to the distribution of phytotoxins in the basin. This position is supported by the fact that mulberry trees have been growing at locations in the basin (Figure 2, quadrant D-6) where the level of contaminants are several fold greater than those present in grass areas (Figure 2, quadrants G-6 and H-3). A more likely explanation for the lack of trees in some portions of the basin is that seasonal flooding kills tree saplings that invade the low areas of the basin and thus grasses remain the dominant form of vegetation. However, this is changing since Chinese tallow trees, a flood tolerant species, have successfully invaded and are growing since 1998 in some grass areas.

The depth of roots in the basin varied and appeared to be a function of both the type of vegetation and the age of plants. The maximum depth of roots associated with trees, forbs, and grasses were 120, 90 and 75 cm, respectively (Figure 3). The deepest roots observed in the basin (> 1m) were associated with one of the oldest mulberry trees (14 yr-old) growing in quadrant F-4 (Figure 2). Younger mulberry trees (5-12 yr old) growing in both heavily and lightly contaminated regions had roots reaching average depths of 90 to 100 cm. Thus, the longer a tree had been growing in the basin the deeper the root had penetrated into the sludge. At several locations (i.e. Figure 2, quadrant D-6) extensive networks of fine roots were observed to have grown into the black sludge material that, upon chemical analysis, proved to be heavily contaminated. Such penetration and robust growth of grass and forb roots into the black sludge material was not observed. These visual observations suggest that mulberry roots are physiologically better suited to penetration into contaminated sludge than Bermuda grass and forbs.

DISCUSSION

When the basin was drained of surface waters in 1982, it is reasonable to assume that the high level of PAHs (averaging 16,854 ppm) now present in the parent sludge was also present in the upper strata of the basin. This contention is supported by the fact that the basin was 1) filled with waste arising from the same continuous chemical manufacturing processes, 2) waste was disposed of in the same manner throughout the period of active basin use, 3) no human effort was made to remediate the site when the use of the basin stopped, and 4) no contaminant-free cover material has been placed on top of the waste in this basin (Olson and Fletcher, 1999). Thus, natural attenuation events appear to have been responsible for the statistically significant difference and decline of PAH compounds in the upper 60 cm of material in the basin, and has led to a 90% reduction in the PAH contaminants that were originally present in the upper strata and still remain in the parent sludge beneath the root zone.

The natural attenuation that has occurred in the upper strata of this basin is believed to have been brought about through the integrated action of numerous abiotic and biotic processes (factors) operating in a dynamic, evolving fashion over the last 16 years in the basin. Perhaps the two most important abiotic factors considered at hazardous waste sites are solubilization and volatilization, both processes that would have facilitated the movement of contaminants within the matrix of the site. Mobility of contaminants can hasten chemical alteration by allowing a contaminant to move to a chemically active zone in a matrix where enzymatic or nonenzymatic reactions result in either mineralization or molecular alteration with the latter often leading to sorption to biogenically produced organic matter. For example, naphthalene located at the top of the water saturated zone of the basin could. through volatilization. enter the aerobic vadose zone where aerobic degradation can proceed at a rate 50-fold faster than anaerobic rates (MacKay. et. al.. 1992). In the event that chemically active zones do not intercept mobile contaminants. these contaminants may escape to the atmosphere or the groundwater. It is unclear what proportion of the contaminants originally present in the upper 60 cm of this basin disappeared via chemical degradation versus escape. However. data collected in this study suggest that degradation was the more influential event since mobile compounds (i.e. acenaphthylene) and immobile compounds (i.e. pyrene and benzo(a)pyrene) all showed similar percentages of disappearance (85 to 88%) when contaminant concentrations in the 0-30 cm stratum are compared with those of the parent sludge (Table 1). Thus. biodegradation appears to be an important process in the natural attenuation of the vadose zone as previously recognized in the natural attenuation of groundwater systems (Wiedemeier. et. al.. 1995; Wiedemeier. et. al.. 1998). However. in groundwater systems. contaminant degradation is considered to be the sole product of microbial processes; whereas. we consider the natural attenuation of the vadose zone described in this study to be the product of the combined influence of plants and microbes in a recovering ecosystem with plants. the primary producer at the heart of the recovery.

A forensic study conducted at this basin has shown that large portions of the basin were invaded and revegetated by grasses and forbs within two years following drainage of surface waters in the basin (Olson and Fletcher. 1999). Many of the pioneer species have now been largely replaced through natural succession by trees with mulberry being the dominant plant although there are a total of 50 other plant species now growing in the basin. During 16 years of plant invasion and succession, an ever increasing proportion of the basin's matrix has been penetrated by roots of a changing community of plants and by microbes of which many have probably never been cultivated in the laboratory since it is estimated that less than 1 % of microbes found in the environment are culturable (Amann, et. al., 1995). The annual expansion of this increasing network of roots along with the turnover of the fine root component serve to provide organic substrates to support microbial growth, and facilitate air diffusion from the atmosphere via soil channels created when fine roots die. These contentions are supported by laboratory investigations completed with mulberry, where it has been shown that 58% of the fine roots turnover annually and contain several flavone compounds, including morusin which has been shown to support the growth of a PAH degrading bacteria isolated from this basin (Leigh, et. al., 1999). Since chemical synthesis and root turnover are a universal feature of plant roots (Curl and Truelove, 1986) it is reasonable to assume that roots of other plant species growing in the basin have also contributed to the natural attenuation of the basin, but additional work is necessary to determine the contribution made by different plant species.

Data collected at this former sludge basin indicate that natural attenuation of the vadose zone has occurred over the last 16 years through the action of a dynamic community of plants and associated root microorganisms. In arriving at this conclusion, it was necessary to make numerous assumptions and as a result it is imperative that controlled follow up investigations be conducted to confirm the interpretation of these data. Future investigations should not only serve to understand natural attenuation, but provide valuable information to develop integrated plant-microbe systems and effective management practices that will accelerate natural processes. The ultimate goal is development of ecologically sound, sustained phytoremediation technology, that once placed in motion leads to an ever-improving ecosystem. With this goal in mind, future studies must be long-term field investigations since natural processes are relatively slow and involve extremely complex communities of plant and microbial organisms that may be impossible to duplicate under laboratory conditions.

References

Amann. R. I.. W. Ludwig. and K. H. Schleifer. 1995. Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. *Microbial Reviews*. 59:143-169.

Curl. E. A.. and B. Truelove. 1986. The Rhizosphere. Springer-Verlag. New York. 288 pgs.

Leeson. A.. and B. C. Alleman. 1999. Natural Attenuation of Chlorinated Solvents. Petroleum Hydrocarbons. and Other Organic Compounds. The Fifth International In Situ and On-Site Bioremediation Symposium. San Diego. CA. April 19-22. 1999. 5(1) ISBN 1-57477-074-8

Leigh. M. B.. J. S. Fletcher. X. Fu. and F. Schmitz. 1999. Influence of season. age and senescence of the phenolic content of mulberry fine roots with reference to phytoremediation. *Plant and Soil*. (In Press).

Mackay. D.. W. Y. Shiu. and K. C. Ma. 1992. Illustrated Handbook of Physical-Chemical Properties and Environmental Fate for Organic Chemicals. Volume II: Polynuclear Aromatic Hydrocarbons. Polychlorinated Dioxins. and Dibenzofurans. Lewis Publishers. Chelsea. Michigan. 597 pgs.

Olson. P. E.. and J. S. Fletcher. 1999. Ecological recovery of vegetation at a former industrial sludge basin and its implications to phytoremediation and ecological risk assessment. *Environmental Science and Pollution Research*. (in review).

Tansley. A. G. 1935. The use and abuse of vegetational concepts and terms. *Ecology*. 16:284-307.

USEPA. (1986a): Method 3540. Soxhlet Extraction. SW-846: Test Methods for Evaluating Solid Waste (Physical/Chemical Methods). United States Environmental Protection Agency: Office of Solid Waste. Washington. D.C.

USEPA. (1986b): Method 8100. Polynuclear Aromatic Hydrocarbons. SW-846: Test Methods for Evaluating Solid Waste (Physical/Chemical Methods). United States Environmental Protection Agency: Office of Solid Waste. Washington. D.C.

Wiedemeier. T. H.. J. T. Wilson. D. H. Kampbell. R. N. Miller. and J. E. Hansen. 1995. Technical protocol for implementing intrinsic remediation with long-term monitoring for natural attenuation of fuel contamination dissolved in groundwater. U.S. Air Force Center for Environmental Excellence. San Antonio. TX.

Wiedemeier. T. H.. M. A. Swanson. D. E. Moutoux. E. K. Gordon. J. T. Wilson. B. H. Wilson. D. H. Kampbell. P. E. Haas. R. N. Miller. and J. E. Hansen. and F. H. Chapelle. 1998. Technical protocol for evaluating natural attenuation of chlorinated solvents in ground water. U.S. Environmental Protection Agency. Office of Research and Development. Washington. D.C.

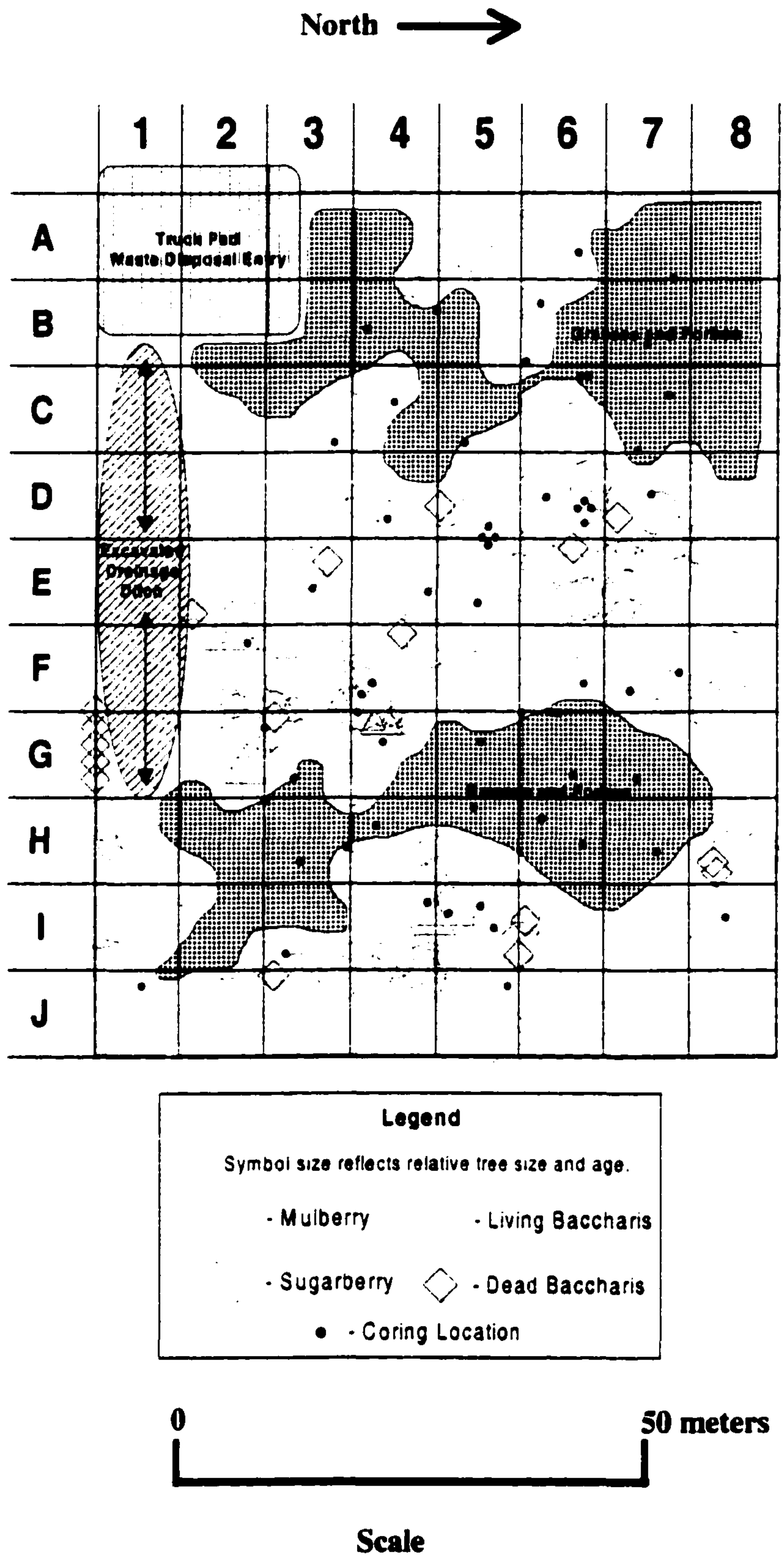

Figure 1. Distribution of dominant vegetation and relative size and age of tree species growing in the former sludge basin in 1998.

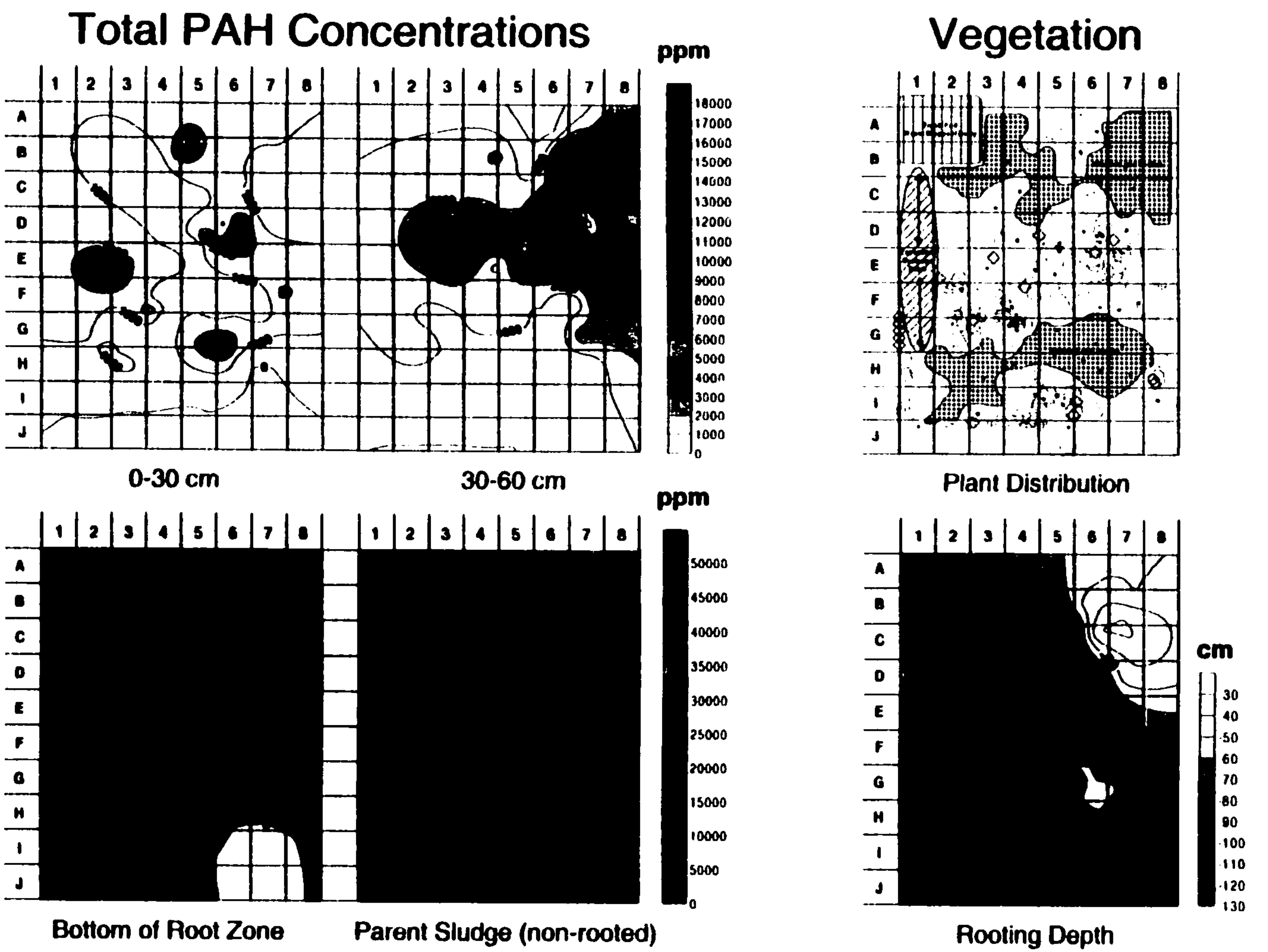

Figure 2. Horizontal distribution of PAH contaminants with reference to plant distribution and root depth in 1998.

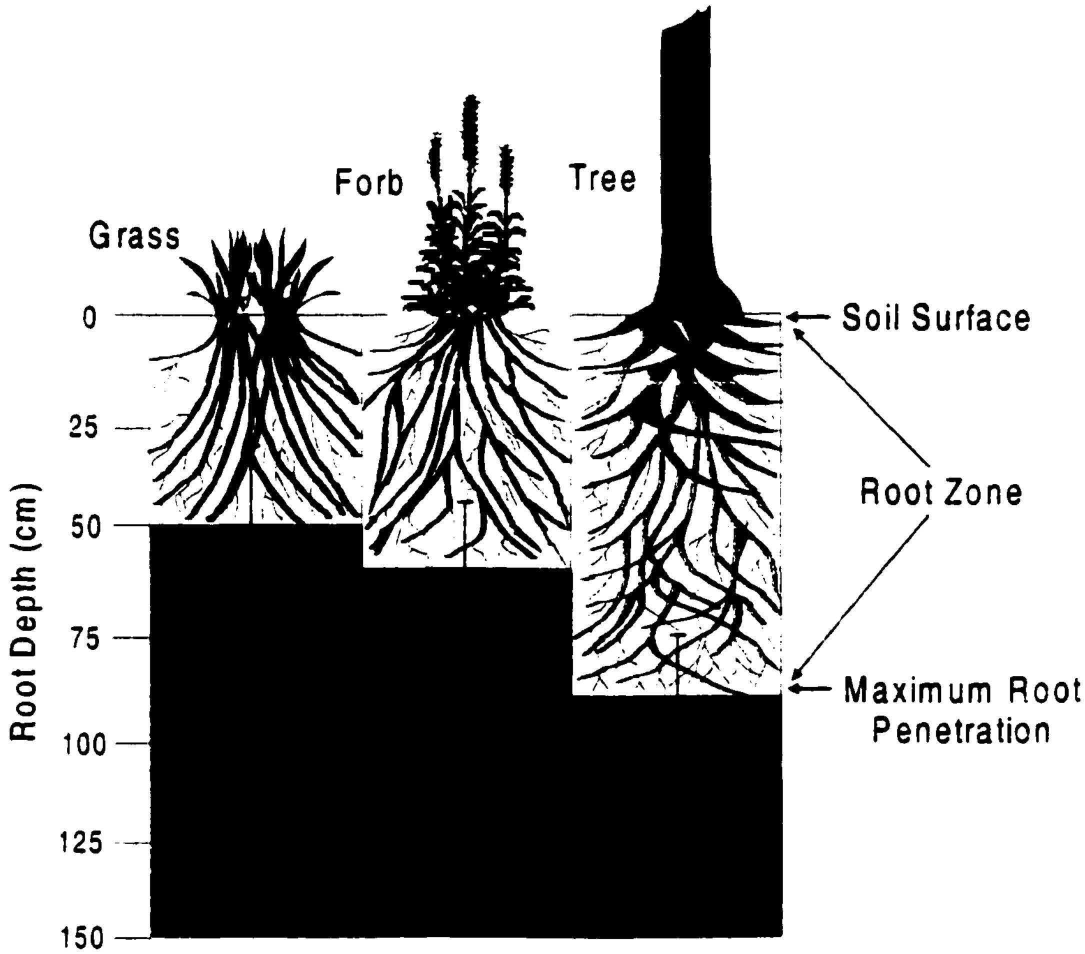

Figure 3. Average root depth of different types of vegetation growing in the sludge basin in 1998.

Table 1. The concentration and vertical distribution of sixteen polycyclic aromatic hydrocarbons (PAHs) in a re-vegetated former sludge basin.

	Vertical Section Analyzed For PAH Content											
	Root Zone: 0-30 cm			Root Zone: 30-60 cm			[a]Bottom of Root Zone			[b]Parent Sludge		
	[c]*Average*	[d]*C.V.*	[e]*M.C.*	*Average*	*C. V.*	*M.C.*	*Average*	*C. V.*	*M.C.*	*Average*	*C. V.*	*M.C.*
Naphthalene	63	229	A	668	259	A	2988	130	B	6098	81	C
Acenaphthylene	197	84	A	269	94	A	779	96	B	1523	75	C
Acenaphthene	34	121	A	176	452	A	424	120	B	558	88	B
Fluorene	83	103	A	160	127	A	714	99	B	1242	76	C
Phenanthrene	239	113	A	440	133	A	1731	109	B	3102	72	C
Anthracene	66	93	A	126	131	A	462	94	B	717	65	C
Fluoranthene	96	111	A	157	128	A	429	111	B	791	75	C
Pyrene	201	108	A	349	145	A	902	111	B	1658	75	C
Benzo(*a*)Anthracene	51	135	A	154	187	A	295	126	B	548	84	C
Chrysene	37	159	A	68	147	A	308	253	B	282	106	B
Benzo(*b*)Fluoranthene	13	183	A	21	192	A	35	221	B	63	112	C
Benzo(*k*)Fluoranthene	[f]n.d.	n.d.	A	n.d.	n.d.	A	n.d.	n.d.	A	n.d.	n.d.	A
Benzo(*a*)Pyrene	21	170	A	34	169	A	70	166	B	135	102	C
Indeno(1,2,3-*cd*)Pyrene	4	365	A	6	301	A	8	331	A	12	247	A
Dibenzo(*ah*)Anthracene	n.d.	n.d.	A	n.d.	n.d.	A	n.d.	n.d.	A	4	497	B
Benzo(*ghi*)Perylene	16	345	A	26	285	A	45	237	A	121	130	B
Total PAH	1121	100	A	2654	156	A	9190	95	B	16854	68	C

[a]Deepest penetration of roots into basin matrix, varied from 30-120 cm in depth (see Figure 3).
[b]Non-rooted sludge material removed below the bottom of the root zone.
[c]Average: Arithmetic average of PAHs (mg / Kg of dry soil or ppm).
[d]C.V., Coefficient of Variation
[e]M.C., Multiple comparisons among PAH means by sample depth. Means within rows having same letter are not significantly different from each other (LSD, p = 0.05).
[f]n.d. Non-detect (< 2 ppm)

Table 2. Concentration ratios for the five most prominent PAH compounds in the non-rooted parent sludge at eight different locations in the basin in 1998.

	Location in Basin							
	B-5[a]	B-7	D-4	D-6	F-3	F-8	H-3	H-6
Naphthalene	2[b]	6	3	6	2	7	8	14
Acenaphthylene	1	4	1	1	1	2	2	2
Fluorene	1	3	1	1	1	2	1	1
Phenanthrene	3	8	3	4	3	5	3	2
Pyrene	1	4	2	2	1	4	2	1

[a]Quadrant on vegetation map shown in Figure 2.
[b]Concentrations rounded to the nearest 1000 ppm.

Chapter 3.

FIELD EVALUATION OF MULBERRY ROOT STRUCTURE WITH REGARD TO PHYTOREMEDIATION

Abstract

This study examined the root structure of a mature 12 year-old mulberry tree growing in a former waste disposal basin, and the relationship of the tree's root system to the distribution of polyaromatic hydrocarbons (PAHs) in soil/sludge beneath the tree. Root excavation showed that large roots were restricted to the upper 60 cm of the root zone, whereas medium-sized (0.3 - 2.5 cm) and fine roots (<0.3 cm) were present throughout the root zone and reached depths up to 110 cm. Comparison of the concentrations of PAHs in the root zone with that present in sludge beneath the root zone (>2.0 m) showed dramatic differences. In the upper 60 cm of the root zone, where roots have prevailed for potentially 12 years, the PAH concentration was approximately 10-20 % of the sludge value. At lower rooting depths 60-100 cm, where fewer roots have been present for a shorter time period, the PAH concentration has decreased but to a lesser degree. These field results provide evidence that the mulberry root system gradually develops over time in a vertical direction with fine roots serving as the leading edge of a moving front. The degradative properties of this biological front (root-microbe combination) towards toxic chemicals holds great importance in successful development of phytoremediation technology designed to degrade deep contaminants (i.e. in former sludge basins).

Introduction

An emerging technology known as phytoremediation is the use of selected vegetation and associated root-microorganisms to immobilize and/or enhance the biodegradation of organic pollutants (Shimp, et al., 1993). Plants have the ability to take up water-soluble chemicals from the soil and either accumulate and/or metabolize them (McFarlane, et al., 1987; Fletcher, et al., 1990; McFarlane, et al., 1990). Plant roots may also foster the degradation of organic pollutants by enhancing the growth of desirable microorganisms in the soil adjacent to the root (rhizosphere). This enhancement may be brought about by the release of certain plant compounds that foster the growth of microorganisms capable of biodegrading environmental pollutants (Fletcher and Hegde, 1995; Donnelly, et al., 1994), and by soil aeration attributed to the air channels provided when roots turn over (repeated growth and death). Thus through long-lived, perennial vegetation, phytoremediation has the potential to serve as a sustained, ecologically and economically-sound method to remediate contaminated soil.

Although there is good laboratory evidence that plants can be used effectively to remediate hazardous waste sites (Aprill and Sims, 1990; Ferro, et al., 1994; Anderson and Walton, 1995) the field studies have been limited to sites with relatively nontoxic agricultural pollutants (Nair, et al., 1993; Burken and Schnoor, 1997) or shallow industrial contaminants (Schwab, et al., 1995; Qiu, et al., 1997). As a result, the proposed use of deep-rooted plants (trees) to treat heavily contaminated, deeply deposited pollutants over extended periods of time (years) is virtually untested. This study capitalized on vegetation that invaded and grew for several years at a former sludge lagoon. heavily laden with polyaromatic hydrocarbons listed by EPA as priority pollutants (Keith and Telliard. 1979).

This communication is the first known effort to determine the structure and depth of mature tree roots growing in heavily contaminated industrial sludge. Observations regarding the apparent changes in the sludge associated with the tree roots provide useful information regarding future use of trees in the phytoremediation of former sludge lagoons.

Methods

The study site is a former waste basin (0.45 hectare) put into use in the 1940s at an industrial site in Texas. River water containing residual sediment (sand. silt. clay) was mixed with waste products from the chemical plant and subsequently disposed of in the basin. The settling of the river sediment and associated chemicals in the basin during its use resulted in an accumulation of sludge with a high level of polyaromatic hydrocarbons (PAHs) averaging 2% dry weight across the basin. The general makeup of material in the basin as determined from a 30 cm deep composite sample was 50% sand. 16% silt. and 34% clay. Soil fertility at the same depth was 1161 total nitrogen. 1150 available phosphate. and 191 available potassium mg/Kg. respectively. In addition. analysis of pH and ion exchange capacity yielded values of 6.9 and 1.3 mmhos/cm. respectively. The disposal of waste materials in the basin stopped in the early 1980s. and the basin was partially drained of water. Since then. the site has become revegetated through the natural invasion of plants growing from seeds dispersed into the basin from mature plants in the vicinity of the chemical plant. At present, the entire basin is covered by dense vegetation with approximately 40% of the site occupied by mulberry trees.

The root structure and depth of a mature, large, healthy mulberry tree growing in the interior of the basin was examined in this study (Fig. 1a). Taxonomic classification of this tree and other mulberry growing in the basin has been difficult. At present, mixed characters suggest that the tree is a hybrid of *Morus alba* and *Morus rubra*, a common occurrence of mulberry growing in the wild (Wunderlin, 1997). The height of the tree was approximately 6 m and its maximum canopy diameter was also approximately 6 m. The diameter of the trunk measured just beneath its point of branching (60 cm from the soil surface) (Fig. 1a) was 40 cm. The age of the tree was estimated to be 12 years old based on examination of trunk corings removed 60 cm from the base of the trunk.

A determination of PAH levels in soil/sludge material beneath the mulberry tree was conducted. Three 2.5 m deep, vertical soil/sludge cores were removed at 0, 1.5, and 3.0 meters along a transect starting at the base of the tree and extending to the perimeter of the canopy (Fig. 2). Recovered cores were divided into four sections according to distance from the soil surface and/or the presence of roots. The four segments analyzed represented the 0-30 cm section, 30-60 cm section, 100-110 cm section or the transition zone (region of deepest root penetration), and the sludge collected approximately 40 cm below the transition zone. The samples were placed in Teflon-capped amber jars and transported to the University of Oklahoma for analysis. Samples were Soxhlet extracted using a 1:1 acetone/hexane solvent system (USEPA, 1986a). The resulting extract was condensed and analyzed for the 16 Priority PAH pollutants using GC-FID with a capillary column (USEPA, 1986b).

Root excavations were conducted starting at the base of the trunk on the west side of the tree where large horizontal roots from the tree protruded above the soil surface (Fig. 2). Layers of soil were removed from a trench measuring 0.8 x1.5 m without disturbing the large roots (> 2.5 cm in diameter) until the trench had reached a depth of 0.8 m. After the roots in the top 0.8 m of soil/sludge had been photographed they were cut off and removed to facilitate excavation of the lower portion of the trench. The trench was dug to a depth of 1.2 m.

Results and Discussion

A visual examination of the mulberry tree growing approximately in the center of the sludge basin indicated that the tree was in good health by virtue of the high density and deep green color of its leaves and absence of dead branches. It was also observed that major roots extended horizontally in all directions from the base of the tree, a typical growth pattern for mulberry roots (Bunger and Thomson, 1938). Soil excavation on the west side of the tree (Fig. 2) uncovered approximately 20 different major roots ranging in size from 15 to 25 cm in diameter. Major roots were restricted to the upper 60 cm of the excavated trench (Fig. 2), and crisscrossed each other in a haphazard fashion (Fig. 1b). Both medium (0.3 - 2.5 cm) and fine (< 0.3 cm) roots were present throughout the root zone and reached depths up to 110 cm. The high concentration of large, major roots in the upper strata is consistent with the mulberry study of Bunger and Thomson (1938) where approximately 70% of the large roots were in the top 60 cm of soil. The absence of any large roots in deeper zones and the lack of root growth below 1.1 m is contrary to the work of Bunger and Thompson (1938) who detected small numbers of both large. medium. and fine roots as deep as 1.8 to 2.4 m.

Both a visual and chemical examination of soil/sludge surrounding the mulberry roots at increasing depths suggests that the roots were associated with a restoration of sludge to soil. Plant roots and associated microorganisms have apparently expedited this restoration in which a "normal soil" with respect to color. texture. and odor has developed from the dense. black. aromatically unpleasant industrial sludge that still remains at lower depths in the basin (Fig. 1c). The chemical concentration of PAHs has also drastically decreased within the root zone as compared to the parent sludge present at deeper depths (Table 1). Total PAH levels in the first 60 cm are several orders of magnitude lower in comparison to the transition zone and deep-sludge. Furthermore. PAH-degrading microorganisms reported in the literature (Cerniglia. 1984) have been isolated from rhizosphere samples collected in the basin (Nagle et al.. 1998).

Interpretation of these data is presented as a conceptual model shown in Figure 3. This 12 year-old tree grew from a seed deposited naturally in the basin. After one year's growth. the root system had developed and explored a shallow zone of the basin. Over time. this zone broadened and became deeper. resulting in an ever increasing zone of root exploration. This interpretation is compatible with the growth and development of tree roots in non-contaminated soil where the increasing demands of the aboveground portion of the tree for inorganic nutrients and water dictates the continued growth and increased dispersal of roots in the soil (Borchert. 1991). As a result. the root portion of a tree or other long-lived perennial vegetation grows both laterally and vertically in an exploratory fashion. Exploration of the soil by the roots is further enhanced by the continuous turnover of fine roots [grow-die-regrow] (Russell, 1977), whereas the larger roots from which the fine roots originate are relatively permanent. The location and direction of fine root growth is random and therefore on a micro-scale different soil particles are contacted each time root turnover occurs (Fitter, 1991). The turnover of the fine roots is of paramount importance in phytoremediation because of certain microbial substrates (i.e. flavonoids and coumarins) are probably only released upon root death (Hedge and Fletcher, 1996) and because air channels are created through the soil by the cavities left following root death. A recent study completed in our laboratory has shown that 30% of the fine roots of mulberry turn over annually and that this root class has a high concentration of phenolic compounds (Leigh, 1997). Thus by the release of select plant compounds and improved soil aeration brought about by root turnover, the activity of indigenous microorganisms capable of biodegrading recalcitrant contaminants is stimulated in the rhizosphere. It is proposed that as sludge is explored by growing plant roots, the combined action of roots and associated microorganisms restore the contaminated sludge to a healthy soil matrix that supports a diversified community of living organisms.

Although generally root growth of the tree examined in this study (Fig. 3) matches the descriptions provided in the literature for mulberry (Bunger and Thomson, 1938), there were two major differences. No large roots (dia > 2.5 cm) were observed to grow vertically beneath a depth of 60 cm, and there were no roots of any size in the heavily contaminated (parent sludge) at the lower depths in the basin. Although mulberry is genetically capable of developing deeper roots, it has not in this basin. It is proposed that penetration of roots into the heavily contaminated parent sludge below 1.1 m was prevented by a high concentration of either single or a combination of phytotoxic compounds. This hypothesis raises the question of how the root system managed to grow to its current average depth of 1.1 m through sludge that 15 years ago possessed the same average concentration and makeup of contaminants as are now present in the deep sludge. A plausible explanation is that the compounds responsible for this phytotoxic barrier at 1.1 m are low molecular weight, volatile compounds [i.e. nitrobenzene (Fletcher, et. al., 1990); trichloroethylene (Newman, et. al., 1997); xylene (Schubert, et. al., 1995), etc.]. These compounds are readily volatilized to the atmosphere and also biodegraded by aerobic microorganisms active in well aerated, shallow soil, and as a result they disappeared quickly from the top 15 cm of sludge when the basin was drained. Free of these compounds, the surface sludge supported seed germination and plant growth that in turn provided improved gas diffusion to lower depths, thereby enhancing both volatilization and microbial activity at increasingly lower depths. It is proposed that the combined bioremediation process of plants and microorganisms continues to edge deeper into the sludge as a moving front that is now approximately 1.1 m deep (Fig. 3). This investigation suggests that there is a narrow layer of biological activity at the edge of this moving front that is conducive to the degradation of a broad spectrum of organic contaminants in the sludge. Numerous abiotic and biological events occur simultaneously for the front to advance downward. Among these events would be the upward diffusion of phytotoxic, volatile compounds from the sludge to higher root zones where their concentrations are diluted to below phytotoxic levels. Fluctuating moisture levels in the basin between dry summer months and wet winter months would also be conducive to the upward movement of water soluble contaminants. The gradual upward migration of phytotoxic compounds clears a thin layer of sludge permitting deeper root penetration, and also places the upwardly mobile contaminants in a more favorable location for aerobic biodegradation. Further analysis of the roots, microbes, and contaminant distribution in this basin is underway to test the concepts advanced as a result of this investigation.

References

Aprill. W.. and R. C. Sims. 1990. Evaluation of the use of prairie grasses for stimulating polycyclic aromatic hydrocarbon treatment in soil. *Chemosphere*. 20:253-265.

Anderson. T. A.. and B. T. Walton. 1995. Comparative fate of [14C] trichloroethylene in the root zone of plants from a former solvent disposal site. *Environ. Toxicol. Chem.* 14:2041-2047.

Borchert. R. 1991. "Growth Periodicity and Dormancy." In: Raghavendra. A. S. (Ed.). *Physiology of Trees*. pp. 221-246. John Wiley & Sons. Inc. New York.

Bunger. M. T.. and H. J. Thomson. 1938. Root development as a factor in the success or failure of windbreak trees in the southern high plains. *J. Forestry*. 36:790-803.

Burken. J. G.. and J. L. Schnoor. 1997. Uptake and metabolism of atrazine by poplar trees. *Environ. Sci. Tech.* 31:1399-1406.

Cerniglia. C. E. 1984. "Microbial Transformation of Aromatic Hydrocarbons." In: R. M. Atlas (Ed.). *Petroleum Microbiology*. Macmillan. New York. pp. 99-128.

Donnelly. P. K.. R. S. Hegde. and J. S. Fletcher. 1994. Growth of PCB-degrading bacteria on compounds from photosynthetic plants. *Chemosphere*. 28:981-988

Ferro. A. M.. R. C. Sims. and B. Bugbee. 1994. Hycrest crested wheatgrass accelerates the degradation of pentachlorophenol in soil. *J. Environ. Qual.* 23:272-279.

Fitter. A. H. 1991. "Characteristics and Function of Root Systems." In: Waisel. Y.. A. Amram. and U. Kafkafi (Eds.). *Plant Roots: The Hidden Half*. pp. 3-24. Marcel Dekker. Inc. New York.

Fletcher. J. S.. and R. S. Hegde. 1995. Release of phenols by perennial plant roots and their potential importance in bioremediation. *Chemosphere*. 31:3009-3016.

Fletcher. J. S.. J. C. McFarlane. T. Pfleeger. and C. Wickliff. 1990. Influence of root exposure concentration on the fate of nitrobenzene in soybean. *Chemosphere*. 20:513-523.

Hegde. R. S.. and J. S. Fletcher. 1996. Influence of plant growth stage on the release of root phenolics by mulberry as related to development of phytoremediation technology. *Chemosphere*. 32:2471-2479.

Keith. L. H.. and W. A. Telliard. 1979. Priority pollutants: a perspective view. *Environ. Sci. Tech.* 13:416-423.

Leigh, M. B. 1997. The release of phenolic compounds into the soil by fine root turnover of mulberry (*Morus rubra* L.). MS Thesis. University of Oklahoma. Norman. OK.

McFarlane, J., C. Nolt, C. Wickliff, T. Pfleeger, R. Shimabuku, and M. McDowell. 1987. The uptake, distribution and metabolism of four organic chemicals by soybean plants and barley roots. *Environ. Toxicol. Chem.* 6:847-854.

McFarlane, J., T. Pfleeger, and J. Fletcher. 1990. Effect, uptake and disposition of nitrobenzene in several terrestrial plants. *Environ. Toxicol. Chem.* 9:513-520.

Nair, D. L., J. L. Schnoor, and L. A. Licht. 1993. Mineralization and uptake of a triazine pesticide in soil-plant systems. *J. Environ. Eng.* 119:842-854.

Nagle, D. P., M. D. Kyle, P. E. Olson, and J. S. Fletcher. 1998. "Bacterial Microflora From a Vegetated, PAH-Contaminated, Industrial Site." In: *Proceedings of IBC's Third Annual International Conference on Phytoremediation*. Houston, TX.

Newman, L. A., S. E. Strand, N. Choe, J. Duffy, G. Ekuan, M. Ruszaj, B. Brook Shurtleff, J. Wilmoth, P. Heilman, and M. P. Gordon. 1997. Uptake and biotransformation of trichloroethylene by hybrid poplars. *Environ. Sci. Tech.* 31:1062-1069.

Qiu, X., T. W. Leland, S. I. Shah, D. L. Sorensen, and E. W. Kendall. 1997. "Field Study: Grass Remediation of Clay Soil Contaminated with Polycyclic Aromatic Hydrocarbons." In: E. L. Kruger, T. A. Anderson, and J. R. Coats (Eds.). *Phytoremediation of Soil and Water Contaminants*. pp.186-199. American Chemical Society, Washington, D. C.

Russell, R. S. 1977. *Plant Root Systems: Their Function and Interaction with the Soil*. pp. 298. McGraw-Hill Book Company (UK) Limited, London.

Schubert, U., L. Wisanowsky, and U. Kull. 1995. Determination of phytotoxicity of several volatile organic compounds by investigating the germination pattern of tobacco pollen. *J. Plant. Physiol.* 145:514-518.

Schwab, P. A., M. K. Banks, and M. Arunachalam. 1995. "Biodegradation of Polycyclic Aromatic Hydrocarbons in Rhizosphere Soil." In: R. E. Hinchee, R. E. Hoeppel, and D. B. Anderson (Eds.). *Bioremediation of Recalcitrant Organics*. pp.23-30. Battelle Press, Columbus, Ohio.

Shimp, J. F., J. C. Tracy, L. C. Davis, E. Lee, W. Huang, and L. E. Erickson. 1993. Beneficial effects of plants in the remediation of soil and groundwater contaminated with organic materials. *Critical Rev. Environ. Sci. Tech.* 23:41-77.

USEPA. (1986a): Method 3540. Soxhlet Extraction. SW-846: Test Methods for Evaluating Solid Waste (Physical/Chemical Methods). United States Environmental Protection Agency: Office of Solid Waste. Washington. D.C.

USEPA. (1986b): Method 8100. Polynuclear Aromatic Hydrocarbons. SW-846: Test Methods for Evaluating Solid Waste (Physical/Chemical Methods). United States Environmental Protection Agency: Office of Solid Waste. Washington. D.C.

Wunderlin, R. P. 1997. "Moraceae (Link): Mulberry Family." In: Flora of North America Editorial Committee (Eds.). *Flora of North America North of Mexico*. pp. 388-399. Oxford University Press. New York: Oxford.

Figure 1a. Mulberry tree growing in a former industrial waste basin.

Figure 1b. Root excavation in the proximity of a 12 year old mulberry tree.

Figure 1c. Sludge-to-soil restoration in the root zone extending to a depth of 110 cm.

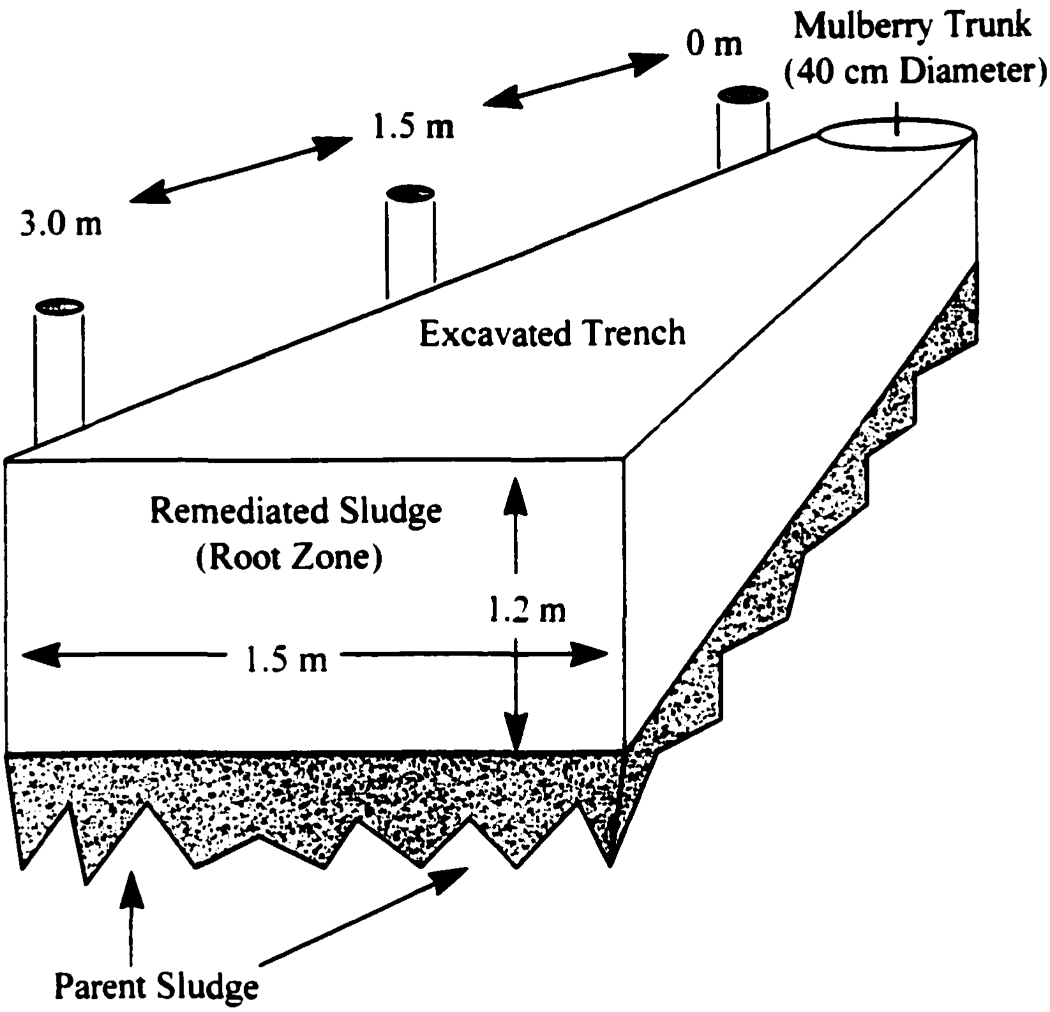

Figure 2. Location and depth of root excavation showing the position of parent sludge, remediated sludge, root zone and cores removed for PAH analysis.

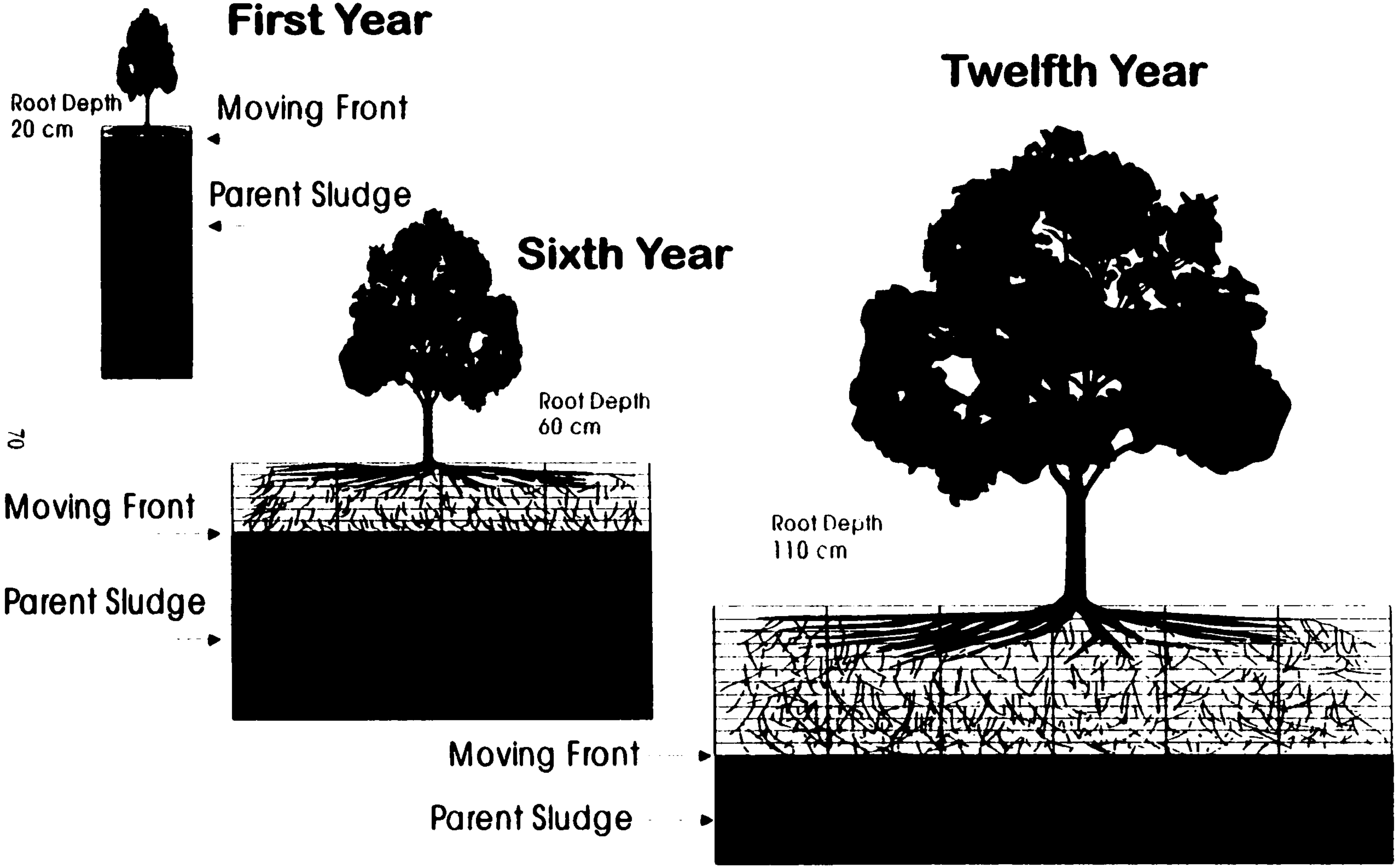

Figure 3. Conceptual model showing the growth of a mulberry tree in industrial sludge over a 12 year period. Zone of remediated sludge is associated with the expanding root system.

Table 1. Concentrations of individual PAHs (ppm) present in corings within the mulberry root zone (0-110 cm in depth) and parent sludge beneath the root zone.

	Section of Core											
	First (0-30 cm)			Second (30-60 cm)			Transition[a] (100-110 cm)			Sludge (>110 cm)		
Contaminant	*0*[b]	*1.5*	*3.0*	*0*	*1.5*	*3.0*	*0*	*1.5*	*3.0*	*0*	*1.5*	*3.0*
Naphthalene	70	30	32	98	122	150	4000	2249	2401	12900	4699	13741
Acenaphthylene	535	197	164	226	289	239	677	1438	881	2049	885	2491
Acenaphthene	139	46	42	43	44	44	227	439	286	766	311	857
Flourene	281	104	74	113	123	122	604	1516	919	1878	883	2490
Phenanthrene	906	366	43	342	365	302	1645	3708	2347	4503	2280	5899
Anthracene	152	66	45	77	116	97	428	918	1758	798	465	1108
Fluoranthene	174	98	51	85	123	82	348	945	574	1252	495	1505
Pyrene	358	206	108	183	270	184	677	1819	792	2674	1059	3093
Benzo(a)Anthracene	69	49	27	41	70	49	165	514	211	775	265	752
Chrysene	56	37	23	41	59	38	159	273	162	269	203	381
Benzo(b)Fluoranthene	31	40	8	16	22	15	11	76	67	124	25	182
Benzo(k)Fluoranthene	nd[c]	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Benzo(a)Pyrene	38	27	16	20	37	27	62	148	110	234	82	237
Indeno(x)Pyrene	nd	nd	nd	nd	nd	nd	nd	nd	nd	82	nd	62
Dibenzo(x)Anthracene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Benzo(x)Perylene	nd	nd	nd	nd	nd	nd	nd	155	nd	410	19	257
Total PAH	2809	1266	633	1285	1640	1349	9003	14198	10508	28714	11671	33055

[a]Transition zone is the boundary between the root zone and the parent sludge.
[b]Distance (m) that core was removed from the base of the mulberry trunk.
[c]nd or non-detect (detection limit < 2 ppm).

Chapter 4.

USE OF ALLOMETRIC MODELING TO ESTIMATE TREE GROWTH AND PHYTOREMEDIAITON PERFORMANCE AT HAZARDOUS WASTE SITES

Abstract

Allometric modeling was used to provide realistic time frames regarding tree growth and phytoremediation performance at terrestrial sites contaminated with organic pollutants. Thirty-one mulberry trees, aged 2-16 years old, growing at a former industrial sludge basin were uprooted with a crane and their roots were washed free of sludge material. The trees appeared healthy by virtue of their appearance and had roots penetrating the contaminated sludge to depths between 30-200 cm. All of the above ground (stems and leaves) and belowground components (different diameter roots) from the harvested trees were sectioned and measured. A logarithmic-logarithmic allometric model of constant differential growth was developed from these datasets and used to conservatively estimate tree growth and phytoremediation performance for trees aged 3-15 years. The combined influence of root growth and changes in microbial communities suggest that a gradual disappearance of organic pollutants at depths of 1-1.5 m can occur over time. Furthermore, increased transpiration rates as a result of tree growth can be instrumental in the reduction of risks associated with the pollutants by maintaining a hydraulic balance and controlling off-site migrations of contaminants via leeching into

groundwater systems. The results of this study suggest that site-tolerant trees, including mulberries, can be effectively used for the phytoremediation and ecological recovery of deeply contaminated soils.

Introduction

Vegetation and root-associated microorganisms perform vital roles in the ecological recovery of physically and chemically disturbed environments (Barbour, et. al., 1987). Soils contaminated with organic pollutants from anthropogenic activities illustrate an extreme example of a disturbed environment (Olson and Fletcher, 1999a). Although the total number of sites contaminated with organic pollutants is unknown, the use of vegetation, or phytoremediation, has been suggested as an economical alternative for the enhanced remediation of chemically distressed soils (Shimp, et al., 1993). Through extensive root penetration and growth (Olson and Fletcher, 1999b), vegetation and root-associated rhizosphere microorganisms were instrumental in the enhanced remediation of soils contaminated with polycyclic aromatic hydrocarbons (PAHs) over 16 years (Olson and Fletcher, 1999c).

Mathematical models have been developed to estimate plant growth and simulate phytoremediation processes from 3 months to 8 years (Chang and Corapcioglu, 1998; Davis, et al., 1993). However, it is difficult to construct accurate models because of all of the complex environmental factors that are subject to dynamic changes in the field over time. Thus, plant growth and phytoremediation models to date are based on data from laboratory results that are then extrapolated to the field. An alternative approach is to use field-collected data from previously revegetated hazardous waste sites to quantify the growth of certain plant species exposed to contamination. Although such data are taken from uncontrolled settings, it is more realistic than laboratory data because it provides the opportunity to evaluate the growth of mature plants that have been subjected simultaneously to both normal environmental stresses as well as hazardous waste chemicals that may be phytotoxic. Since root penetration and lateral growth at contaminated sites is essential for the phytoremediation of soil contaminants, both root and shoot growth must be evaluated. Due to the extreme difficulty of studying roots in the field, root growth has been estimated from *in-situ* soil corings with almost no attention given to contaminated sites.

The objective of this study was to quantify the biomass of both the above and belowground organs of mulberry trees that have grown for a variable time period (2-16 years) in a former industrial waste basin contaminated with PAHs. A dataset derived from measurements of 31 trees uprooted from this former sludge basin were used to formulate allometric relationships. These relationships were used to estimate the growth and phytoremediation potential of mulberry trees planted in PAH-contaminated waste.

Methods

Study Site

The study was conducted at a former industrial waste basin at a chemical manufacturing facility in S.E. Texas. The waste basin was previously used for the disposal of wastewater containing high levels of PAHs and residual river sediments from primary clarifier water. The basin was taken out of use in the late 1970's, and the resulting sludge has become naturally revegetated with an assortment of grasses, forbs, and mulberry trees. This site is found adjacent to an extensively studied basin located in the center of the actively manufacturing chemical plant and therefore has been subject to the same environmental conditions as reported previously (Olson and Fletcher. 1999a: Olson and Fletcher. 1999b: Olson and Fletcher. 1999c).

Tree Selection and Harvesting

Mulberry trees growing at the site were a result of naturally deposited seeds. The trees were healthy in appearance and grew together as a dense canopy. Mulberry trees. covering the size range of trees growing at the site. were marked for sampling prior to removal. Thirty-one mulberry trees were completely unearthed with roots attached using a 150-ton industrial crane (Figure 1). The tree harvesting was conducted in April 1998 when soil/sludge was moist to facilitate complete removal of the root systems. The roots of the uprooted trees were washed immediately with a pressure hose and growth measurements were subsequently made.

Aboveground Measurements

Typical in tree allometry studies. the diameter of a tree is measured at "breast height" and related to other growth-dependent parameters. However. due to abrupt branching of mulberry trees close to the ground surface at this site. it was difficult to accurately measure the diameter at breast height. Therefore. in this study. the diameter (mm) of individual tree trunks was measured at a trunk location just above root swell. The easily measured diameter above root swell (DARS) is correlated with tree age. other aspects of tree growth in contaminated soils (i.e. leaf biomass: rooting depth), and phytoremediation performance.

Aboveground growth parameters were measured for all uprooted trees. The tree height (cm) was measured at a distance from the DARS to the top of the canopy. The maximum tree canopy spread (cm), reflective of trees growing in a dense canopy at this site, was measured for each tree. Next, the shoot portion of an individual tree was separated from the roots at a region on the tree trunk equivalent to soil surface level. The entire shoot was sectioned and weighed in the field with a swinging balance. Reference samples were removed and taken back to the laboratory and dried in a plant oven at 50° C for 4 days to obtain fresh to dry weight ratios. On average, the wood samples contained 12.5% water and was used as a correction factor to report the biomass measurement (g) on a dry weight basis. Finally, age was determined by counting annular tree rings from the tree trunk.

Branch and Leaf Measurements

The majority of leaves resided on branches with a diameter between 20-50 mm. In an effort to estimate the total leaf biomass and leaf surface area for each tree, the total number of branches (20-50 mm diameter) and their actual diameters were recorded and correlated with the DARS. For younger trees, smaller branches (< 20 mm diameters) that possessed the majority of leaves were also included.

To estimate the leaf biomass per branch, thirty-five branches with diameters between 20-50 mm were removed from undisturbed trees in the basin and all leaves were removed for fresh weight measurements. Collected leaves were next placed into paper sacks and dried in the laboratory. On average, the fresh leaves contained 37.5% water by weight which was used to make dry weight corrections for leaf biomass (g). The resulting data were used to correlate branch diameter and leaf biomass in relation to the entire tree.

Total leaf surface area (cm^2) for a given tree was estimated from total leaf biomass measurements. One hundred and twenty leaves were collected at the site, placed flat and dried in a plant press. The perimeter of each individual leaf was carefully traced on graph paper to determine the individual leaf surface area, and subsequently weighed. The weight of each leaf was correlated to the total surface area, resulting in a strong linear relationship ($r^2 = 0.99$). Total leaf biomass and surface area was estimated from total branch diameter measurements in relation to the DARS for each tree.

Belowground Measurements

Due to the importance of plant roots in phytoremediation efforts, special attention was given to the belowground growth parameters from the uprooted trees. The rooting depth (cm) was measured as the distance between the tree trunk at soil surface and the approximate maximum depth of the root system for each of the unearthed trees. This depth was confirmed by measuring the depth of the hole left after trees were uprooted. The maximum rooting spread (cm) was measured from the longest lateral root extending from the tree trunk and combining this measurement to an appropriate lateral root on the other side of the tree trunk in the same plane. The orthogonal rooting spread (cm) was measured from lateral roots extending from the tree trunk orthogonal to the previously measured maximum rooting spread. The rooting depth and patterns of trees growing in contaminated soils provides important information regarding the potential of rhizosphere remediation.

The entire tree root system for each individual tree, separated from the aboveground shoot portion, was labeled and placed into plastic bags to reduce moisture lost from the root tissue. Roots were transported back to the lab, subsequently sectioned and separated into various root classes based on individual root diameter. The root classes included roots having a diameter of 1) < 1.5 mm, 2) 1.5-5 mm, 3) 5-20 mm, 4) > 20mm, or 5) large supporting trunk roots. Separated roots were placed into paper bags and dried in a plant oven at 50° C for 4 days to obtain fresh to dry weight ratios. Root biomass (g) for the individual size classes and total roots was recorded on a dry weight basis and related to the DARS of the tree.

Total root length was estimated for all roots < 20 mm in diameter (excluding root hairs). A total of 100 randomly selected dried root sections were collected from each of three root size classes (< 1.5 mm, 1.5-5mm, and 5-20 mm). These root sections represented the spread in size variation found within each root class. The root sections were measured in a linear manner to determine the overall length (cm) of each root section and subsequently weigh (mg) to estimate the average length (cm) to root weight ratio. This information was used to approximate the total root length of each tree for < 1.5 mm, 1.5-5 mm, 5-20 mm root classes, and summed for the total tree length of roots measuring < 20 mm in diameter based on the DARS of a tree.

Root surface area and total volume was also estimated for each of three root size classes (< 1.5 mm, 1.5-5mm, and 5-20 mm). An assumption was made that sectioned roots approximated a geometric cylinder, and each root size class having an average radius of 0.75 mm, 3.75 mm, and 12.5 mm, respectively for each of the three size classes. This information was related to the approximate root length previously determined. Total root surface area (cm^2) total root volume (cm^3) in contact with contaminated soils was estimated for each tree in terms of the < 1.5 mm, 1.5-5 mm, 5-20 mm root classes, and summed for the total tree length of roots measuring < 20 mm in diameter.

Statistical Approaches

Scaling approaches and dimensional analyses of tree growth with respect to DARS and age were determined using Huxley's model of constant differential growth ratios applied in many biological sub-disciplines (Huxley, 1924; Huxley, 1932; Niklas, 1994). The simplest allometric model assumes that a logarithmic-logarithmic relationship exists between one growing component of an organism in relation to another part. In the case of trees, a simply measured character, such as trunk diameter, is correlated with other more difficultly measured variables (i.e. leaf area, root biomass, etc.). The model used in this study approximates a log-linear line equation as follows: $\log_{10} y = m \log_{10}(x) + b$, where x = DARS and y = another tree character related to differential growth. In addition to estimates of the regression parameters [slope of the linear model (m) and y-intercept (b)], uncertainty of the estimates [coefficients of determination (r^2) and standard error of the model estimate (e)], and statistical measures of the "goodness-of-fit" employing an ANOVA and estimated probability were calculated. All tree growth characters were ultimately described using Huxley's model, with the exception of tree age and DARS (which remained a simple linear model).

Allometric models could be used to predict tree growth and contaminant fate for trees planted at other sites. Although a bias (i.e. underestimating the parameter of interest) exists in log-transformed data used for allometric analyses and regression estimates (Beauchamp and Olson, 1973; Whittaker and Marks, 1975), this bias usually accounts for less than 10% of the residual error (Sprugel, 1983), and as a result was not accounted for or corrected in the final tree growth simulations. Our intent is to report allometric models that provide a conservative estimate of projected tree growth, phytoremediation potential, and contaminant fate to be applied at other sites.

Chemical Analyses

The concentrations of PAHs in the basins were estimated in the tree rhizosphere and below the rooted zone in the basin. The soils, in association with 6 trees ranging in age between 7 and 16 years of age, were selected for PAH analysis. Approximately 500 grams of soil, representing a composite sample, was collected from each of the tree's root zone. Another composite sample was recovered at each sampling location below the root zone. The contaminated soils were transported to the labs at the University of Oklahoma and subsequently extracted (USEPA, 1986a) and analyzed for the 16 priority PAH pollutants using gas chromatography (USEPA, 1986b). Polycyclic aromatic hydrocarbon concentrations were expressed mg/Kg dry weight of soil (ppm).

Results and Discussion

Chemical analyses at six locations in the grove of mulberry showed that the basin was contaminated with PAH compounds. All of the 16 Priority PAH compounds were detected in the rooted and non-rooted sludge with the exception of benzo(*k*)fluoranthene. indeno(1.2.3-*cd*)pyrene. and dibenzo(*ah*)anthracene. The average total PAH concentration in the root zone (samples recovered at approximately 60 cm in depth) was 2.395 ppm and in the non-rooted sludge (approximately 140 cm in depth) was 12.825 ppm. The difference in PAH concentrations between the rooted and non-rooted sludge was comparable to that reported earlier in a more comprehensive chemical study conducted in an adjacent former sludge basin (Olson and Fletcher. 1999c).

Of the 31 uprooted trees growing in this PAH-contaminated soil/sludge. 29 mulberries were found to be growing as a single-trunked tree. and were used to develop allometric models of relative tree growth in contaminated soils (see Table 5). In addition to the 29 trees. two large mulberries were later determined to be a "cluster" of more than one tree growing together as a multi-trunked tree. The large. multi-trunked trees were included in this study to illustrate the genetic potential of mulberry tree growth at contaminated sites. but they were not used for the development of subsequent allometric models.

The mean dimensional relations of mulberry tree growth in PAH-contaminated sludge were determined by harvesting 29 single-trunked trees (Table 1 & 2). The average age of these trees was 7.3 years and ranged in age from 2 to 15 years. For all variables measured. the smallest trees in terms of above and belowground biomass. tree height. leaf area. and rooting patterns were observed from trees beginning their second year of growth. Conversely. the maximal measured variables were recorded from the older trees growing at the contaminated site. For example. aboveground shoot biomass. leaf biomass. and total leaf surface area were found highest for the oldest. 15 year-old tree measured tree. Rooting depth for the trees averaged 77.6 cm. and ranged from 30 to 200 cm. The maximal rooting depth and spread was not observed from the oldest tree. but from a single. 9 year-old tree. This deep-rooted tree was located growing among several smaller trees in a dense canopy. and hence may have been under greater competition for available water and nutrients and thus was forced to have greater root growth.

Total belowground root biomass was found to be maximal in the older. larger trees (Table 2). Smaller diameter roots (< 20 mm) had higher total weights for the large trees compared to younger. smaller trees. Although the percentage of these smaller diameter roots in relation to the total root biomass accounted for 3.3% for the fine roots (< 1.5 mm). 16.6% for 1.5-5 mm diameter roots. and 31.0% of 5-20 mm. the greatest proportion of these smaller diameter roots were generally observed in younger aged trees. These smaller diameter roots are considered very important in regards to phytoremediation as root-turnover and release of plant-derived pollutant analogs that have the capability to promote growth of indigenous pollution-degrading microbes (Hegde and Fletcher. 1996: Leigh. et. al.. 1999). The root-to-shoot ratio averaged 27.8% in this study. and corresponds to previously published information that mature tree root biomass averages 25% by weight of the aboveground shoot biomass (Ovington. 1962).

The total root volume and root surface area for roots < 20 mm in diameter is generally highest in older trees (Table 2). Estimations of root volume and surface area are important to phytoremediation in terms of microorganisms colonizing the root surface (rhizoplane). and the total impact on contaminated soils from the influence of roots within the first few millimeters away from the root surface (rhizosphere). Total root length was estimated to average 315 m for the smaller roots. Estimations of total root volume, surface area, and length did not include the very small root hairs associated with water and nutrient uptake.

Two very large mulberries, aged 15 and 16 years, were also uprooted from the former industrial sludge basin to illustrate the maximal growth potential of mulberries growing in contaminated soils (Tables 3 & 4). Because it was impossible to distinguish if these trees were actually a single tree or a composite of more than one tree growing at the same location, they were not used during regression analyses to develop conservative, allometric models of tree growth. In general, they both were growing alone without the influence of other trees as reflective of the tree height and large canopy spread. The shoot biomass, leaf biomass, and total leaf surface area averaged approximately 10-20x the average of the other 29 sampled trees, and were 2-4x greater than the largest sampled tree for regression analysis which was aged 15 years. The maximum rooting depth of these large mulberries ranged from 100-150 cm, and the large maximum rooting spread suggests that large volumes of contaminated soils are under the direct influence of these two trees. Total root biomass averaged 10-25x more than the average of the other 29 trees and 3-6x greater than the maximum biomass sampled for the other set. Based on the root-to-shoot ratio, these trees were well rooted, having a tremendous amount of root volume and total root surface area in direct contact with the contaminated soil. These trees, as well as the other 29 trees sampled, have influenced the visual appearance and chemical concentrations of the contaminated soil.

The dimensional relations of 29 variously aged mulberry trees growing in PAH-contaminated sludge revealed a strong correlation to logarithmic growth over time. The information collected during this study in regards to 26 growth-dependent variables were analyzed through regression analysis against an easily measured variable, diameter of the mulberry trunk above root swell (DARS), in the field (Table 5). Growth-dependent variables included above and belowground biomass, tree height, rooting patterns, and volume and area estimations of leaf and root parameters. The allometric model predicts growth-dependent variables based on DARS measurements as follows: $\log_{10} y = (m) \log_{10} + b$: where y = growth-dependent variable, b = y-intercept, and x = DARS (mm). Estimates of the strength of the growth relationships (r^2 values) indicate that mulberry growth in contaminated soils is highly predictable in this study. Estimates of the uncertainty regarding these relationships are reported as standard errors (e) that can be used to correct for potential bias in allometric models (Baskerville, 1972; Whittaker and Marks, 1975; Sprungel, 1983). Results from analysis of variance (ANOVA) suggest that the regression analysis of the 29 trees provide a good estimate of mulberry tree growth in contaminated soils as demonstrated by the low probability values. Furthermore, because two of the largest, multi-trunked trees showing the genetic capability of mulberry growth were eliminated from these regression analyses, a reliable, yet conservative model simulating mulberry tree growth in terms of phytoremediation potential and contaminant fate at other sites has been developed.

The allometric models proposed in this study estimate vegetative growth of a moderately fast growing tree, such as mulberry, inhabiting PAH-contaminated soils under a long growing season (250-300 days) and mesic conditions (100 cm precipitation). To provide appropriate time frames, the age of the 29 individual mulberries were regressed against their DARS (Figure 2). The strong relationship between tree age and DARS allows for reliable estimates of mulberry tree growth over time. However, these models underestimate tree growth for trees less than 3 years of age. Furthermore, caution should be exercised when using allometric models that include older trees, as logarithmic relations are not always maintained as a tree matures towards the end of its life cycle (Whittaker and Marks, 1975). As a result, the allometric models from this study are relevant to predict mulberries growing in contaminated soils under warm, mesic climates for trees aged 3-20 (25) years. To further utilize these models, the growth of woody vegetation in other contaminated sites can also be estimated. Various species of shrubs and trees are "linked by allometric trends in common" (Whittaker and Woodall, 1968). Therefore, an "interspecies regression" for the dimensional relations of woody vegetation suggests log-arithmetic allometric growth can be predicted for similar and dissimilar woody vegetation from a single species study.

This study capitalized on naturally occurring mulberry trees growing in contaminated soils. The dimensional relations of tree growth provide a useful means to predict the growth and phytoremediation dependent performance of vegetation similar to mulberries. Time scales should be adjusted for the selected vegetation in relation to mulberry, a moderately fast growing tree. Furthermore, the length of growing season precipitation, and individual site characteristics should also be adjusted for during model development. Local knowledge of vegetation growth and climatic conditions at other sites will strengthen the predictability of the models.

Simulated Tree Growth and Phytoremediation Potential

The growth of individual mulberry trees planted in contaminated soils was simulated from allometric models previously described (Figure 3). The simulation estimated various growth-related parameters for trees aged 3, 5, 10, and 15 years. Growth-related variables could be used to conservatively predict the phytoremediation potential of mulberry trees and other deciduous woody vegetation growing in soils contaminated with organic pollutants.

Dry weight biomass (g) estimations increased as the trees aged during the simulation (Figure 3). Total aboveground biomass and leaf weight increased logarithmetically as the trees aged, maintaining a relative dimensional structure. Transpiration rates are a direct function of the total leaf biomass and ultimately the total leaf surface area. As the trees grow, transpiration rates will proportionally increase as a result of structural changes associated with growth.

The role of transpiration during phytoremedial efforts can be important in several ways. The control of off-site migration of organic contaminants, and thus the reduction in risks associated with the chemical, may potentially be achieved through the transpiration process and a water balance at a given site. Furthermore, plants may have the ability to take up water-soluble organic pollutants (i.e. TCE and atrazine), and potentially metabolize and degrade them within the plant itself (Burken and Schnoor, 1997; Newman, et. al., 1997). Mulberry trees had a greater than 130-fold increase in total leaf biomass and leaf surface area as the tree growth was simulated from 3 to 15 years in age (Table 6). By taking an average midsummer transpiration rate for a variety of trees and shrubs growing in three geographic locations (Kramer and Boyer, 1995), the average maximal water loss / day for an individual mulberry tree was estimated to increase 138-fold. A 3-year-old mulberry tree transpired approximately 0.4 L / day, while the larger 15-year-old tree can transpire approximately 55.1 L / day. These predictions based on mulberries are comparable to transpiration rates reported for similarly sized, but not necessarily aged, hybrid poplars known for high water uptake (Hinckley, et. al., 1994), and indicate that the general allometric relations of plant growth and physiological function are similar across a variety of taxa.

As the trees grow in height and expand its canopy, the overall rooting patterns in relation to their growth change (Figure 3). Rooting depth increased from 45 cm for a 3-year-old tree to approximately 105 cm for the older trees in this simulation (Table 7). In addition to increasing rooting depth, lateral roots also spread away from the tree trunk leading to an ever-increasing radial zone of influence. Mulberry root biomass increased greater than 620-fold and the rooting depth was nearly 2.5x greater as tree growth was simulated for the 12 year period. For roots having a diameter of less than 20 mm, total root length increased approximately 175-fold, and the total root surface area and root volume increased greater than 200-fold and 300-fold, respectively as the tree grew from 3 to 15 years of age. This increase in root length, surface area, and volume of the roots should provide an ample opportunity for a significant interaction between the contaminated soil and the tree root.

Changes in rooting patterns over time will ultimately lead to more of the contaminated soil in direct contact with the root or under the influence of the root through changes in the physical, chemical, and biological environment in the soil (Curl and Truelove, 1986). Rhizosphere degradation of recalcitrant organic pollutants is a potential benefit from using a plant/microbe phytoremediation approach. The total number and species richness of microorganisms inhabiting the root surface (rhizoplane) and soils under the immediate influence of the root (rhizosphere) are usually much higher than the surrounding bulk (non-rooted) soil. The rhizosphere-to bulk soil (R/S) ratio is typically 2-20(100) in terms of total bacterial numbers. Based on the average number of rhizoplane and rhizosphere bacterial numbers for a variety of crop plants (Rouatt and Katznelson. 1961). total root associated bacteria increased dramatically as tree growth was simulated (Table 7). In relation to higher overall bacterial numbers. a greater proportion of PAH-degraders to the total heterotrophs within the rhizosphere of various plant species roots have been isolated from a similarly PAH-contaminated site (Nagle. et. al.. 1998). Plant derived compounds that serve as natural analogs to organic pollutants may stimulate metabolic pathways for microbial metabolism of the pollutant and ultimately select for an increase in the relative proportion of indigenous bacteria capable of degrading pollutants (Donnelly. et. al.. 1994: Fletcher and Hedge. 1995: Gilbert and Crowley. 1997: Sicilano and Germida. 1998). Furthermore. mulberry roots with a diameter of 1-2 mm and < 1 mm contained approximately 3% and 5%. respectively. of ringed phenolic compounds on a root dry weight basis (Leigh. et. al.. 1999). These phenolic compounds are released into the rhizosphere as roots turnover. approaching 50% turnover / growing season. and have been shown to act as a sole carbon source for previously isolated PAH-degraders from a mulberry root zone growing in PAH-contaminated soils. With prevailing R/S ratio. it is conceivable that a plant/microbe phytoremediation approach can have a significant effect on recalcitrant pollutants and the overall reduction in risks associated at contaminated sites.

The allometry relationships derived in this study from trees growing in hazardous waste material provide a means of estimating phytoremediation potential as well as monitoring its progression at a newly planted site. This is of great importance because for the first time it will be possible to make realistic estimates of the length of time required for this slow sustained technology to reclaim contaminated ecosystems. Further information regarding phytoremediation performance can be acquired from long-term growth studies of a variety of plants at contaminated sites.

References

Barbour. M. G.. J. H. Burk. W. D. Pitts. 1987. Terrestrial Plant Ecology. Second Edition. Benjamin/Cummings Publishing Company. Inc. Menlo Park. CA. Pgs. 634.

Baskerville. G. L. 1972. Use of logarithmic regression in the estimation of plant biomass. *Canadian Journal of Forest Research*. 2:49-53.

Beauchamp. J. J.. and J. S. Olson. 1973. Corrections for bias in regression estimates after logarithmic transformation. *Ecology*. 54:1403-1407.

Burken. J. G.. and J. L. Schnoor. 1997. Uptake and metabolism of atrazine by poplar trees. *Environmental Science and Technology*. 31:1399-1406.

Chang. Y. Y.. and M. Y. Corapcioglu. 1998. Plant-enhanced subsurface bioremediation of nonvolatile hydrocarbons. *Journal of Environmental Engineering*. 124:162-169.

Curl. E. A.. and B. Truelove. 1986. The Rhizosphere. Springer-Verlag. New York. 288 pgs.

Davis. L. C.. L. E.. Erickson. E. Lee. J. F. Shimp. and J. C. Tracy. 1993. Modeling the effect of plants on the bioremediation of contaminated soil and ground water. *Environmental Progress*. 12:67-75.

Donnelly. P. K.. R. S. Hegde. and J. S. Fletcher. 1994. Growth of PCB-degrading bacteria on compounds from photosynthetic plants. *Chemosphere*. 28:981-988.

Fletcher. J. S.. and R. S. Hegde. 1995. Release of phenols by perennial plant roots and their potential importance in bioremediation. *Chemosphere*. 31:3009-3016.

Gilbert. E. S.. and D. E. Crowley. 1997. Plant compounds that induce polychlorinated biphenyl biodegradation by *Arthrobacter* sp. Strain B1B. *Applied and Environmental Microbiology*. 63:1933-1938.

Hegde. R. S.. and J. S. Fletcher. 1996. Influence of plant growth stage on the release of root phenolics by mulberry as related to development of phytoremediation technology. *Chemosphere*. 32:2471-2479.

Hinckley. T. M.. J. R. Brooks. J. Cermak. and R. Ceulemans. 1994. Water flux in a hybrid poplar stand. *Tree Physiology*. 14:1005-1018.

Huxley. J. S. 1924. Constant differential growth-ratios and their significance. *Nature*. 114:895-896.

Huxley, J. S. 1932. Problems of Relative Growth. Dial Press. New York, NY. Pgs. 276.

Kramer, P. J., and J. S. Boyer. 1995. Water Relations of Plants and Soils. Academic Press. New York, N.Y. 495 pgs.

Leigh, M. B., J. S. Fletcher, X. Fu, and F. Schmitz. 1999. Influence of season, age and senescence of the phenolic content of mulberry fine roots with reference to phytoremediation. Plant and Soil. (In Press)

Nagle, D. P., M. D. Kyle, P. E. Olson, and J. S. Fletcher. 1998. "Bacterial Microflora From a Vegetated, PAH-Contaminated, Industrial Site." In: *Proceedings of IBC's Third Annual International Conference on Phytoremediation*. Houston, TX.

Newman, L. A., S. E. Strand, N. Choe, J. Duffy, G. Ekuan, M. Ruszaj, B. B. Shurtleff, J. Wilmoth, P. Heilman, and M. P. Gordon. 1997. Uptake and biotransformation of trichloroethylene by hybrid poplars. *Environmental Science and Technology*. 31:1062-1069.

Niklas, K. J. 1994. Plant Allometry: The Scaling of Form and Process. The University of Chicago Press. Chicago, Ill. Pgs. 395.

Olson, P. E., and J. S. Fletcher. 1999a. Ecological recovery of vegetation at a former industrial sludge basin and its implications to phytoremediation and ecological risk assessment. *Environmental Science and Pollution Research*. (In review).

Olson, P. E., and J. S. Fletcher. 1999b. Field evaluation of mulberry root structure with regard to phytoremediation. *Bioremediation Journal*. 3:1-7.

Olson, P. E., and J. S. Fletcher. 1999c. Natural attenuation of a former industrial sludge basin with reference to phytoremediation. *Environmental Science and Pollution Research*. (In preparation).

Ovington, J. D. 1962. Quantitative ecology and the woodland ecosystem concept. *Advances in Ecological Research*. 1:103-192.

Rouatt, J. W., and H. Katznelson. 1961. A study of the bacteria on the root surface and in the rhizosphere soil of crop plants. *Journal of Applied Bacteriology*. 24:164-171.

Shimp, J. F., J. C. Tracy, L. C. Davis, E. Lee, W. Huang, and L. E. Erickson. 1993. Beneficial effects of plants in the remediation of soil and groundwater contaminated with organic materials. *Critical Reviews in Environmental Science and Technology*. 23:41-77.

Siciliano, S. D., and J. J. Germida. 1998. Mechanisms of phytoremediation: biochemical and ecological interactions between plants and bacteria. *Environmental Reviews*. 6:65-79.

Sprugel, D. G. 1983. Corrections for bias in log-transformed allometric equations. *Ecology*. 64:209-210.

USEPA. (1986a): Method 3540. Soxhlet Extraction. SW-846: Test Methods for Evaluating Solid Waste (Physical/Chemical Methods). United States Environmental Protection Agency: Office of Solid Waste. Washington, D.C.

USEPA. (1986b): Method 8100. Polynuclear Aromatic Hydrocarbons. SW-846: Test Methods for Evaluating Solid Waste (Physical/Chemical Methods). United States Environmental Protection Agency: Office of Solid Waste. Washington, D.C.

Whittaker, R. H., and P. L. Marks. 1975. "Methods of Assessing Terrestrial Productivity." In: H. Lieth, and R. H. Whittaker (Eds.). *Primary Productivity of the Biosphere*. pp.55-118. Springer-Verlag. New York, N.Y.

Whittaker, R. H., and G. M. Woodall. 1968. Dimension and production relations of trees and shrubs in the Brookhaven Forest, New York. *Journal of Ecology*. 56:1-25.

Figure 1. Representative trees uprooted from the former industrial sludge basin with a crane.

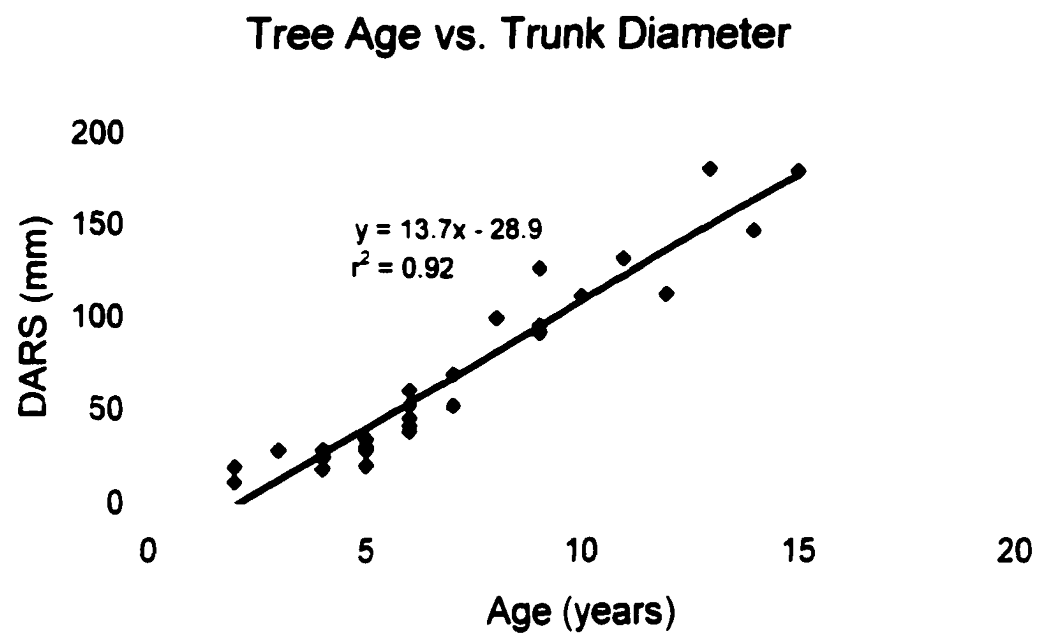

Figure 2. Tree age vs. trunk diameter simple linear regression. DARS = diameter above root swell.

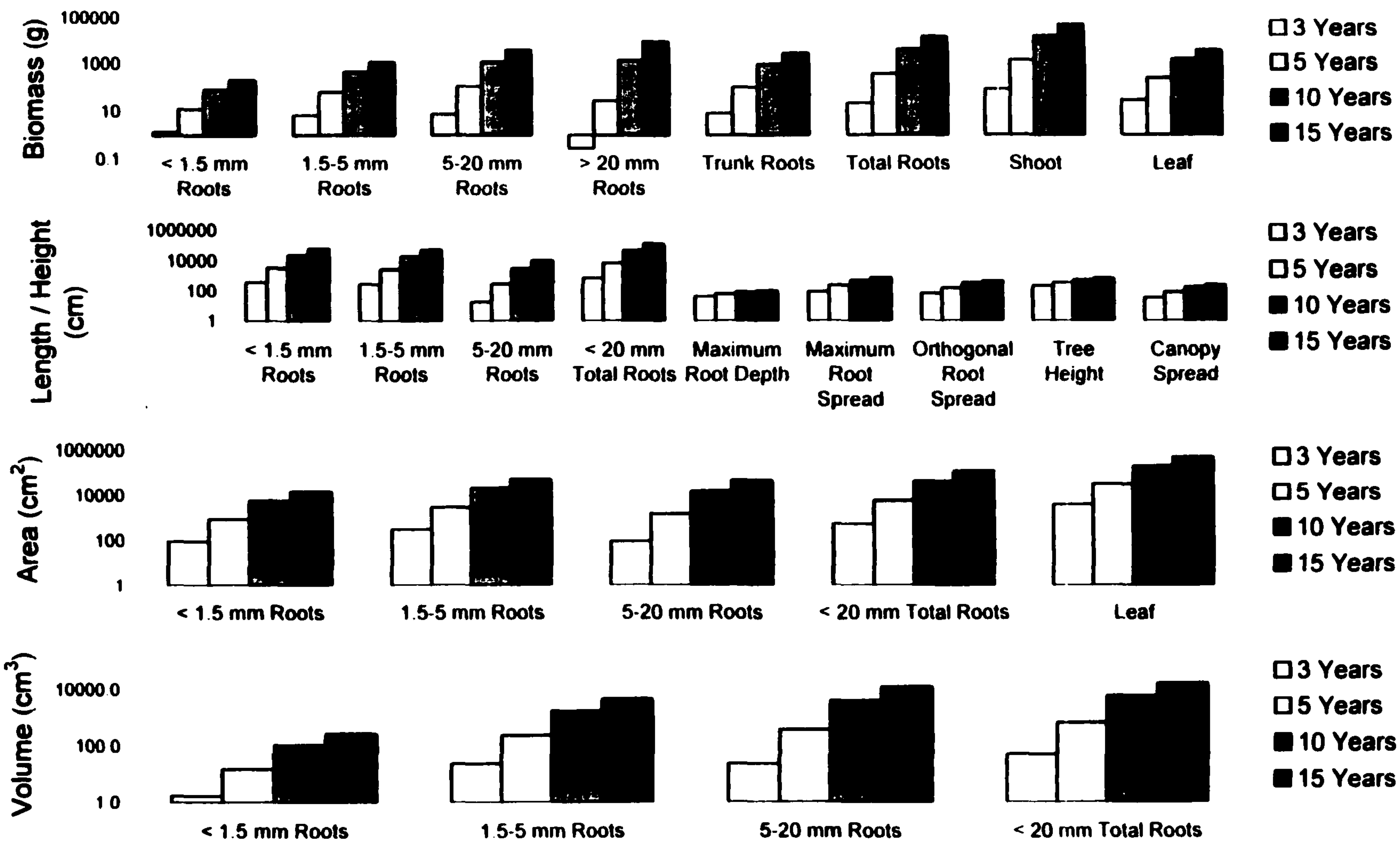

Figure 3. Projected mulberry growth in contaminated soils.

Table 1. Mean dimensional relations of mulberry trees growing in PAH-contaminated sludge (n=29).

	DARS[a] (mm)	Tree Rings (age)	Tree Height (cm)	Canopy Spread (cm)	Shoot Biomass Dry Weight (g)
Mean	70.8	7.3	430.0	120.0	9425.8
Std. Dev.	49.2	3.4	130.0	70.0	11413.3
Min.	11.0 (*2 y*)[b]	2.0	140.0 (*2 y*)	30.0 (*2 y*)	39.7 (*2 y*)
Max.	180.0 (*13 y*)	15.0	580.0 (*14 y*)	280.0 (*12 y*)	45882.4 (*15 y*)

	Leaf Biomass Dry Weight (g)	Leaf Area (cm^2)	Maximum Root Depth (cm)	Maximum Root Spread (cm)	Orthogonal to Maximum Root Spread (cm)
Mean	937.8	122289.3	77.6	369.5	227.7
Std. Dev.	1074.3	140083.8	33.0	224.6	137.6
Min.	15.9 (*2 y*)	2078.0 (*2 y*)	30.0 (*2 y*)	75.0 (*2 y*)	45.0 (*2 y*)
Max.	4632.3 (*15 y*)	604056.6 (*15 y*)	200.0 (*9 y*)	960.0 (*9 y*)	540.0 (*9 y*)

[a]Diameter of tree trunk Above Root Swell (DARS)
[b]Age of individual tree (years) sampled

Table 2. Mean dimensional relations of mulberry tree roots growing in PAH-contaminated sludge (n=29).

	< 1.5 mm Roots	**1.5-5 mm Roots**	**5-20 mm Roots**	**> 20 mm Roots**	**Trunk Roots**	**Total Roots**
	Root DW[a] (g)	Root DW (g)	Root DW (g)	Root DW (g)	Root DW (g)	Root DW (g)
Mean	55.8	286.4	718.2	967.8	606.8	2635.0
Std. Dev.	68.9	316.6	838.0	1330.7	739.6	3165.7
Min.	1.1 (*2 y*)[b]	8.2 (*2 y*)	3.3 (*2 y*)	0.0 (*2,3,4,5 y*)	8.2 (*2 y*)	21.1 (*2 y*)
Max.	278.3 (*12 y*)	1198.1 (*12 y*)	2708.6 (*14 y*)	4617.6 (*15 y*)	2782.5 (*15 y*)	10844.4 (*15 y*)
	% of Total	% of Total	% of Total	% of Total	% of Total	Root:Shoot Ratio
Mean	3.3	16.6	31.0	21.4	27.7	27.8
Std. Dev.	1.9	8.5	9.4	17.4	9.2	7.6
Min.	0.6 (*6 y*)	5.3 (*9 y*)	15.6 (*2 y*)	0.0 (*2,3,4,5 y*)	14.8 (*6 y*)	15.5 (*5y*)
Max.	8.1 (*5 y*)	41.5 (*4 y*)	54.5 (*4 y*)	52.0 (*9 y*)	48.9 (*2 y*)	53.1 (*2y*)
	Volume (cm^3)	Volume (cm^3)	Volume (cm^3)	Total Volume (cm^3) of < 20 mm Roots		
Mean	74.2	1063.4	2320.5	3458.2		
Std. Dev.	91.7	1175.7	2707.6	3876.2		
Min.	1.5 (*2 y*)	30.5 (*2 y*)	10.7 (*2 y*)	43.0 (*2 y*)		
Max.	370.2 (*12 y*)	4448.8 (*12 y*)	8751.6 (*14 y*)	11464.9 (*12 y*)		
	Area (cm^2)	Area (cm^2)	Area (cm^2)	Total Area (cm^2) of < 20 mm Roots		
Mean	3958.5	13135.5	9188.4	26282.5		
Std. Dev.	4889.8	14522.3	10720.9	28999.4		
Min.	78.1 (*2y*)	376.1 (*2 y*)	42.2 (*2 y*)	517.7 (*2 y*)		
Max.	19745.3 (*12 y*)	54953.3 (*12 y*)	34653.0 (*14 y*)	101014.0 (*12 y*)		
	Length (cm)	Length (cm)	Length (cm)	Total Length (cm) of < 20 mm Roots		
Mean	16798.4	12818.6	1890.9	31507.9		
Std. Dev.	20750.3	14171.9	2206.3	36074.5		
Min.	331.2 (*2 y*)	367.1 (*2 y*)	8.7 (*2 y*)	797.2 (*2 y*)		
Max.	83791.4 (*12 y*)	53627.4 (*12 y*)	7131.5 (*14 y*)	142834.4 (*12 y*)		

[a]Dry Weight (g)

[b]Age of individual tree (years) sampled

Table 3. Dimensional relations of multi-trunked mulberry trees growing in PAH-contaminated sludge (n=2).

	Diameter at Soil Level (mm)	Tree Rings (age)	Tree Height (cm)	Canopy Spread (cm)	Shoot Biomass Dry Weight (g)
#10[a]	610	16	620	600	191275.9
#31[b]	620	15	500	560	100305.6

	Leaf Biomass Dry Weight (g)	Leaf Area (cm^2)	Maximum Root Depth (cm)	Maximum Root Spread (cm)	Orthogonal to Maximum Root Spread (cm)
#10	16082.6	2097175.7	150	1200	960
#31	7499.3	977913.4	100	1200	1200

[a]#10 Diameter at soil level = 610 mm; diameter above root swell (DARS) consisted of three main trunks (256 mm, 175 mm, 138 mm).
[b]#31 Diameter at soil level = 620 mm; diameter above root swell (DARS) consisted of two main trunks (339 mm, 231 mm).

Table 4. Dimensional relations of multi-trunked mulberry tree roots growing in PAH-contaminated sludge (n=2).

	< 1.5 mm Roots	**1.5-5 mm Roots**	**5-20 mm Roots**	**> 20 mm Roots**	**Trunk Roots**	**Total Roots**
Tree ID	Root DW[a] (g)	Root DW (g)	Root DW (g)	Root DW (g)	Root DW (g)	Root DW (g)
#10	698.5	3188.1	9512.5	38790.5	15328.6	67518.2
#31	534.6	2647.2	8615.7	17408.6	6801.5	36007.6
Tree ID	% of Total	% of Total	% of Total	% of Total	% of Total	Root:Shoot Ratio
#10	1.0	4.7	14.1	57.5	22.7	35.3
#31	1.5	7.4	23.9	48.3	18.9	35.9
Tree ID	Volume (cm^3)	Volume (cm^3)	Volume (cm^3)	Total Volume (cm^3) of < 20 mm Roots		
#10	929.1	11838.1	30735.3	43502.5		
#31	711.1	9829.6	27837.7	38378.4		
Tree ID	Area (cm^2)	Area (cm^2)	Area (cm^2)	Total Area (cm^2) of < 20 mm Roots		
#10	49558.5	146228.7	121700.1	317487.2		
#31	37929.8	121419.2	110226.7	269575.7		
Tree ID	Length (cm)	Length (cm)	Length (cm)	Total Length (cm) of < 20 mm Roots		
#10	210306.5	142700.5	25045.4	378052.4		
#31	160959.0	118489.7	22684.2	302132.8		

[a]Dry Weight (g)

Table 5. Dimensional relations and regression summaries of mulberry trees growing in PAH-contaminated sludge (n=29). Log transformed linear regression model: $\log_{10} y = m \log_{10} (x) + b$, where x = DARS[a] (mm).

Biomass Dry Weights (g)

	Roots							
	< 1.5 mm	*1.5 - 5 mm*	*5 - 20 mm*	*> 20 mm*	*Trunk*	*Root Total*	*Leaf*	*Shoot*
m	1.885	1.937	2.340	3.883	2.190	2.406	1.820	2.401
b	-1.925	-1.291	-1.688	-4.764	-1.482	-1.276	-0.526	-0.697
r^2	0.872	0.954	0.944	0.900	0.967	0.969	0.970	0.964
e	0.247	0.145	0.194	0.441	0.137	0.147	0.109	0.158
p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Length / Height (cm)

	Roots								
	< 1.5 mm	*1.5 - 5 mm*	*5 - 20 mm*	*< 20 mm Total*	*Maximum Depth*	*Maximum Spread*	*Orthogonal Spread*	*Tree Height*	*Canopy Spread*
m	1.885	1.937	2.340	1.927	0.319	0.782	0.709	0.428	0.721
b	0.554	0.360	-1.268	0.767	1.303	1.127	1.049	1.860	0.751
r^2	0.872	0.954	0.944	0.931	0.412	0.841	0.769	0.746	0.815
e	0.247	0.145	0.194	0.179	0.130	0.116	0.133	0.085	0.117
p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0002	<0.0001	<0.0001	<0.0001	<0.0001

Area (cm^2) / Volume (cm^3)

	Roots Area					Root Volume			
	< 1.5 mm	*1.5 - 5 mm*	*5 - 20 mm*	*< 20 mm Total*	*Leaf Area*	*< 1.5 mm*	*1.5 - 5 mm*	*5 - 20 mm*	*< 20 mm Total*
m	1.885	1.937	2.340	2.028	1.820	1.885	1.937	2.340	2.147
b	-0.074	0.371	-0.581	0.497	1.590	-1.802	-0.721	-1.179	-0.621
r^2	0.872	0.954	0.944	0.962	0.970	0.872	0.954	0.944	0.964
e	0.247	0.145	0.194	0.137	0.109	0.247	0.145	0.194	0.141
p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[a]DARS: diameter above root swell.

Table 6. Projected growth and transpiration potential of mulberry trees growing in contaminated soils.

Tree Age	Leaf Biomass[a]	Leaf Area[b]	Transpiration Rate[c]	Water Loss / Day
3	28.3	3690.7	0.115 ml cm^{-2} day^{-1}	0.4 L
5	241.1	31495.2	0.115 ml cm^{-2} day^{-1}	3.6 L
10	1500.2	195951.0	0.115 ml cm^{-2} day^{-1}	22.5 L
15	3665.5	478780.0	0.115 ml cm^{-2} day^{-1}	55.1 L

[a]grams dry weight
[b]total leaf surface area (cm^2)
[c]average midsummer transpiration rates for various trees and shrubs in three geographic locations (Krammer and Boyer, 1995)

Table 7. Projected growth, rooting patterns, and root-associated bacteria of mulberry trees growing in contaminated soils.

Tree Age	Root Biomass[a]	Root Depth (cm)	Root Length[b]	Root Surface Area[c]	Root Volume[d]	Rhizoplane Bacteria[e]	Rhizosphere Bacteria[f]	Root Bacteria Totals[g]
3	21.8	44.6	725.0	501.3	51.4	7.89×10^{10}	2.61×10^{10}	1.05×10^{11}
5	370.4	65.0	7018.2	5465.3	645.3	1.34×10^{12}	4.44×10^{11}	1.79×10^{12}
10	4151.0	89.5	48618.5	41903.5	5575.9	1.50×10^{13}	4.97×10^{12}	2.00×10^{13}
15	13522.9	104.7	125198.8	113391.4	15996.0	4.90×10^{13}	1.62×10^{13}	6.52×10^{13}

[a]total root biomass (g)
[b]total root surface area (cm^2) having a diameter of < 20 mm, excluding root hairs
[c]total root volume (cm^3) having a diameter of < 20 mm, excluding root hairs
[d]total root length (cm) having a diameter of < 20 mm, excluding root hairs
[e]rhizoplane bacteria numbers colonizing root surface (Rouatt and Katznelson, 1961)
[f]rhizosphere bacteria found in soil directly adhering to the roots (Rouatt and Katznelson, 1961)
[g]total of rhizoplane and rhizosphere numbers (Rouatt and Katznelson, 1961)