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This work and its defense approved by:

Chair: Dr. Jodi R. Shann

Dr. Brian K. Kinkle

Dr. Steven H. Rogstad

Dr. Joseph A. Caruso

Dr. N. V. K. Kumar and Dr. Lin Liu

Natural Revegetation of an Aged Petroleum Landfarm Impacted with PAHs and Heavy Metals: Ecological Restoration, Remediation and Risk

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Heather Fort Henry

B.S., University of Rochester, 1997
M.S., University of Cincinnati, 2002

Committee Chair: Dr. Jodi R. Shann

ABSTRACT

Ecological restoration of hazardous waste sites is a potential remediation strategy that has not been well documented. Here, we assessed natural plant community development and soil remediation on an aged petroleum refinery land treatment unit (LTU) containing recalcitrant environmental pollutants. Preliminary assessment of phytotoxicity using bioassays (*Lactuca sativa* L. and *Solidago canadensis* L.) indicated that some tolerant phenotypes would grow on LTU soil. Fourteen permanent plots (37 m²) were then established onsite to assess actual plant succession and remediation: 11 for study of natural succession and 3 to act as a control by removal of vegetation. Two soil cores were removed annually from each plot, analyzed for edaphic factors and then sequentially extracted for metals and PAHs. Analysis of contaminants indicated a 50% reduction of total petroleum hydrocarbons (TPHs) and polycyclic aromatic hydrocarbons (PAHs) in surface soil of vegetated and unvegetated plots. There were no significant changes in total metal loadings. Metal content in plant root and shoot tissue was highly variable between species, but still low relative to soil levels, verifying the low bioavailability estimated from soil extracts. Plots were subsampled (1 m^2) monthly for cover and abundance during the growing season, and for biomass at the end of the season. Monthly measurements of plant variables indicated that species richness increased from 28 to 57 species, cover increased from 33 to 79%, and biomass increased by a factor of four over three years. Plant growth was correlated to spatial and microclimatic factors, but contaminant loading showed no correlation. In fall of the following year, both LTU and a nearby unpolluted plant community of comparable size and successional stage were sampled as before: cover and abundance were measured in triplicate subplots (1 m^2) within eleven plots. There were no significant differences in richness and percent cover between the sites. State-listed invasive

species were less abundant onsite than offsite. Broader implications of these results suggest that other abandoned waste sites may be candidates for ecological restoration by natural succession. This study is unique in its field-scale demonstration of the potential of natural plant revegetation (passive ecological restoration) as a means of phytoremediation and phytostabilization of aged contaminated sites.

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CHAPTER 1

LITERATURE REVIEW:

NATURAL REVEGETATION AS A MEANS OF PHYTOREMEDIATION

HEATHER F. HENRY AND JODI R. SHANN

Department of Biological Sciences ML6, University of Cincinnati, Cincinnati, OH 45221-0006

INTRODUCTION

Phytoremediation (the use of plants to reduce environmental contamination) is a long-term treatment process that has traditionally been managed using high-maintenance, short-term agricultural techniques including the planting of selected species. However, natural plant communities undergo a process known as secondary succession. If allowed to proceed without input or maintenance, succession may provide the cover and stability for eventual ecological restoration (the return of ecosystem structure and function following disturbance) without the need for such costly plantings. This thesis is a case study of ecological restoration by natural revegetation of a hazardous waste site. The study site is a "landfarm" that received petroleum refinery waste, consisting of various heavy metals and hydrocarbons, specifically: chromium, copper, lead, nickel, zinc, and polycyclic aromatic hydrocarbons (PAHs). PAHs form when hydrocarbons are heated at very high temperatures, conditions that occur during petroleum processing. Metals, present in small abundance in crude oil, become concentrated in sludges as petroleum is processed (Deuel & Holliday, 1998). PAH compounds, characterized by fused benzene rings, are often carcinogenic (Brady & Weil, 1999). Heavy metals (e.g. lead, chromium, and zinc) cause health problems in a variety of organisms by interfering with metabolic pathways (Kahn et al., 2000). Both PAHs and metals have been shown to negatively impact both flora and fauna (Erickson et al., 1999; Lepp et al., 1997; McBride, 1994; Porta et al., 1999). Although both pose health risks individually, they are also thought to be comutagenic (Feng et al., 2003). Nonetheless, on many aged sites, the contaminants are sequestered to soil particles and so risk is greatly reduced. On such sites, allowing the

establishment of vegetation from the surrounding plant community may be a low risk management strategy that will lead to ecological restoration.

SOIL AND CONTAMINATION

We depend on soil for our health and survival. Despite its pivotal role, there is much we do not understand about this complicated matrix. All soils, though unique and individually complex, have similar components: they are a mixture of solid, liquid (water) and gas (air). The solid components are the weathered minerals of the underlying parent rock and accumulation of organic matter from plant and faunal decay. Because of this physical structure, soils have the ability to retain, release, and sequester nutrients and matter that include uncharged materials as well as those that may be more or less hydrophobic or hydrophilic. Soils, especially those high in clay and organic matter, can create tight chemical bonds with contaminants which become locked in the interstices of the soil matrix (Brady & Weil, 1999). The sequestration of many contaminants is known to increase with time as soils "age". This aging process is thought to decrease the physical mobility and with that, the bioavailability to living systems (Nam & Alexander, 2001). As mobility and bioavailability decrease, so does the risk of exposure.

There are thousands of "heritage" contaminated sites in the U.S. These abandoned, aged sites (such as Superfund, RCRA and brownfields) have become the taxpayers' burden since the responsible party is no longer available (Bradshaw, 1997). With an increased awareness in the link between untreated hazardous waste sites and human diseases, there has been a demand for treating these otherwise forgotten sites. Since hauling and removal of these aged soils often puts more people at risk through

inhalation or by mobilizing contaminants through altered hydrology, *in situ* (in place) methods of remediation are preferable for these aged sites. The goal of *in situ* remediation is to decrease contamination (extraction or degradation) or risk (stabilization), and perhaps restore the ecological integrity of the site. Numerous *in situ* methods exist for the treatment of aged contaminated soil (Hamby, 1996). Within an acceptable risk scenario, the chosen technique depends on the stakeholders and cost.

PHYTOREMEDIATION

Phytoremediation is an *in situ* remediation strategy that is gaining support for its cost effectiveness and popularity with stakeholders (Bradshaw, 1997; Glass, 1999). Phytoremediation is the use of plants and plant-associated microbes to reduce or stabilize contaminants in soils. One of the advantages of phytoremediation is that no new hazardous waste is created (as would be the case with chemical extraction) and the contamination is gradually decreasing (as opposed to cement capping) (Hardisty & Brown, 1999). Communities, when given the chance, often choose to use phytoremediation because of the greenspace it creates. Phytoremediation has been used to remediate all classes of contaminants: organic, inorganic and radioactive. One of the greatest advantages of phytoremediation is that it is capable of treating mixtures of contaminants. Most actual sites are contaminated with soils that have both metals and organics hence phytoremediation has been explored for mitigation of many of these sites (Forstner, 1995).

Phytoremediation emerged from the practice of landfarming: a waste management strategy that involves maintaining a vegetative cover above waste materials to prevent

erosion. It was soon realized that the cover crops actually aided in contaminant loss by providing a soil environment for microorganisms that enhanced biodegradation. The zone of soil under the influence of plant roots is the rhizosphere. The rhizosphere is almost always aerobic, and is known to contain greater magnitudes of bacteria numbers than that found in non-rhizosphere soil (Rovira & Davey, 1974). Plant roots release carbon substrates and retain moisture both thought to stimulate microbial growth. All of these characteristics aid in the microbial degradation of contaminants. Hence, the use of plants on these sites became a remediation strategy as opposed to simply a management strategy (Hejazi et al., 2003).

Initially it was assumed any plant species could be used for phytoremediation, as long as it provided adequate cover. Since all terrestrial plant rhizospheres have the same general qualities (with a few exceptions), it was thought any plant would work. However, numerous studies have revealed the importance of plant species selection to attain a particular remediation objective such as degradation, extraction, or stabilization (Fletcher & Hedge, 1995; Freitas et al., 2004; Hedge & Fletcher, 1996; Huang & Cunningham, 1996; Macek et al., 2000; Prasad, 2003; Shann, 1995; Shann & Boyle, 1994; Yanqun et al., 2003; Ye et al., 2000). The objectives can be used to select the dominant remediation mechanism suitable (phytodegradation, phytostabilization and phytoextraction, to name a few) and the most appropriate vegetation.

Phytodegradation refers to plant-assisted biodegradation of organic contaminants. Plant species with root systems that accommodate large bacterial communities are often associated with high soil degradation rates (Gunther et al., 1996). The quantity of microbes is not always the most important factor. Plants appear to select for unique

degrading microbial communities through the release of root exudates into the rhizosphere (Yoshitomi, 2001). Exudates take the form of a wide variety of compounds (amino acids, sugars, and phenolics) and can be species-specific (Alexander, 1977). In some plant species, exudates resemble the chemical nature of the soil pollutant and therefore, naturally support a microbial community adapted to utilizing the pollutant as a food source (Leigh et al., 2001). In a similar fashion, plant species with high root turnover stimulate populations of bacteria/fungi that feed on recalcitrant organic material (Reilley et al., 1996).

Phytoextraction refers to using plants that accumulate harvestable quantities of contaminants in aboveground tissue, that is then removed, leading to a net reduction of the contaminant in soil. This process is generally reserved for metals and radionuclides since organic molecules, such as PAHs, are not translocated into aboveground tissue (Porta et al., 1999). These plant species, called hyperaccumulators, are rare (Whiting et al., 2004). Many plant species that hyperaccumulate metals (i.e. metal content is significant portion of total biomass) were discovered by surveying the plant community naturally established on metalliferous soil. Most plants can accumulate (and may translocate) available metals to a lesser extent than the hyperaccumulators. Certain plant families seem to possess similar metal-accumulating attributes, indicating that the mechanism is conserved. For example, members of the Brassicaceae and Asteraceae have been the focus of research for use in metal phytoremediation because of numerous examples of metal accumulators in each family. Phytoextraction by these species may not be efficient enough to warrant harvesting – but may instead serve as an entry point in the food chain, and, therefore, an increased exposure risk (Whiting et al., 2004).

A similar process, called phytostablization, optimizes growth of plant species that sequester contaminants without translocation into aboveground tissue. Especially where herbivory may lead to food-chain contamination, it is not desirable to grow plants that bioaccumulate metals. Plants used for phytostabilization detoxify metals through immobilization or alteration of the oxidation state to a less toxic form. Stabilizing may involve living plants or the soil organic matter that forms as plant tissue decomposes and humifies.

LIMITATIONS TO PHYTOREMEDIATION

Phytoremediation may be limited by contaminant bioavailability. The degree of uptake, degradation, or toxicity depends on the bioavailability of the contaminant. The most simplistic definition of "bioavailablity" is the proportion of total contaminant in a form (physical or chemical) that is capable of interacting with biota. However, since organisms vary in their ability to access material in soil, the proportion of any compound that is bioavailable depends on the organism at hand. Solubility of the contaminant is a dominant factor in most models of bioavailability. Water soluble compounds likely partition into the soil solution (pore water) where they would be available to living systems. An empirical assessment of fate, the K_{ow} (octonol-water partition coefficient), is the proportion of a compound that dissolves into water relative to octanol. Octanol is a surrogate measure for partitioning into soil organic matter or lipid membranes. The K_{ow} estimates, for example, the capability of a contaminant to enter a microbial cell, the real limitation of bioavailability of a compound to microbes in soil is contact between the organism and the compound (Verstraete & Top, 1999). Water soluble contaminants

move through water passively by plant-, gravity- or precipitation-driven diffusion and may therefore increase contaminant contact with indigenous plants and microorganisms.

In phytoremediation, any toxic effect of the contaminants on the microorganisms or plants (a phenomenon called biotoxicity) may hinder the rate of remediation. For essential and nonessential compounds alike, there exists a threshold level above which natural processes are impeded. Numerous bioassays can be used to assess how toxic a compound is to particular organisms, but in field settings, the degree of toxicity of the contaminant depends on its bioavailability to the organism. This is usually only of minor concern for sites that are contaminated with organic contaminants alone. For the most part, PAHs are not phytotoxic with the exception of the 2-ring PAH, naphthalene. However, despite the acute toxicity of naphthalene, its volatility precludes any long-term detrimental effects (Henner et al., 1999). In any case, it is unlikely that such volatile compounds would persist on aged sites. Of real concern, therefore, is the toxicity of metals to plants and organisms. Various aspects of metal and PAH bioavailability and biotoxicity (both individually and as mixtures) are addressed below.

HEAVY METALS: Bioavailability, Toxicity and Remediation

Heavy metals are elemental contaminants classified as having density greater than 6 g/cm^3 (Davies, 1994). Because of their chemical structure, metals have the ability to form complex molecules. Hence, living systems have evolved to use metals, at low "trace" concentrations, as co-enzymes for important biological reactions (Nies, 1999). Zinc, for example, is essential to all living systems. Lead, on the other hand, has no known biological function. In some cases, biological mechanisms to acquire essential

metals from the environment also bring in non-essential metals. Immobilizing the non-essential metals comes at a metabolic cost. Even essential metals, if present at high concentration, can be toxic. Not surprisingly, plants and microbes have evolved mechanisms to regulate metal uptake to fulfill biological functions without causing toxic effects. As such, the environmental fate of metals in soil is complicated by many factors: soil composition, the metal valence and oxidation state, and plant or microbe-mediated metal transport.

Metal Bioavailability

Soil properties have a large effect on metal bioavailability. Relatively speaking, the higher the pH, clay content and organic matter, the less available metals are to plants (Greger, 1999). Clays and organic matter generally have negative charged surfaces that act to bind and immobilize metals in the soil matrix. The charges on clay are not changed by pH, while those on organic matter arise from acids found on fulvic (FA) and humic. At a pH above 4.5, fulvic and humic acid groups dissociate and carry a negative charge. As pH is lowered, the excess H^+ ions compete for and replace metal cations adhering to soil or organic matter.

The fate of a metal in soil depends on its structure and affinities. Unlike the other metals in this study, zinc remains a divalent cation under normal soil conditions. Zinc tends to persist in soil solution, a property that facilitates movement into groundwater or into living systems. Lead and copper, on the other hand, readily complex with fulvic acids and therefore have a shorter retention time in soil solution (Greger, 1999).

Chromium (especially Cr^{3+}) readily precipitates in soil solution and therefore is most likely to be adsorbed onto clay particles (Peters & Shem, 1995).

Metal Toxicity

As noted before, plants and microbes have evolved mechanisms to alter metal (or cation) availability in soil. Metals, if present in toxic levels, can interfere with normal enzyme functions in plants and microbes which leads to decreased metabolism and growth (Doelman & Haanstra, 1979; Evdokimova & Mozgova, 2003; Forstner, 1995; Meager, 2000). In both plants and microbes, the level at which a particular metal is toxic varies widely from species to species. Previous experiments using soil from the site used in this study found a negative correlation between microbial growth and heavy metals in aged soil (Gomez, 2001). This is typical of findings by Giller (1998) and Nordgren (1988). Metal resistance genes are commonly found on mobile genetic elements. Hence, it is possible for microbes to transfer metal resistance by plasmids or through mutation, processes that are known to occur in soil microbial communities (Silver et al., 2001). However, the extent to which this occurs in the microbial community and/or the influence it may have on a community level is not fully understood.

The coevolution of plants with microbial communities has led to a variety of microbial-mediated metal resistance mechanisms. It has been suggested that mycorrhizae (fungi associated with plant roots) act as a protective barrier against what would otherwise be phytotoxic metal concentrations in soil (Aggangan et al., 1998; Gadd et al., 2001; Jacquot et al., 2000; Kidd et al., 2004; Leyval et al., 1997). This is not only true for obligate mycorrhizal plants: the inoculation of arbuscular mycorrhizal fungi (AM) to

metal-rich soil also improved the growth of non obligate-mycorrhizal plants (Hetrick et al., 1994). Without a metal-tolerant strain of *Rhizobium*, white clover growth is limited when planted in metal enriched soils (Zn, Cd, Zu, Ni) (Giller et al., 1998). Though the known role of AM in mobilizing plant nutrients complicates the interpretation of these results, it is clear that metal toxicity is less acute in their presence.

Since metals can clearly inhibit microbial function and plant growth, most groups have evolved tolerance mechanisms. Certain exudates bind to metals to prevent uptake while other exudates bind to metals specifically to increase uptake. Metallothionins (MT) and phytochelatins (PC) are cysteine-based molecules produced by plants to immobilize metals both in plant tissue and in the environment. The size and charge affinities of these molecules make for marginal metal specificity, though many are nonspecific (Meager, 2000). They effectively immobilize toxic cations within the plant. There is evidence these metal-complexes, sequestered in the vacuole, aid in regulation of membrane action potential. Plants also respond to elevated soil metal content by increased suberization or increased production of phenolic compounds (Piechalak et al., 2003).

Metal Remediation

Metals cannot be degraded, therefore remediation efforts require either removal or stabilization of metals at a given site. Due to the metal-binding capabilities of organic matter, it is generally believed that any plant community with considerable biomass will increase the soil litter layer and aid in the stabilization of metals. Studies have found a correlation between organic matter and loss of metal extractability in sequential

extractions (Kabata-Pendias & Pendias, 2001; Narwal & Singh, 1998). Sequential extractions gauge the extractability of metal from various components of the soil matrix: ion exchangeable, organic bound and surface sorption (Sposito et al., 1982; Tessier et al., 1979). Hence it can be used as a proxy for bioavailability. Total metals can then be determined separately or with additional extractions performed on the same soil used for bioavailability.

PAHs: Bioavailability and Remediation

PAH hydrophobicity and environmental persistence tend to increase with the number of rings. PAHs result from incomplete combustion of organic material from natural sources such as volcanoes, the burning of biomass, and from anthropogenic sources such as car exhaust and industry. These compounds are common air, water, soil and sediment pollutants. Because of the stability of the benzene structure, these compounds are recalcitrant and have half lives in soil from 16 to 535 days depending on the PAH.

PAH Bioavailability

As a class, PAHs, have high K_{ow} values and a very limited water solubility (between 4.7 to 6.8) (ASTDR, 1995). Hence PAHs are not generally found in solution and are unavailable to plants (Pearlman et al., 1984; Reilley et al., 1996; Sims & Overcach, 1983). Phytotoxicity from PAHs is only reported for low molecular weight compounds (2-ring volatile) (Henner et al., 1999). On an aged site, it is unlikely that

such volatile compounds would persist unless partitioned into other hydrophobic portions of soil. The real concern, then, is the toxicity of metals to plants and microorganisms.

In general, the higher the log K_{ow}, the more likely a compound partitions into the organic components of soil and sediments. The degree of adherence of a PAH to the soil substrate is important. As previously noted, this increases with time, a process known as aging. The sorption of PAHs is a function of the soil itself. It is dependent on the organic carbon content and surface area of the sorbent particles. Sorption also varies with the properties of the individual PAHs. Klotter and Anderson (2001) found a trend which suggests that the degree of sequestration increases with molecular size and K_{ow} (Kottler et al., 2001). Half-lives positively correlate with log K_{ow} and negatively correlate with water solubility.

Often residual tar-like materials associated with petroleum-based contamination make the prediction of PAH half-lives in field studies challenging. These asphaltenes are not specifically regulated. Given their hydrophobic nature, it is likely that PAHs are partitioning within these hydrophobic compounds within soil micropores. These residues have shown a negative impact on PAH degradation, due to a loss of PAH extractability within asphaltene complexes (Uraizee et al., 1997).

PAH Remediation

PAHs have been shown to degrade under both aerobic and anaerobic conditions in soils. Anaerobic degradation is considerably slower. Bench scale mineralization studies provide evidence of the microbial degradation of PAHs; however less is known about degradation *in situ*. In some studies, bacterial degradation has been shown to account for

almost all PAH transformation but white rot fungi, decomposers of lignin, also degrade PAHs (Šašek et al., 1999). Since monitoring endpoints on actual contaminated (and regulated sites) require reporting of the parent PAH compound and not necessarily the residual byproducts, not much is known beyond the parent compound, especially in field conditions.

Of all PAHs, the pathways of degradation of anthracene, benzo[a]pyrene and phenanthrene by microorganisms are the best characterized (Sims & Overcach, 1983). However, the pathways vary depending on whether the degradation is attributed to bacteria or fungi: the former forms a cis-dihydrodiol through dioxetane intermediates; whereas eukaryotic pathways produce arene oxide intermediates (Sims & Overcach, 1983). Microbial biomass, diversity and activity have been found to play a role in PAH degradation; the importance of having at least one PAH degrading bacteria present in soil may outweigh other microbial community factors (Grosser et al., 1991). Microbes that possess surfactants that mobilize PAHs, have been under investigation, though it is not yet clear whether they actually degrade PAHs (Dua et al., 2002; Willumsen & Karlson, 1997).

The plant rhizosphere, high in microbial diversity and biomass, is a soil environment rich in oxygen, nutrients and cofactors. Hence, the presence of plants creates conditions favorable to biodegradation. Many studies have shown evidence of higher degradation in the rhizosphere than in bulk soil (Liste & Alexander, 2000; Yoshitomi, 2001). However, there are specific aspects of plants associated with increased degradation such as root size, plant phenology and plant exudation patterns. In many studies, a fine, fibrous root system with a high turnover is critical for

phytoremediation (Olson et al., 2003). This is, apparently, more important than crown or root biomass. Plant lifecycle stage is also important. In clover, reduction of contaminants was greatest at the time of senescence and root turnover, indicating that the easily degradable carbon was consumed first, leaving the more complex, recalcitrant forms of carbon for later degradation in the plant-microbe-soil lifecycle. This demonstrates the importance of root turnover (Banks et al., 2000). The composition of exudates released from plant roots also plays a significant role in PAH degradation (Leigh et al., 2002; Shann, 1995). Exudates (sugars, amino acids and other photosynthates) may act as growth substrates for PAH degraders. Some phenolic secondary compounds, released as exudates, have been shown to select for microbial communities that degrade PAHs, indicating further that plant specificity plays an important role in stimulating degradation (Leigh et al., 2002).

MIXTURES OF PAHs AND METALS

As indicated earlier, one advantage of phytoremediation is that it can simultaneously stabilize metals while aiding in the degradation of organic compounds. The interactive effect of mixtures of contaminants for degradation is not fully understood. Microbial communities have wide ranges of metal resistance and often the metal concentration that causes an inhibitory effect in one community is within the resistance range of another. There may not be any loss of PAH degradation capability if the degrading population is resistant to the metal contaminant. However, metal toxicity reduces microbial degradation of plant organic products such as glucose, straw, plant residues, and cellulose (Giller et al., 1998). These organic substrates usually lead to

exponential growth of microorganisms. However, in metal contaminated soil, this exponential growth phase may be delayed for years in aged or freshly added metal enriched soils (Doelman & Haanstra, 1979; Haanstra & Doelman, 1984; Nordgren et al., 1988). Similarly, it has been observed that metal-toxicity may slow PAH biodegradation (Forstner, 1995). Other studies present the argument that when initial biodegradation is slow, relatively more organic compounds become sequestered and may prevent full degradation for an even longer duration (Nam & Alexander, 2001). As such, it is thought that the residence time of organic contaminants may be longer at hazardous waste sites where other inorganic pollutants may have a toxic effect on microbes (Bossert & Bartha, 1986).

PHYTOREMEDIATION MANAGEMENT

In the preceding discussion of soil contamination and phytoremediation it is clear that factors such as bioavailabity and toxicity may limit the rate of biodegradation. Relative to other forms of remediation, phytoremediation is a slow process taking years as opposed to months. Over time, contaminant sequestration and the concentration of the bioavailable pool decrease. As concentrations of bioavailable contaminants decrease, so do their rates of degradation. Removal of all contaminants, therefore, is not usually achieved. Nonetheless, if sustainable for the time period required, phytoremediation could result in reduced contamination, increased stabilization and lowered risk. Sustainability, then, becomes important to the effectiveness and cost of the system. In general, cultivars chosen for phytoremediation have qualities such as root length or drought resistance, but are often inbred and lack the genetic plasticity to adapt to local

conditions. Because they are planted as a monoculture, these species strip the soil of nutrients and are sensitive to pestilence (Ewel, 1999). Expensive irrigation, fertilization, and reseeding on plots being remediated are often required to maintain the 90% plant cover required by the U.S. EPA (Condit & Doherty, 2000). Furthermore, the heavy farm equipment used for maintaining the monoculture can damage vegetation, adding stress to the cultivars. While these inefficiencies can be overcome using standard agricultural practices, the resulting system is not self-sustaining or cost effective.

This study introduces an alternative management strategy for future phytoremediation efforts: to allow self establishing *in situ* species to revegetate contaminated soil. Thus the need for mowing, tilling, irrigating, fertilizing, and reseeding would be eliminated or minimized. Self-establishing species are tolerant of the microclimatic conditions and should have, collectively, greater genetic plasticity than agriculturally-obtained plants (Ewel, 1999). It is likely that species colonizing contaminated sites will tolerate the soil and participate in remediation and stabilization of contaminants (Cunningham & David, 1996).

The establishment of *in situ* vegetation would naturally occur through the process of secondary succession. Secondary succession, the changes in plant community following a disturbance, generally follows a predictable pattern. In the first few years following disturbance the community that forms will be dominated (both in number and cover) by species that are particularly tolerant of harsh conditions. They are acclimated to intense heat, light, nutrient-limitations, and poor soil making them more competitive in extreme environmental conditions. These early succession species are sometimes referred to as r-selected individuals, investing a majority of their energy into reproduction. As a

consequence, less input is given to root production, or other long-lasting vegetative growth. These species provide low biomass at a high turnover rate, reseed easily and generally improve soil conditions (increased carbon, moisture and shading). Annual grasses and weedy forb species are particularly common in early succession. Given their reduced biomass, and lifespan, these early colonists are gradually replaced by species with greater biomass and longer lifespan referred to as middle to late succession species (Odum, 1963). The latter exhibit a K-selected life strategy, meaning the rate of growth of each individual is slower with more input into long-lasting vegetative growth. Tree seedlings, biennials and perennials are examples of mid to late succession species. Though present at the beginning of succession, they become more dominant given their continually expanding crown cover and root extent. If secondary succession proceeds, the established community should provide the long term cover necessary for the duration of land treatment.

It is common for plant communities to develop on abandoned hazardous sites, but very little is known about the ecology of these systems (Barazani et al., 2004; Freitas et al., 2004; Olsen & Fletcher, 2000). Relative to non-contaminated communities, it has been reported that plant communities growing in contaminated soil have decreased vegetative cover, increased exotic species, and lower plant diversity (Forstner, 1995; Galbraith et al., 1995; Porta et al., 1999; Riley & Banks, 1996). The presence of potentially toxic contaminants, and the impact of environment and time on these chemicals may alter establishment and community development from normal patterns (Depledge, 1999). Essentially, the community is not progressing through the stages of

succession. Such communities may become dominated by invasive species which may further prevent later successional species from establishing and/or reaching maturity.

There are many reasons why plant communities growing on contaminated sites may show reduced growth and/or succession. In cases where metals are present, often the concentration in plant tissue is higher than in offsite species (Barazani et al., 2004). The metabolic toxicity cost for immobilizing metals in above-ground tissue may result in reduced biomass and loss of floral production. Further, germination of seeds may be reduced due to soil toxicity. Toxicity of metals to mutualistic bacteria or fungi may prevent or reduce host plant growth.

Ecological restoration refers to the passive or assisted return of disturbed communities to their original pre-disturbance state. Restoration and succession may be identical processes, or restoration may be a result of intervention and management. As mentioned in the previous section, plants have developed strategies to deal with metals; however, the metal levels found in contaminated soils are often much higher than in natural systems (Forstner, 1995).

In many ways, ecological restoration and phytoremediation are similar. In particular, both share the common goal of reestablishing ecosystem quality - the former, in terms of plant systems, the latter in terms of environmental systems. Ecological restoration of hazardous waste sites is a desirable endpoint, but the dominant processes that govern that restoration have not been clearly identified (Doelmann et al., 1999). It is important to document the existing vegetation at a site locale (species, growth characteristics, prevalence) to select species that will most likely thrive in the given climate and soil conditions with the least input. Though this step is commonly

disregarded, assessing the naturally establishing species should theoretically be the most sustainable choice for site restoration. It is possible that metal tolerant species are present in other nearby plant communities but, by random processes, seeds did not reach the contaminated site. For this reason, it is important to know which early successional species tolerate conditions and may be used at other restoration locations. Hence restoration efforts are beginning to pay more attention to the natural community that establishes on disturbed sites (Kearney et al., 1999; Palmer et al., 1997; Parrotta & Knowles, 2001).

Despite obvious advantages, more natural approaches to phytoremediation have not been adopted for three major reasons: 1) regulations, 2) lack of knowledge of ecotoxicological risks or beneficial effects, and 3) time. Currently, allowing revegetation is not an option for many regulated sites, though there are a few exceptions where this has been permitted. As discussed previously, ecological risks are minimized on aged sites, where natural processes have sequestered contaminants. Nonetheless, there is no distinction between aged and new sites because total contamination (as opposed to labile levels) is used to gauge site regulations. This conservative approach, though intended to be protective of human lives, results in an overestimation of risk associated with aged sites. Though ecological risk can be assessed using bioassays (e.g. in a lab setting), few hazardous waste site custodians wish to investigate *in situ* flora and fauna due to the liabilities they may encounter with negative results. Regulations may change as more is learned about the reality of ecotoxicological risks. Lastly, natural succession, the process by which plant communities form following disturbance, takes decades (Barbour et al., 1999). Hence, the systematic study of succession is limited, yet riddled with complicated

models proving and disproving the latest successional theories. Even less is known about succession on contaminated sites. This poses a challenge in terms of regulating possible "natural revegetation" sites: it is not clear what success criteria should be used given succession may take well over fifty years.

REVIEW OF DISSERTATION

As current research has been lacking on the use of natural successional vegetation in soil remediation, this study is a first step toward determining if natural revegetation is an effective alternative to managed phytoremediation. This field study investigated natural succession on an aged contaminated site following the lift of cover management. The general plan of research was to allow encroachment of the local vegetation onto contaminated soil. Initial contamination of a land treatment unit was assessed shortly after a final tilling event and at intervals across the duration of research. The plant community that colonized the contaminated soil was monitored to assess changes in plant community structure and function.

In the chapter that follows (Chapter 2), the potential for natural community establishment on the landfarm was assessed. Phytotoxicity tests were used as a first indication of the potential for natural plant populations to become established. In addition to a typical phytotoxicity bioassay, a common early succession species was tested on soil collected from the study site grown under greenhouse conditions.

In Chapter 3, the patterns of natural revegetation of an aged site were tested against environmental and contaminant loading. Plant diversity, richness, cover, and

biomass were measured as an indication of normal secondary succession. Richness, cover, and invasive incidence were compared to an offsite unpolluted site.

In Chapter 4, evidence of phytoremediation of soil contamination during natural revegetation of the site is presented. Bioavailability changes in metals as well as the degradation of organic compounds were assessed in relation to changes in the plant community.

Finally, Chapter 5 addresses the practical issues of the risk of metal uptake on contaminated sites undergoing ecological restoration. The plant community established on the contaminated site was compared to a non-contaminated site with equal time for revegetation. Differences in metal uptake and growth characteristics were compared between the onsite and offsite location. Finally, a summary of the findings of this study in the context of the feasibility of natural plant succession for the remediation of aged contaminated sites was discussed.

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CHAPTER 2

POTENTIAL FOR NATURAL REVEGETATION ON AN AGED PETROLEUM LANDFARM CONTAMINATED WITH HEAVY METALS AND PETROLEUM HYDROCARBONS

HEATHER F. HENRY AND JODI R. SHANN

Department of Biological Sciences ML6, University of Cincinnati, Cincinnati, OH 45221-0006

ABSTRACT

Contaminated sites approved for natural revegetation often use bioassays to gauge the phytotoxicity of remaining pollutants and, therefore, the necessity for a clean soil cap. One would expect plant species that appear at the early onset of succession to be more informative about a natural population's response to contaminants than a highly sensitive species typically used for bioassays. Soil from an aged petroleum refinery landfarm was assessed for phytotoxicity to determine if contaminants might inhibit natural revegetation of the site. Soil was separated by layers to determine the relative phytotoxicity of soil from the original sludge material (lower) layer and the overlying soil that had been altered from the original by years of landfarming activities. Phytotoxicity was assessed using soil from a highly contaminated area of the landfarm. A standard bioassay was used to measure the germination and root elongation response of a sensitive (*Lactuca sativa* L.) species subjected to water extracted soil supernatant from the upper, lower, and a topsoil control. Phytotoxicity to a local native species (*Solidago canadensis*, L.) was assessed by growing, directly in soil, seedlings that were collected from perimeter of the landfarm. Total extraction of total petroleum hydrocarbons (TPH) and polycyclic aromatic hydrocarbons (PAHs) and sequential extraction of metals were used to determine their content in the test soil. Sequential extractions were divided into the mobile (readily available to plants), labile (potentially available to plants), and residual (not available). The layers differed in metal extractability. The lower layer had higher chromium and nickel in the mobile fraction. The upper layer labile fraction had higher levels of copper and lead. Phytotoxic PAHs were not present in either layer; however, TPH was three times higher in the lower layer. For both the lettuce and goldenrod

bioassays, the lower layer of soil produced phytotoxic responses. The upper layer soil marginally inhibited measured growth variables in goldenrod, but did not decrease lettuce germination and root elongation. The low bioavailability and minimal phytotoxicity of contaminants did not preclude the use of natural revegetation for phytoremediation and site restoration. The establishment of goldenrod was monitored for three years as validation of the bioassay assessment. Initially, goldenrod was found in only two perimeter plots; however, by the end of the three year study period, it had become established throughout the entire site and within all of the study plots.

INTRODUCTION

In the United States, there are 450,000 brownfields, former industrial areas that have been abandoned for years but still contain a considerable amount of contaminants (EPA, 2004). Although redevelopment on these sites is limited by current regulations, management is more flexible. Allowing natural vegetation to establish on these contaminated sites could provide tremendous benefits. As organic matter increases with revegetation, stabilization of contaminants would reduce risk. With time, the process of community succession may lead to ecological restoration and increased habitat quality. Wildlife habitat, or "green corridors", would be created where previously none existed. The success of this strategy depends on the ability of natural revegetation to establish and continue to grow on a given contaminated site. The potential for natural revegetation would optimally be evaluated prior to management decisions, such as whether or not to apply a clean soil cap.

On aged soils, contamination may be significant, but unavailable due to sequestration of pollutants onto soil particles (Nam & Alexander, 2001). On sites where there are multiple metals present (and/ or other contaminants), overall risk is calculated as the sum of the risk due to each individual contaminant (Levert et al., 1995). Risk equations typically use total levels of contaminants. While this conservative strategy is protective of human health, it is well known that in aged soils, contaminants may have become tightly bound to organic matter and clay particles. Therefore, the use of total levels of contaminants may overestimate the real risk of the site by several orders of magnitude (Kottler et al., 2001).

In order to determine a more realistic estimate of biotic exposure to contaminants, sequential extraction can be used (Doelmann et al., 1999). Sequential extraction methods determine the proportion of labile contaminants, that is, those likely available to biota. For metals, a variety of solutions can be used for extraction of different fractions considered labile: exchangeable, organically bound, clay-oxide bound (LeClaire et al., 1984). Generally, the most available are the exchangeable and soluble metals (Kabata-Pendias & Pendias, 2001). However, plants may possess mechanisms to mobilize metals held in the labile form (Ernst, 1996). Metals that extracted in the final step of the sequential procedure (4M HNO_3) are thought to be unavailable to plants in natural settings.

Standard bioassays tend to utilize species of plants, microbes, or animals that are particularly sensitive to the contaminant at hand (Greene et al., 1989; Keddy et al., 1995). In other words, for a very conservative determination of metal toxicity to plants, one would use the lettuce bioassay since lettuce has a low tolerance to metals compared to

many plants. The advantage of measuring the response of an organism over a simple analysis of the toxins present, is that the synergism or antagonism of multiple stressors can become evident. Together with analysis of compounds found in sequential fractions, the bioassay gives an idea of potential toxic effects that might be anticipated in the field.

The lettuce bioassay is good for a general assessment of phytoxicity, but for assessing the potential for natural revegetation, it is important to evaluate the tolerance of typical pioneer species. Natural populations have considerable genetic plasticity allowing for successful establishment in a variety of environments (Ewel, 1999). Given the random process of seed arrival in the early successional community on the contaminated site, one would expect some individuals to be sensitive while others may be resistant to elevated levels of metal and petroleum hydrocarbons (Crosby, 1998). Hence, it is likely these early successional species may have a different response than those typically used in bioassays.

This study evaluated the potential for success of passive natural revegetation on an aged petroleum refinery landfarm. An initial assessment of the range of total metal and petroleum levels was made throughout the selected 2.2 hectare site. Soil was then collected from an area of high contamination and used in bioassay studies to determine bioavailability and phytotoxicity. Establishment and growth response of two species, lettuce (*Lactuca stativa* L.) and goldenrod (*Solidago canadensis* L.) were compared. The goldenrod is a common early succession species found in the region. Finally, the validity of the goldenrod bioassay was verified by subsequent survey of goldenrod establishment onsite.

MATERIALS AND METHODS

Study Site History. The soil used for this study was collected from the Chevron Corporation Land Treatment Unit (LTU) located in Hooven, Ohio. The LTU was built in 1980 on a 2.2 hectare site by excavation, compaction of a natural clay liner and construction of a peripheral berm (Figure 2.1). The site received 10.6 million liters of petroleum refinery wastes from the nearby refinery, currently out of service. As waste was added, it was tilled into the limestone-based soil. Stratification in the substrata occurred with weathering, producing two distinct layers (Figure 2.2). The top layer extends down about 30 cm and overlies a layer characterized by a black, sticky consistency and has a strong phenolic smell. The depth and thickness of the lower layer, though variable, occurs primarily between the depth of 30 to 45 cm. Below the zone of incorporated materials (at depth of 60 – 90 cm) is a clay layer which extends for many meters to the Fairview limestone/shale formation.

Figure 2.1. Aerial photo of excavation efforts at the Chevron-Texaco Land Treatment Unit, 1980. The landfarm is located 17 miles west of Cincinnati, OH near the banks of the Great Miami River.

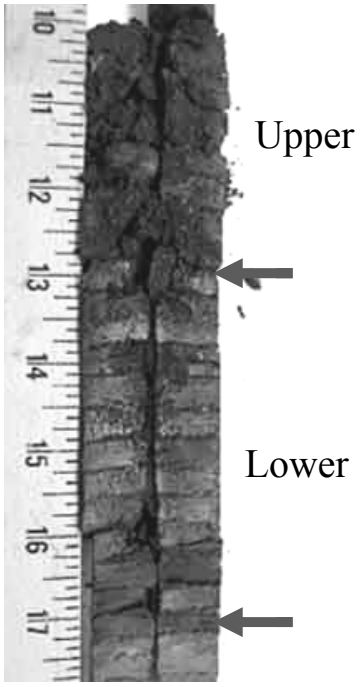


Figure 2.2. Soil stratification in LTU soils: the upper, planted layer and lower, presumably similar to the original waste materials (petroleum sludges from refinery processes). Depth is shown in inches.

Site-wide Contamination Assessment. In 1999 the site was subdivided into 4 quadrats. Fourteen plots approximately 6 m x 6 m (37.21 m²) were delineated using flagging tape. GPS (Global Positioning System) was used to locate latitude, longitude, elevation of plots and the site periphery (the berm) (Figure 2.3). Two replicate 75 cm cores were removed from each plot in June and October of 2000. Replicate cores were taken 50 cm apart. These cores were placed in a plastic liner, sealed, and stored in a -14.4° C freezer. Cores were analyzed according to physical characteristics and rooting depth. Duplicate cores within plots were analyzed separately as opposed to compositing in order to have a more realistic understanding of within plot variation. Total PAHs were extracted using accelerated solvent extraction (4 g soil in 22 mL of v:v, acetone:methylene chloride) and were analyzed using gas chromatography coupled to a flame ionization detector (GC-FID) (Richter, 2000). Integration of the nonspecific hydrocarbon peaks between 18 and 55 minutes (roughly the retention range of diesel) was calculated for each chromatogram to account for total petroleum hydrocarbon (TPH) concentration (EPA, 1996b).

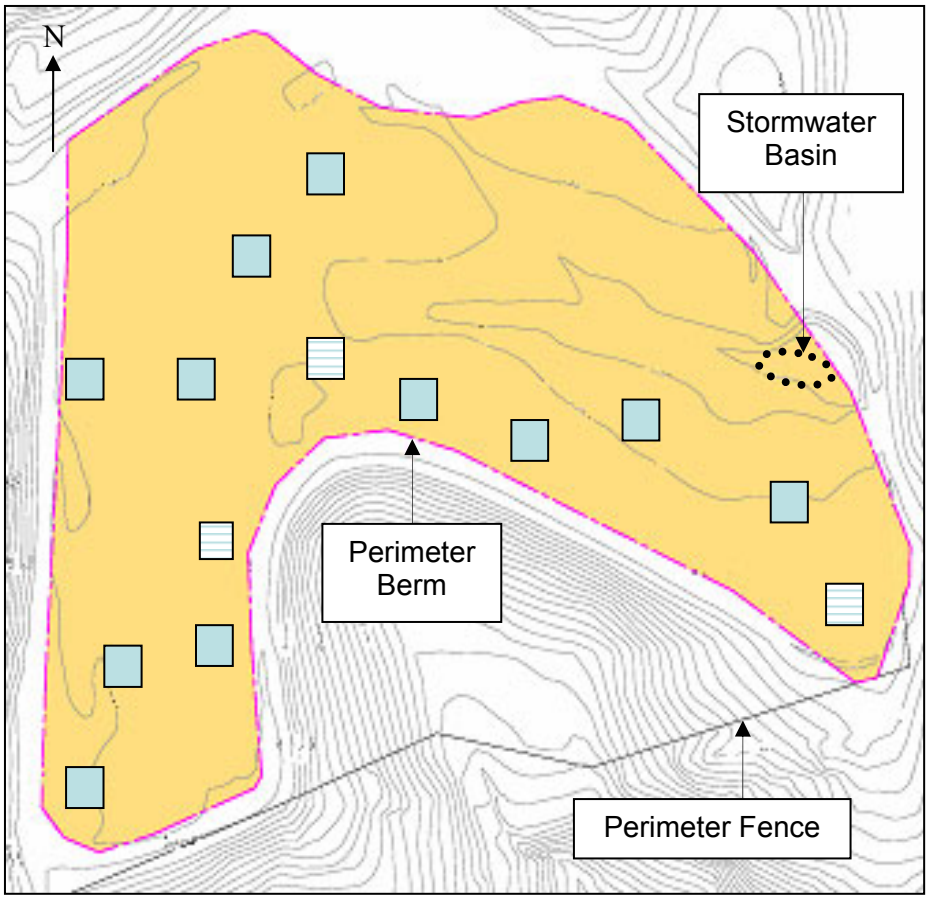


Figure 2.3. The pie-shaped shaded area was leveled and filled with truckloads of petroleum refinery sludges. Study plot arrangement throughout the LTU site. The boxes represent study plots where total metal levels were analyzed. The shaded boxes were monitored monthly for *S. canadensis* revegetation. The striped boxes represent additional plots where biomass was measured. The perimeter berm is raised 1 m above the rest of the LTU to prevent runoff of contaminants. The LTU was constructed to slope towards the stormwater basin were water is collected and pumped back to the main plant. The perimeter fence (the south portion shown below) circles around the entire site, restricting access to the LTU.

Sequential extraction was used to assess the degree of bioavailablity of soil metals (Tessier et al., 1979). Approximately 2 g of sifted, oven dried soil was extracted with 25 mls of 0.5M KNO_3 for 16 hrs on a shaker (100-150 cycles per minute) and then centrifuged for 15 minutes at 5100 rpm. The supernatant was poured through a Whatman #42 filter paper and brought up to 25 mls with extractant solution. The process was repeated with DDI water (2 hr), 0.5M NaOH (16 hr), 0.05M Na_2EDTA (6 hr) and finally, 4M HNO_3 (16 hr at 70°C). Three replicates of each layer per core were extracted and then analyzed for metals using inductively coupled plasma atomic emission spectroscopy (Thermo Jarrel Ash Corporation ICAP 61E Plasma Emission Spectrometer, Agilent 7500c ICP-MS and Perkin-Elmer Elan 6000 ICP-MS). The extracts of KNO_3 and H_2O were combined and concentrated to 25 ml and are referred to as the mobile fraction, or readily available fraction (Sposito et al., 1982). The labile fraction is the sum of the mobile, the NaOH (bound to organic material) and Na_2EDTA (clay-oxide bound) concentrations.

Soil Sample and Collection. Soil was taken from upper and lower layers from a central section of the LTU with high levels of metal and PAHs as determined above (693139N, 4340162E in UTM). The soil was excavated, separated by layer, sifted (2 mm) and stored in an airtight container at -14.4° C. Soil was analyzed using methods of sequential extraction for metals and total extraction for PAHs.

Lettuce Bioassay. Lettuce seeds (*Lactuca sativa* L. var. *"Black-Seeded Simpson"* from Gurney's) were soaked 20 min in 2% Clorox solution and then rinsed 5 times with distilled water, and placed on 9 cm Whatman #41 filter paper in 10 cm Petri dish. Six replicates were made of soil separated by layer and, for a control, topsoil (sterilized

commercial topsoil with equal parts peat and sand). Sieved soil was weighed out to 10 g (5% wet weight) into a centrifuge tube. To the soil, 10 ml DDI H_2O (adjusted to pH 6.5) was added and agitated overnight (18 h) then centrifuged 15 min at 5100rpm. A volume of 2.5 ml of supernatant solution was poured onto the filter paper. Ten seeds per paper were placed in the Petri dish and spaced evenly, incubated in dark (at room temp) for 5 days. Percent germination was monitored daily. Dishes were remoistened with 1 ml of supernatant solution on the third day. On the fifth day, each seedling radicle length was measured to the nearest 0.1mm using a hand caliper.

Goldenrod Bioassay. Early establishment of *S. canadensis* L. was noted in a region of the LTU close to edge. This species is common in early successional stages in this region. The goldenrod assay compares growth of a common *in situ* species in the two soil layers compared to clean topsoil. Seedlings (1mm) were removed from an area of the LTU with moderate contamination from the same one individual. Because these seedlings had sprouted and initiated root elongation, a repeat of the lettuce bioassay was not conducted. Instead, nine seedlings were transferred to 4" pots containing sifted soil brought up to field water capacity (25% DDI on weight basis) in three treatment groups: LTU upper soil, LTU lower soil, and control (commercial topsoil). There were four replicates for each treatment. Seedlings were grown in a controlled growth room (15/20 °C, 8h dark/16h light, 520 lux). Cellophane covered the treatments to hold in moisture to reduce the need of watering. Rotation of treatments was conducted periodically to ensure equal light exposure. Plants grew for 2 months after which time they were removed from their pots and the number of roots (defined as branching from the base of the leaves), and stolons (root-like projections emerging above the basal leaves) were counted. The

longest two leaves, roots, and stolons were measured using a caliper. Plants were then dried and weighed.

Monitoring Establishment of Goldenrod on LTU. The fourteen permanent plots were sampled for *Solidago canadensis* growth five times (at 3 week intervals) during the growing season of 2000, 2001 and 2002. Triplicate random subplots (1 m^2) were sampled for abundance and cover of goldenrod within each study plot. Abundance (the actual number of individuals) was measured as the ramet emerging from the ground within subplot. Percent cover was determined by vertical projection of crown cover within the 1 m^2 subplot. Goldenrod biomass was determined at the end of the year by collecting, drying, and weighing aboveground plants in 0.33 m^2 subplots placed along the perimeter of each plot. GIS Imaging (ArcView GIS 3.2, ESRI) was used to create exploratory maps of vegetation and contaminants. Point data from each plot were used as the z-value for grid interpolation using IDW method, nearest neighbor (12), and power (2).

Metal Analysis of Goldenrod Tissue. Plants from each of the four quadrats were collected in 2003 and then processed for metal content in plant tissue using an acid digestion procedure. Acid digestion of plant material was conducted according to (Keane et al., 2001). Plant tissue was soaked in distilled water for approximately 3 hours. Leaves were then rinsed 10 times with distilled water, oven dried at 60°C for 48 h and ground in a mill. Powdered leaf tissue (2g) was ashed in a muffle furnace at 550°C for 5 h to maximize for the metals in question (Azcue & Mudroch, 1994). The ash was dissolved in 2 ml concentrated HCl and heated to boiling on a hot plate to extract total leaf metals. Samples were filtered (Whatman 41), brought to 20 ml with 4 M HNO_3 and

stored in plastic bottles at 4°C until analysis. Leaf digests were analyzed for Cr, Cu, Zn, Pb and Ni by inductively coupled plasma atomic emission spectrometry (ICP-AES, Elan). Each instrument was standardized on a curve using stock metal concentrations in a 4 M HNO_3 solution (Azcue & Mudroch, 1994).

RESULTS

The total metal concentrations found across the study site are given in Table 2.1. Of the five metals, zinc and copper are the only ones considered essential for plant growth. Nonetheless, at such elevated levels, phytoxicity of even these essential elements would be expected. The petroleum components from refinery wastes are characteristically complex mixtures of aliphatic and aromatic hydrocarbons. While soil polycyclic aromatic hydrocarbons (PAHs) are suspected carcinogens, these compounds are not considered phytotoxic. The soil used for the bioassays was analyzed for metal availability (by sequential extraction) and total levels of petroleum hydrocarbons and PAHs. That data are given in Table 2.2 as well as the results of sequential and total extraction of contaminants. Total PAHs and TPH were higher in the lower layer whereas metals were higher in the upper layer. Specifically, the lower layer soil mobile fraction had elevated chromium, nickel, and zinc compared to the upper layer. However, in the labile fraction, lead and copper were significantly higher than the upper layer.

Table 2.1. Geometric mean and range of total soil heavy metal concentration on the study site and the role in plant nutrition.

Element	Essential for plant growth*	Clean Silty-Loam Soil, (µg/g)	Upper Layer geometric mean (µg/g)	Lower Layer geometric mean (µg/g)
Cr	No	51	493 (299 – 649)	326 (199 – 471)
Ni	Possible	26	71 (85 – 58)	57 (34 – 95)
Cu	Yes	23	89 (59 – 105)	69 (47 – 91)
Zn	Yes	60	362 (243 – 439)	274 (190 – 360)
Pb	No	28	644 (406 – 1045)	292 (126 – 455)

From *(Kabata-Pendias & Pendias, 2001).

Table 2.2. Concentration of Contaminants from Soil Layers Selected for Toxicity Tests. Numbers are listed in µg/g. RPDs for petroleum hydrocarbons were 30%. Standard deviations of triplicate soil sequential extractions are in parenthesis. The mobile metal detection limit was 0.01 µg/g.

	Upper Layer µg/g D.W.	Lower Layer µg/g D.W.
Total Polycyclic Aromatic Hydrocarbons	28.8 (± 4.1)	122.7 (± 17.3)
Total Petroleum Hydrocarbons	2616.4 (± 672.1)	14850.9 (± 2602.7)
Mobile Metals (Total)	3.12 µg/g	3.72 µg/g
Cr	0.12 (± 0.01)	0.31 (± 0.22)
Ni	0.93 (± 0.13)	1.19 (± 0.24)
Cu	1.19 (± 0.15)	1.18 (± 0.17)
Zn	0.88 (± 0.24)	1.04 (± 0.13)
Pb	< D.L.	< D.L.
Labile Metals (Total)	435.3 µg/g	355.3 µg/g
Cr	16.7 (± 0.8)	20.2 (± 0.6)
Ni	16.6 (± 1.0)	18.1 (± 0.5)
Cu	36.5 (± 1.3)	24.1 (± 1.6)
Zn	76.4 (± 2.6)	102.1 (± 6.1)
Pb	289.0 (± 14.9)	190.8 (± 14.8)
Total Metals	2072.1 µg/g	1918.8 µg/g
Cr	673.0 (± 13.0)	694.0 (± 10.7)
Ni	77.5 (± 4.3)	88.3 (± 4.1)
Cu	97.8 (± 3.2)	101.5 (± 3.4)
Zn	446.4 (± 26..6)	480.9 (± 3.1)
Pb	777.3 (± 15.2)	554.1 (± 24.4)

For the lettuce bioassay, germination in the lower layer treatment was significantly lower than the control for day 1 only ($p = 0.002$, 1-way ANOVA Bonferroni hypothesis test) (Figure 2.4). Root elongation was significantly different between all soil types ($p < 0.0005$, 1-way ANOVA Bonferroni hypothesis test) (Figure 2.5).

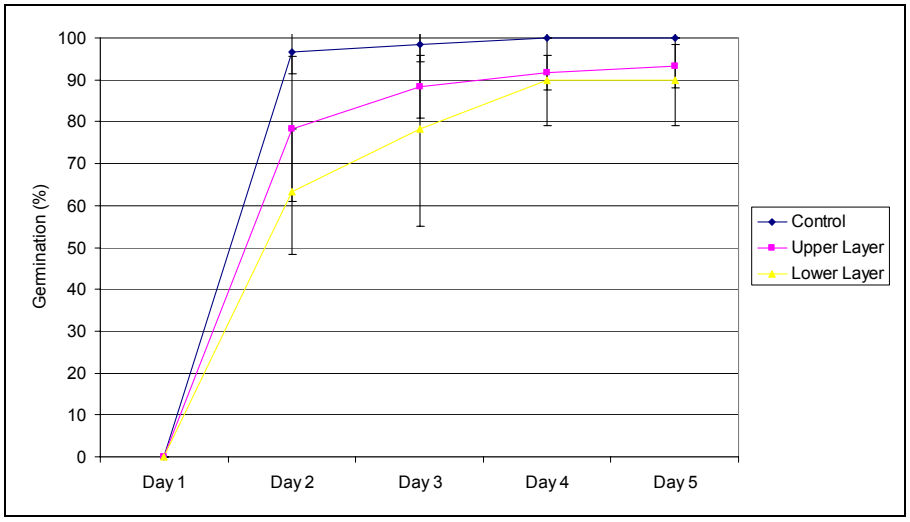


Figure 2.4. Percent germination of lettuce seeds. Lines represent the mean of six dishes (n = 10). Lower layer treatment was significantly lower than the control treatment for the first day ($p = 0.002$, 1-way ANOVA, Bonferroni).

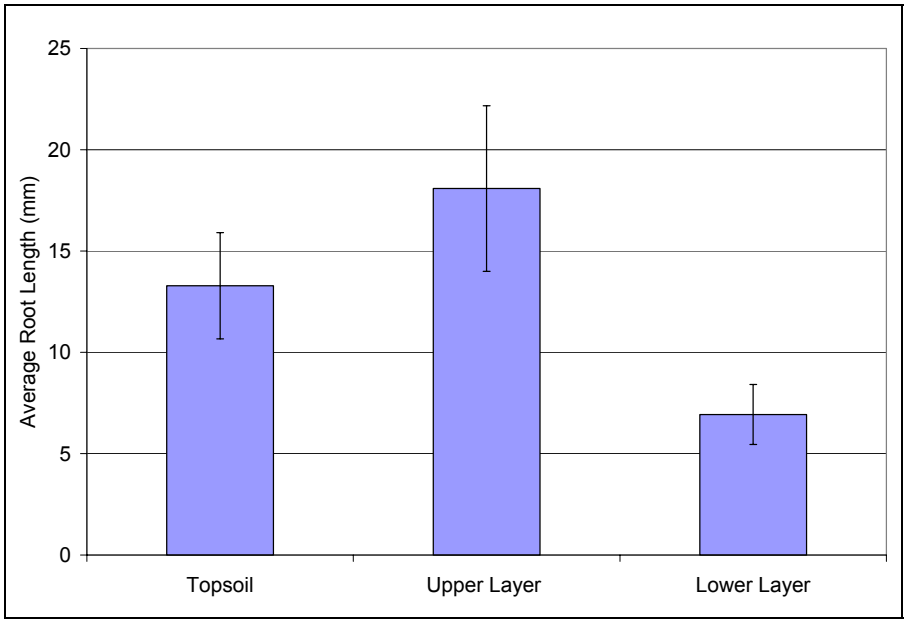


Figure 2.5. Average root elongation of lettuce bioassay. One-way ANOVA Bonferroni post-hoc test revealed significant differences ($p < 0.0005$) between all treatments.

Though lower layer soil had half the average root elongation as the control, root elongation was actually higher in upper layer soil relative to control. It was also noted that root hair production was reduced in upper layer soil relative to the control (Figure 2.6).

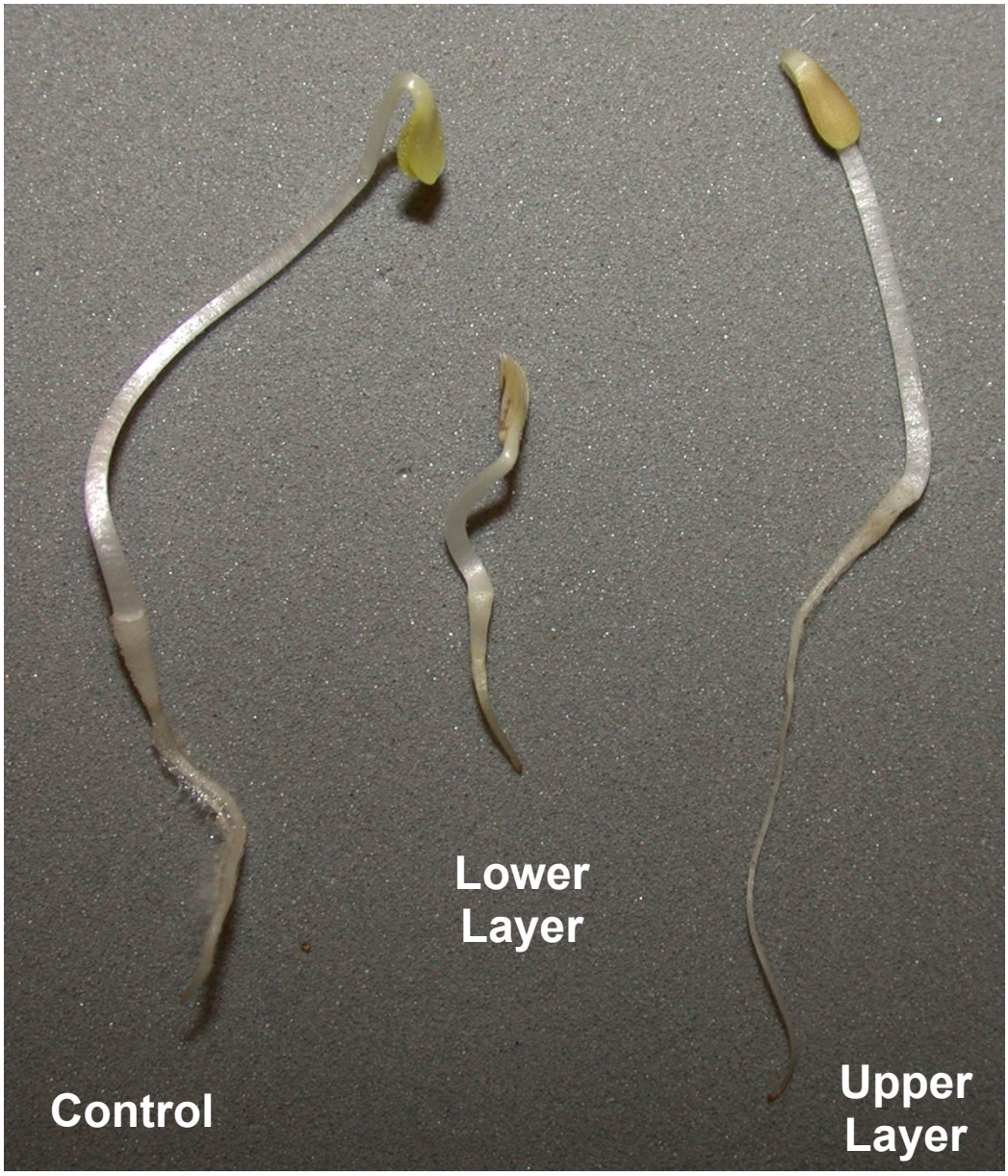


Figure 2.6. Representative individuals showing the general trend between treatments in the lettuce bioassay. Seedlings grown in the water extract of the upper layer contaminated soil (far right) had longer radicle extension than the control (clean topsoil); however, fine root hairs were absent.

The results of the goldenrod bioassay are reported in Table 2.3. There was a significant difference between treatments with regard to total weight (ANOVA, $p < 0.0005$). Root number and length as well as leaf length were significantly different for control and LTU soils ($p < 0.0005$, Bonferroni), but not significantly different between LTU layers. Stolon number was not significantly different between groups. Metal uptake was analyzed but was not detectable given the small amount of material weight (< 0.05 g) in the groups. The survivorship of goldenrods growing in upper layer soil was lower than for the control ($p = 0.018$, 1-way Bonferroni) and lower layer soil ($p = 0.027$, 1-way Bonferroni), noting that for each pot, one or two grew almost as large as the control goldenrods (Figures 2.7 and 2.8). This is also evidenced by the rather high standard deviation for upper layer plants measured variables.

Table 2.3. Goldenrod Growth. Numbers are the averages of surviving goldenrods. Significant differences ($p < 0.05$, 1-way ANOVA, Bonferroni) between groups denoted with different letters.

	Average Weight (mg)	Average Leaf Length (cm)	Average Root Length (cm)	Average # Roots	Average % Survivors
Control	22 (± 19)[a]	2.4 cm (± 0.9)[a]	4.9 ± 2.2[a]	6.6 ± 2.2[a]	86%[a]
Upper Layer	5 (± 8)[b]	0.7 cm (± 0.7)[b]	2.5 ± 2.4[b]	3.1 ± 2.5[b]	50%[b]
Lower Layer	1 (± 0.4)[c]	0.2 cm (± 0.1)[b]	1.3 ± 0.6[b]	1.8 ± 0.8[b]	83%[a]

Figure 2.7. Dot density distribution of of *S. canadensis* log transformed weight (mg) for C). control, L). lower, and U). upper layer soil treatments.

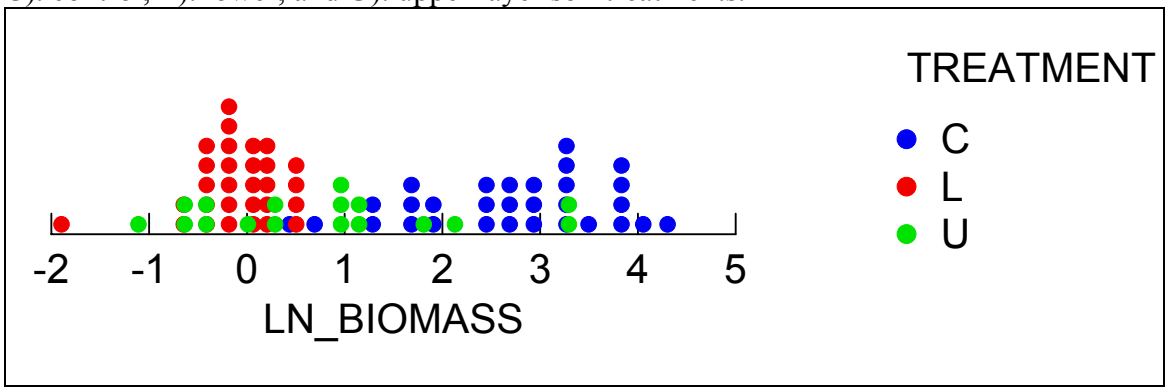
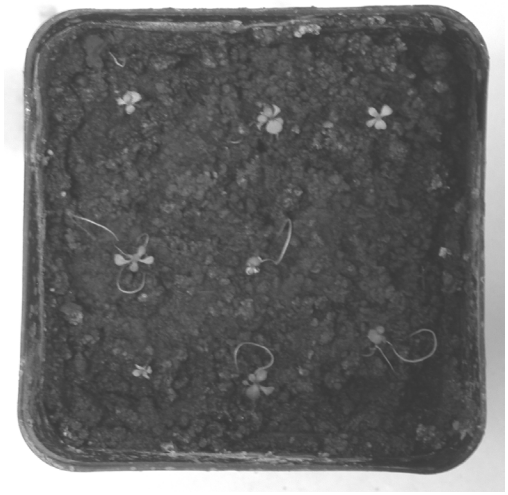


Figure 2.8. Photos of goldenrod seedlings after two months in soil treatments. a). *S. canadensis* seedlings in upper layer of LTU soil, b). *S. canadensis* seedlings in lower layer of LTU soil, c). *S. canadensis* seedlings in sterilized topsoil (control) and d). *S. canadensis* across treatments (Control, Lower, Upper).

a.

b.



c.

d.

Despite slight reduction of growth seen in the lettuce bioassay and goldenrod bioassay, the actual goldenrod population became established throughout the entire site. Establishment of goldenrod began in edge plots but by the third year, it had become established in all plots (Figures 2.9, 2.10, 2.11). In 2003, goldenrod species were collected for tissue metal analysis (Figure 2.12). Though the concentration of metals in the root tissue is higher for all metals (except Ni) in LTU goldenrod compared to the offsite goldenrod, shoot tissue concentrations are comparable.

Figure 2.9. Establishment of *S. canadensis* on site. Legend displays the range of values of the average biomass (in grams dry weight per m^2) harvested at end of each growing season.

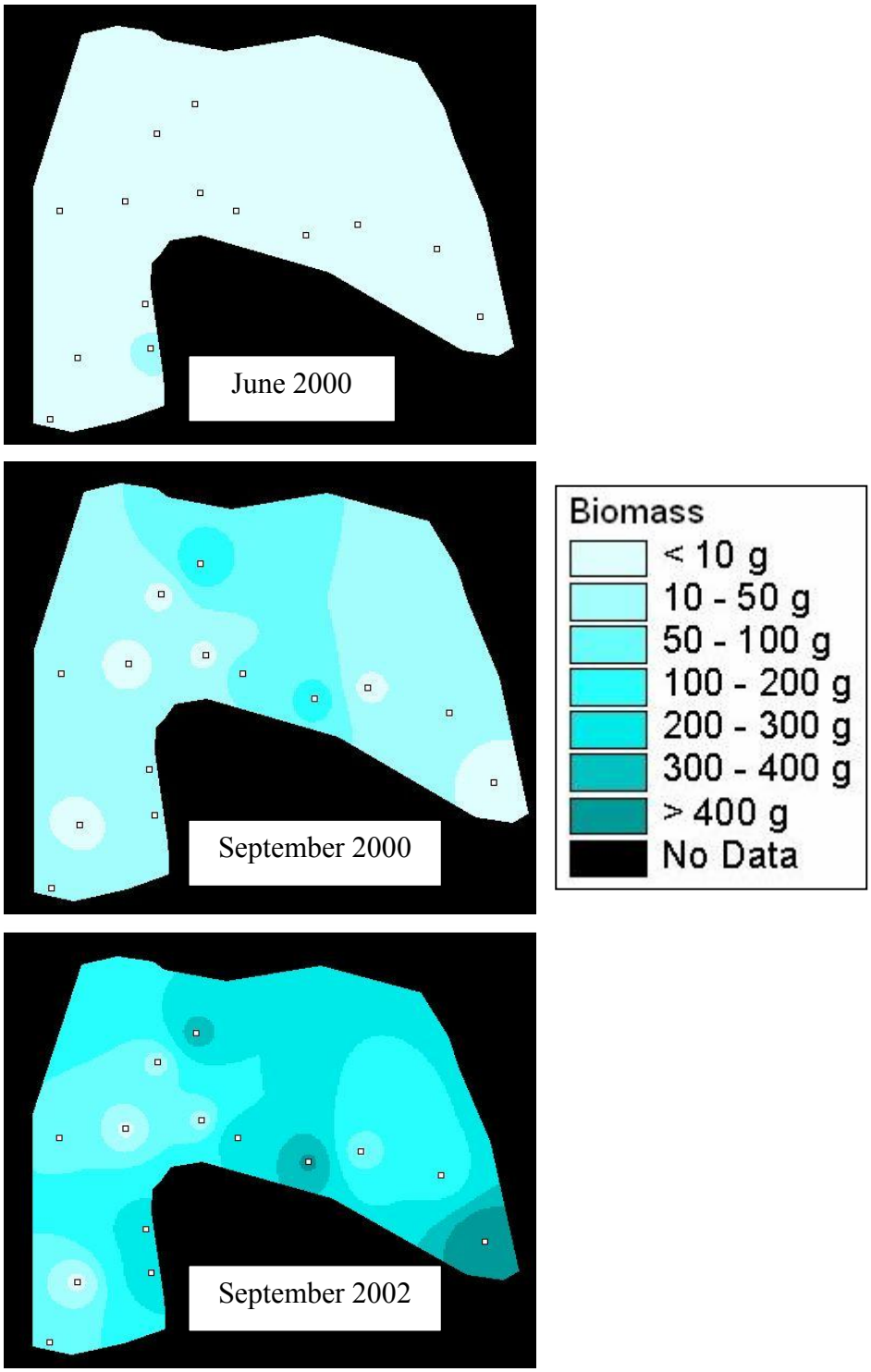


Figure 2.10. Establishment of *S. canadensis* on site. Legend displays the range of values of the average percentage cover.

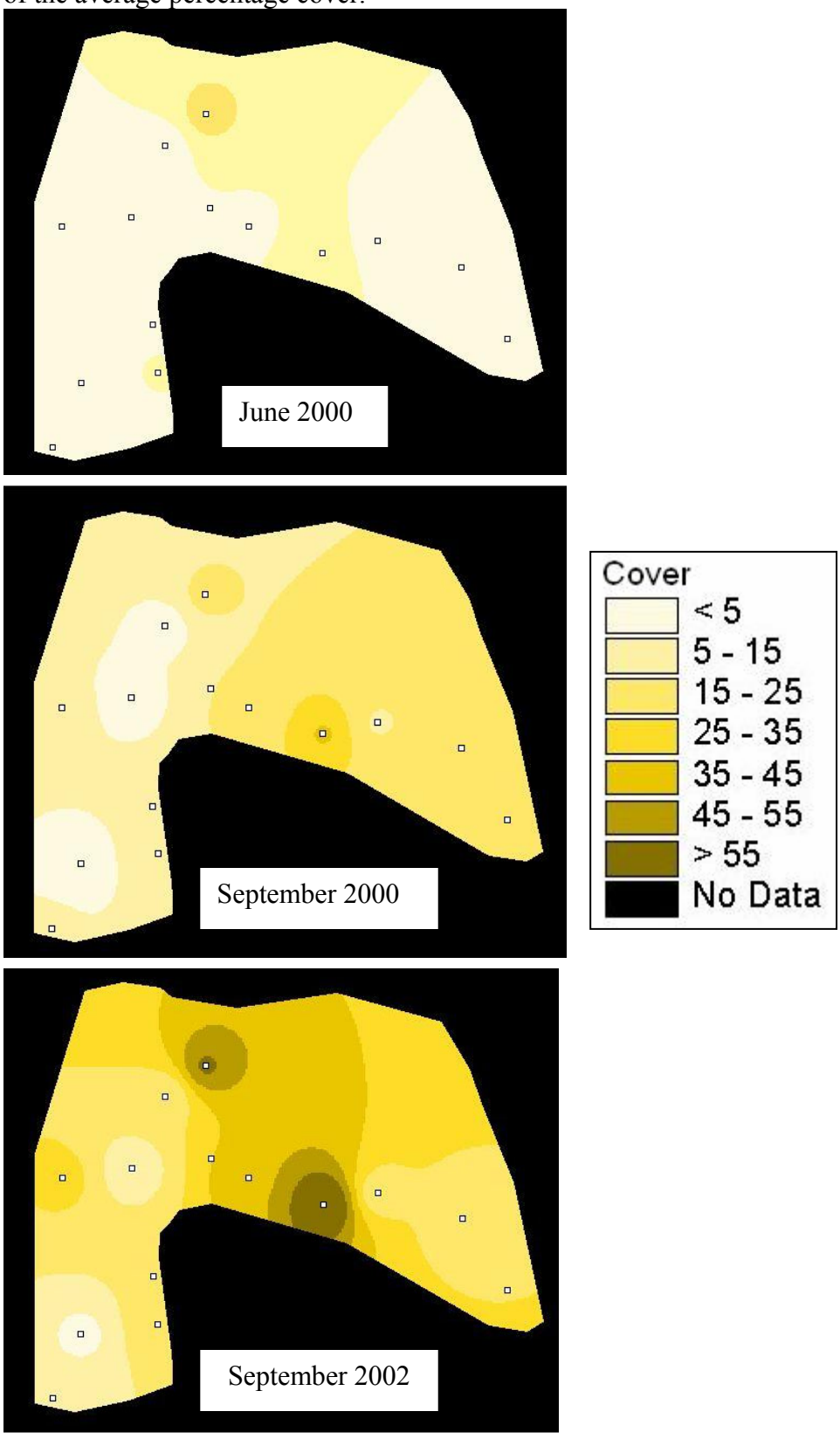
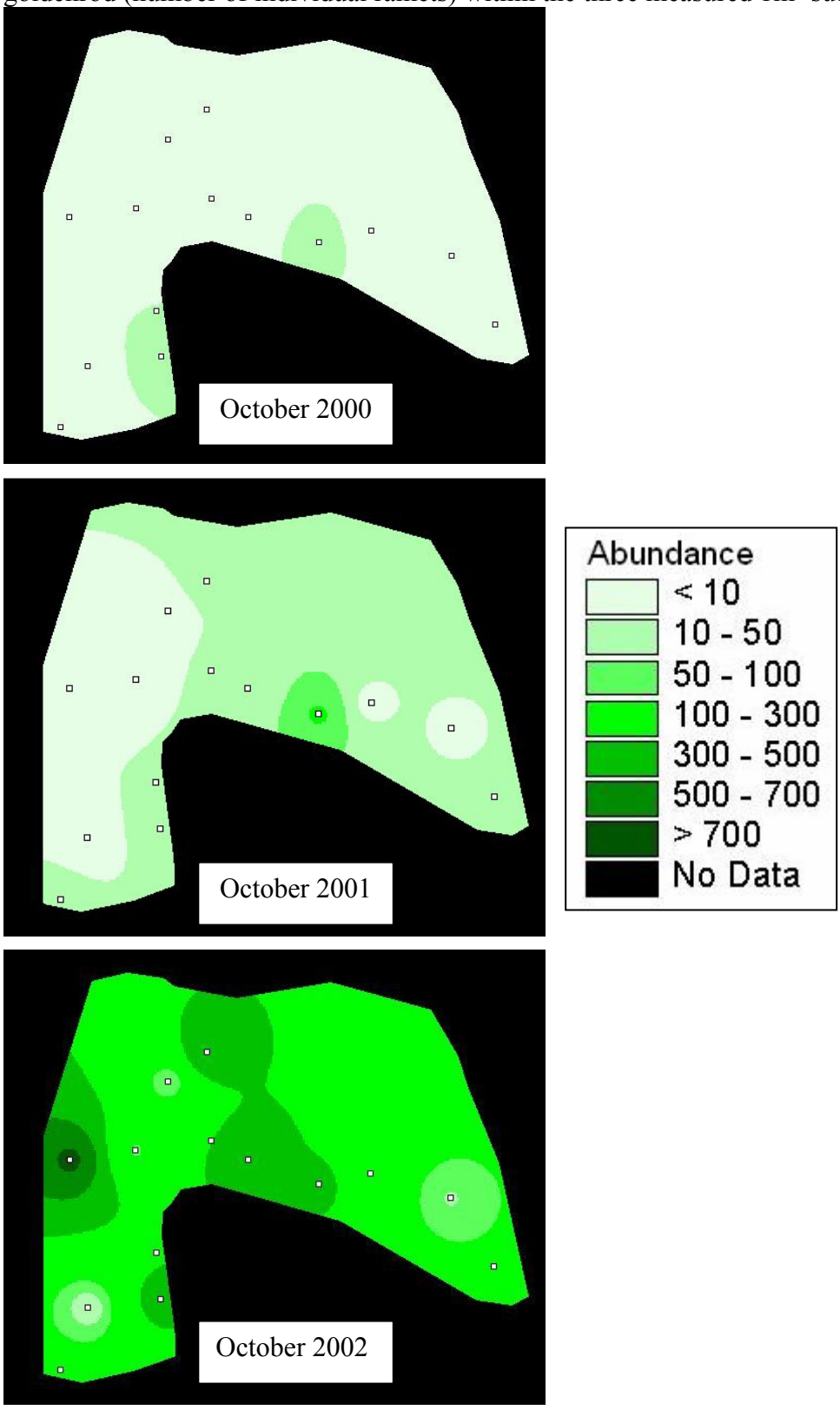


Figure 2.11. Establishment of *S. canadensis* on site. Legend displays the abundance of goldenrod (number of individual ramets) within the three measured $1m^2$ subplots.



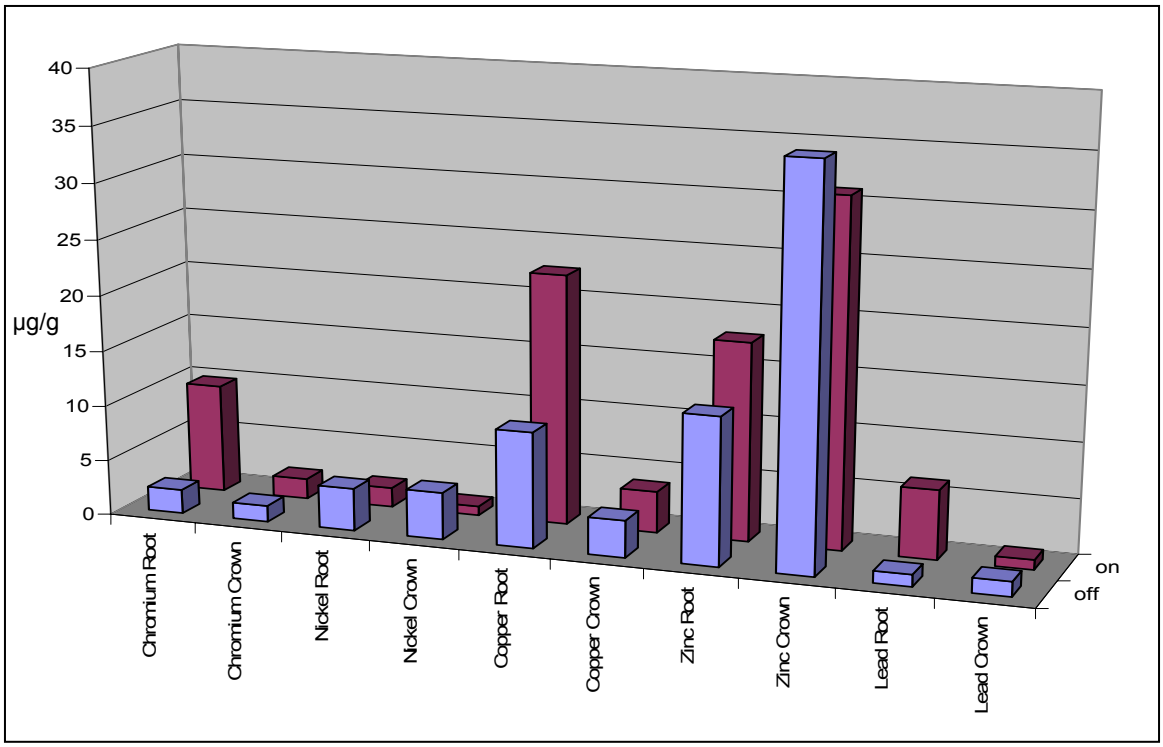


Figure 2.12. Metal concentration (µg/g) in onsite *S. canadensis* and those collected from an unpolluted site.

DISCUSSION

Results from the two bioassays suggest that the aged LTU soil is not as phytotoxic as the total contamination levels would suggest. Water-extracted contaminated soil reduced the rate of germination of lettuce seeds relative to the control; however, only the lower layer soil resulted in stunted root elongation. The upper layer soil actually resulted in greater root elongation relative to the control; however, the lack of fine roots (Figure 2.6) may have been a resulted in resource allocation to root extension. In the goldenrod bioassay, plants were clearly inhibited in both the upper and lower layers of soil relative to the growth of the control. This was more pronounced in lower layer soil.

As expected, the two bioassays yielded slightly different results. In terms of germination and root elongation, the goldenrod test appeared to be more sensitive than the lettuce test. Essentially, all of the lettuce seeds germinated; the ones given upper layer extracts actually grew longer roots. Since goldenrod should be less sensitive than the lettuce, one would assume that all of the seedlings planted in the upper layer soil would also grow well – however, they did not. Why? First, in the goldenrod test, plants were potentially exposed to (or could access) higher metal and TPH levels than in the lettuce bioassay where only the water extractable metals would be present. Secondly, the site soil, as a growth media, is very different than the topsoil control used to evaluate "soil growth". The materials differed in their texture and nutrient availabilities – both of which would impact plant performance. The minimal period of growth in the lettuce assay was not as vulnerable to nutrient limitation and water availability was controlled.

Bioassays cannot confirm the mechanism of toxicity; but the presence of contaminants with known phytotoxic activity (nickel, lead, chromium and petroleum

compounds) is suggestive. Bioavailability of nonessential metals in the upper layer soil was low, therefore it is not surprising that the upper layer soil did not drastically impact lettuce. Bioavailability of chromium and nickel were twice as high in the lower layer soil. These metals are known to affect membrane integrity which may cause the sensitivity seen in the radicle extension (and/or reduced of root hair production) (Kabata-Pendias & Pendias, 2001). Total petroleum hydrocarbons were higher in the lower layer soil. The components of petroleum refinery byproducts that make up the TPH concentration may be any number of compounds with various water solubility and volatility (Wrenn & Venosa, 1996). In order to identify all of the compounds in the unresolved TPH chromatogram, a GCxGC instrument would need to be employed. Petroleum hydrocarbons, particularly diesel hydrocarbons, are phytotoxic but studies have shown that microbial degradation of diesel diminishes the phytotoxic effect. (Green et al., 1996; Siddiqui & Adams, 2002; Siddiqui et al., 2001). The upper layer had been under the influence of plants for nearly 20 years, but the phytotoxic compounds in the lower layer may not have degraded to the extent necessary to accommodate plant growth (Gomez, 2001). The retention window used to determine TPH in this study was that of a purified diesel standard, so it is likely that there are components present in the lower layer of the soil that are constituents of diesel fuel. Given these results it appears that components of the TPH, and possibly in combination with higher metal levels, contributed to the stunted growth seen in the lower layer soil treatments of the lettuce and goldenrod bioassay.

Another clear difference between the tests was the uniformity of response in the lettuce bioassay versus the higher variation in response in the goldenrod study. For

goldenrods growing in the upper layer (higher in metals), there was high variation in the measured variables within in each pot (Figure 2.8a). In the four replicates, only half survived with the observation that two or three in each replicate grew very well (Figure 2.8d and 2.8b). These were siblings, not clones and it is possible the observed variation reflects individual resistance to contaminant toxicity or to soil conditions. At the time these individuals were collected, the onsite goldenrod population would have had several generations to reach flowering stage without the interference of site maintenance. Prior to maintenance cessation, seed arrival, growth and vegetative reproduction would have occurred naturally for the twenty years of site operational. Though these individuals germinated in the soil (this is one level of selection for toxicity on contaminated soil), it is possible that only individuals tolerant of soil contaminants or condition survived. Such "ecotype" formation on mild to highly contaminated soil has been observed in many studies (Brown & Amacher, 1999; Collier, 2003; Kidd et al., 2004; Shu et al., 2002). Natural selection for tolerance to the lower layer soil would not have occurred as the lower layer was not exposed at the site. This experiment could be repeated using clones to account for genetic differences and by incorporating individuals from offsite to see if a local selection has occurred on site.

Establishment of goldenrod began in edge plots, but by the third year had become established in all plots (Fig. 2.9, 2.10, 2.11). Despite slight reduction of leaf and root growth evidenced in both bioassays, goldenrod became established throughout the entire site. This colonization was certainly by individuals that were able to tolerate the soil – as was seen in the bioassay. It is not uncommon for ruderal species to have a high tolerance of industrial sites (Barazani et al., 2004). The population became dominant in the LTU

community and filled the entire study area. Since the bioassay tested individuals collected from a low contamination area against soil with a much higher level of contamination, the bioassay probably provided a conservative estimate of the population level tolerance of the entire site.

The contaminants present on this petroleum refinery land treatment unit were phytotoxic to some individuals of the wild population. However, it was assumed that in a natural setting, intolerant individuals would be out-competed by species that tolerate the soil conditions. This early assumption was validated in the three year follow-up study that showed a ten-fold increase in abundance of goldenrod throughout the entire site. The use of early emergent native onsite plant populations may serve as an early indication of the potential for the natural community to tolerate site conditions. On sites where tolerant individuals are present and risk is low, passive revegetation may be effective without clean soil capping.

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CHAPTER 3

PATTERNS OF NATURAL PLANT SUCCESSION ON AN AGED PETROLEUM LAND TREATMENT UNIT

HEATHER F. HENRY AND JODI R. SHANN

Department of Biological Sciences ML6, University of Cincinnati, Cincinnati, OH 45221-0006

ABSTRACT

Ecological restoration of hazardous waste sites is a potential alternative to *ex situ* treatment that has not been well documented. Although ecological restoration can be accomplished through seeding, most post-disturbance un-manipulated plant communities return to their original condition naturally through secondary succession. Theoretically, contaminated sites should follow the typical course of secondary succession; however, this has not yet been demonstrated. This three year field study investigated natural succession on an aged contaminated site following the lift of cover management. Indicators of ecological succession (biodiversity, productivity and soil quality) were monitored within a complete block design. During the growing season, 11 permanent plots were subsampled each month for species cover, richness and abundance. Soil coring was used to determine edaphic quality changes and out-of-plot collections were used to establish biomass (productivity). Over the three year study period, species diversity increased as did biomass and cover; richness went from 28 to 57 species, cover increased from 33 to 79%, and biomass, by a factor of four. Seeded during landfarming management, perennial ryegrass was dominant at the start of the study, but the biomass (productivity) increases in subsequent years were due to the self-establishing species. Plant community biomass and diversity were negatively correlated with increasing distance to edge. Following the three year study, onsite plant cover, richness, and invasive species incidence were compared to a clean site of the same area in approximately the same year of succession. Richness and percent cover were not significantly different between sites. Though the three year study indicated an increase in invasive cover onsite, the incidence of invasives (measured the following year) was lower

than on the uncontaminated site. Despite clearly differing contamination patterns, there were no relationships between soil contamination (heavy metals and petroleum compounds) and the various plant measures. This was true within each year, and over the three year period. As expected for a clean site, variation in plant growth on a plot basis was related to variation in site microclimatic factors. The natural community formed a self-sustaining, and diverse community within three years. This study suggests similar aged contaminated sites, where there is low risk initially, may be candidates for ecological restoration through natural revegetation and successional processes.

INTRODUCTION

Treatment of hazardous waste sites can be a challenge, and selecting the appropriate technology for the risk and extent of contamination is important. On many aged sites in the U.S., contamination may be above regulatory standards, but actual risk is low due to sequestration of pollutants onto soil particles. On sites where risk is low, *in situ* treatment strategies may be the most economical and safe way to address contamination. Phytoremediation is an economical *in situ* process that uses plants to contain, sequester, and/or aid in degradation of contaminants. The typical process calls for management of horticulturally available species maintained by irrigation, reseeding, and plowing. Many phytoremediation studies mention the presence of volunteer vegetation, but few systematic ecological studies of these natural communities have been conducted (Hegazy, 1997; Olsen & Fletcher, 2000; Olson & Fletcher, 2000).

Restoring the natural plant community in disturbed ecosystems is known as ecological restoration. Contaminated sites where the compounds have become stable in

the soil may be candidates for ecological restoration (Brown & Amacher, 1999; Kearney et al., 1999; McCutcheon, 2002; McCutcheon & Schnoor, 2003; Mitsch & Mander, 1997; Parrotta & Knowles, 2001). Ecological restoration efforts can take the form of reseeding, soil amendments, etc. However, if the contamination disturbance is minimal (as may be the case with aged sites) a more economical and less destructive way to achieve ecological restoration would be to allow natural revegetation to proceed on the site (Brown & Amacher, 1999). Essentially, plants from the surrounding natural community would be allowed to colonize and become established on abandoned waste sites. If adequate cover is achieved by a natural assemblage of species, the community is likely to be more sustainable than a managed system. Through the process of secondary succession, the gradual yearly changes in a plant community following a disturbance, species from the local community adapted to the local conditions form a self-sustainable community. Natural communities are more nutrient retentive, disease resistant, resilient, and long-lasting than managed systems (Ewel, 1999).

The process of secondary succession has predictable endpoints. Usually, the first species to become established are disturbance tolerant pioneer species, generally annuals. These species are gradually replaced by longer lived species with more allocation of energy toward increasing biomass, as opposed to the investment in fecundity in r-selected reproductive strategies. In a matter of years, the community shifts from short-lived to long lived-species with increasing biomass and cover (Odum, 1963). It is generally accepted that the plant community in a given region forms in response to light, moisture, and nutrient gradients (Tilman, 1988). Beyond that, the exact species composition may be unpredictable. For that reason, indices are used to quantify the increasing community

complexity. Richness (the number of species) and diversity (a function of species richness and abundance) are the most common indices (Fortin & Gurevitch, 2001; McCune & Grace, 2002).

Richness and diversity are expected to increase during secondary succession; conversely, abundance and dominance are likely to decrease as a function of self-thinning and replacement of r-selected individuals. This increase in richness and diversity makes it likely the plant community will be more resilient (that is, possess species tolerant of environmental perturbations) (Connell & Slatyer, 1977; Ewel, 1999). Hence, the objective of restoration efforts is to restore diversity in order that the community might be self sustainable (Brown & Amacher, 1999; Mitsch & Mander, 1997). Other factors, such as distance from a founder population, may affect the rate of species establishment during secondary succession (MacArthur and Wilson, 1967).

Soil contaminants can have a community-level effect on plants (Depledge, 1999). This is due, in part, to the strong selective pressure associated with pollutant tolerance. In general there is some metabolic cost associated with tolerance mechanisms, often resulting in reduced individual biomass (Collier, 2003; Schat & Verkleij, 1998). Relative to non-contaminated communities, plants existing with soil contamination are correlated with decreases in vegetative cover, increases in cover by exotic species, and lower plant diversity (Galbraith et al., 1995; Riley & Banks, 1996). In other cases, later successional species typical of the region and community type, do not become established (Berube & Lavoie, 2000). Hence, the course of succession may be altered as a result of high levels of plant available contamination.

The objective of this study was to determine if the typical course of secondary succession is occurring on a site historically contaminated with metals and PAHs. It is during the first few years of succession that the direction of succession is most apparent. During this time it will be apparent whether there is an increase in species number, biomass, and cover as would be expected from a recovering ecosystem. Alternatively, the plants may not naturally revegetate this site that, prior to this study, consisted of a monoculture maintained through irrigation, seeding and fertilization. Instead, the system may have a high incidence of invasive species, low richness, or loss of cover – symptoms of a community under pollution stress (Rapport et al., 1998). As previous studies of plant revegetation on contaminated sites did not include environmental gradients as a contributing factor in plant establishment on contaminated soils, this study will investigate environmental characteristics to determine if secondary succession is responding more to environmental factors than to contamination.

MATERIALS AND METHODS

Study Site History. This study was conducted on a 2.2 hectare RCRA-certified landfarm located near Cincinnati, Ohio. Over a 10 year period, the site received a total of 2.8 million gallons of petroleum refinery waste (sludges and oils with a range of organic compounds and heavy metals) (Figure 2.1, previous chapter). Active landfarming (tilling) occurred throughout the summer months and as a winter vegetation cap, perennial rye grass (*Lolium perenne*) was seeded and maintained from 1992 until 1998. The post-closure plan, initiated in 1998, allowed natural revegetation without a clean soil cap, making it the only U.S. site where unmanaged phytoremediation was approved as a

cover strategy. In Spring of 1998, the LTU was tilled and left to revegetate naturally (Condit & Doherty, 2000) (Figure 3.1). Under the post closure plan, the LTU was not seeded, mowed, irrigated, fertilized or plowed (Condit & Doherty, 2000).

Figure 3.1. Early self-establishment (non-seeded) of *L perenne* on LTU after final tilling event in April 1998. The ryegrass had been seeded periodically since 1992 as a winter cover to stabilize soil after summers of active landfarming (tilling).

Field Study Design. Plots for this study were arranged in a random block design; the LTU was divided into four quadrats, each containing four (6 m x 6 m) plots (Figure 3.2). One plot per quadrat was maintained as an unvegetated control by continual hand removal of seedlings. The remaining plots were allowed to revegetate without disturbance or management. The latitude, longitude, and elevation of all plots were located by GPS as was the perimeter of the site. Plots were sampled during the growing seasons of 2000, 2001 and 2002. Plant community composition and species abundance was monitored throughout the season (June-September). Each of the 11 vegetated plots

was sampled every three weeks between the summer months of June through September. The monthly sampling maximized inclusion of seasonal species and cover development.

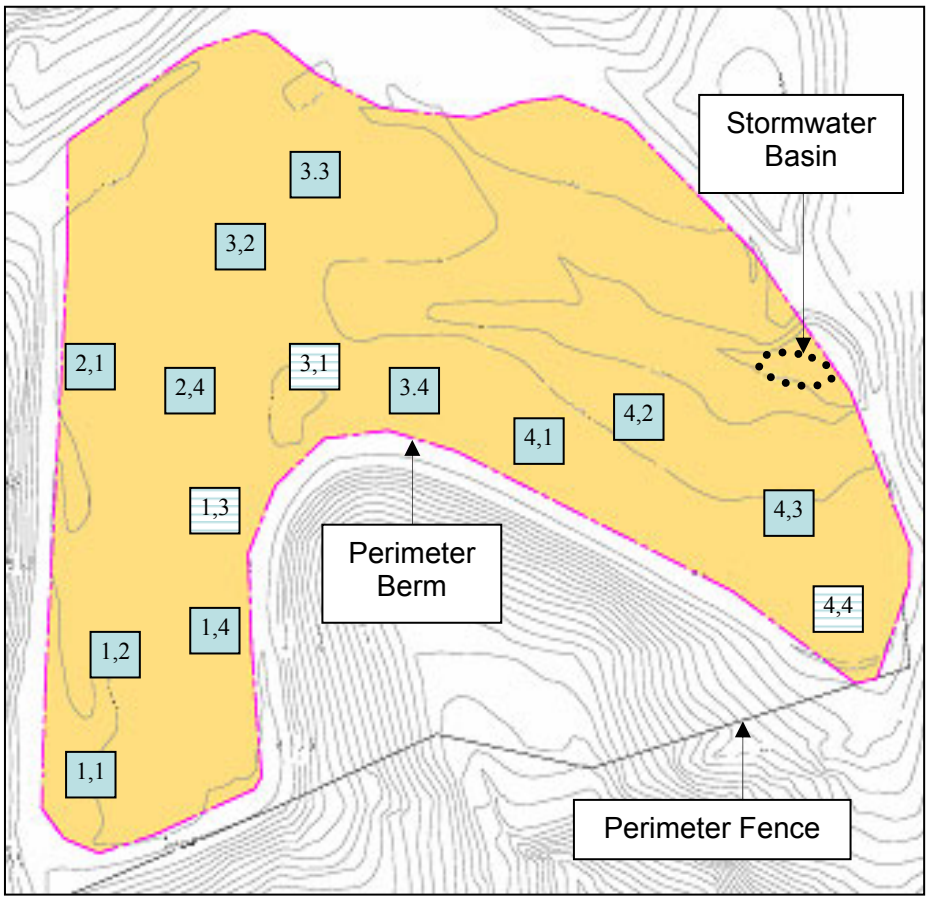


Figure 3.2. Study plot arrangement throughout the LTU site. The filled boxes represent study plots where natural revegetation was occurring. The striped boxes represent plots where plant growth was inhibited by weeding. These plots were not measured for cover or diversity, but biomass was collected from the perimeter (as was for the other revegetated plots).

Sampling. Species abundance and cover were determined in triplicate subplots (1 m^2) located randomly within each plot. Species abundance, the actual number of individuals in a species, was measured as the number of ramets emerging from its own rooting mass within subplots. All seedlings were counted. Percent cover was determined by vertical projection of crown cover with forbs and basal area with grasses within the 1

m^2 subplot. Only in the final two years was percent cover per species determined during sampling. In the first year, digital images were taken and archived.

Plant dry weight per unit plot area was used as a measure of productivity through years. At the end of the growing season, vegetation was harvested along the perimeter of each plot. Three subplots ($0.33m^2$) per plot were selected and stratified with respect to the nearest edge. Plants were cut at the soil-level and dried at 45º C to a constant weight.

Plant establishment was further subdivided into specific categories of interest. Plants were grouped in classes according to ecological function: lifespan (annual, biennial, perennial), origin (native/introduced), and growth type (forb, grass, tree/shrub) using the USDA Plants Database. Invasive status was based on the Ohio Department of Natural Resources Invasive Species list that identify species threatening wild areas in the state (ODNR, 2000; USDA, 2002). Plot data were analyzed as described below in statistical analysis section.

Environment and Contamination Assessment. Environmental data (light, temperature, soil moisture) were obtained for initial and later successional conditions to account for plant-induced changes in light and temperature. These variables were light intensity and temperature at 1 m aboveground. Light intensity was measured in Lux using a light meter (Extech Instruments) and averaged from triplicate readings taken in the plot center at midday. A composite of three (0-10 cm depth) soil grab samples were collected from each plot during the summer to assess variation in moisture. The percent moisture was calculated from the difference in wet and dry over the initial wet weight. Soil samples collected for field soil moisture from summer 2001 and 2002 were combined by plot and sent to an agricultural station for nutritional status of the soil for

plant growth. The qualities measured were as follows: pH, percent organic matter, phosphorus, potassium, magnesium, calcium, CEC, percent K, percent Mg, and percent Ca. Temperature (CVG airport) and precipitation (NOAA) archived data were used to assess overall climate during the study period.

Plot contamination was determined from annual soil cores in 2000 and 2001. Total petroleum hydrocarbons (TPH) and polycylic aromatic hydrocarbons (PAHs) were extracted using accelerated solvent extraction (50:50, acetone:methylene chloride) and were analyzed using gas chromatography coupled to a flame ionization detector (GC-FID). Metals (Cr, Ni, Cu, Zn and Pb) were extracted sequentially to determine the total concentration of mobile and labile metals (Sposito et al., 1982). Details are reported in Chapter 4 (of this dissertation) and are summarized in Table 3.1.

Table 3.1. Average Soil Contaminants are listed below. Values represent the average upper layer of all plots (n = 14) from analysis years 2000 (initial sampling) and 2001. Mobile metals are the concentration of metals in the water soluble and exchangeable fraction. Labile metals are the sum of mobile metals and metals from the organic and carbonate-bound fractions. Total metal is the sum of labile and residual (non-labile) metals.

	Average (μg/g)	Standard Deviation
Total Petroleum Hydrocarbon	9649	5895
Polycyclic Aromatic Hydrocarbons (Total)	106	39
Mobile Metals (Total)	7.9	
Cr	0.7	0.2
Ni	0.6	0.1
Cu	2.6	0.4
Zn	3.5	0.9
Pb	0.6	0.3
Labile Metals (Total)	388	
Cr	25	3
Ni	7	1
Cu	38	5
Zn	70	13
Pb	248	69
Total Metals	1681	
Cr	495	88
Ni	82	7
Cu	93	10
Zn	368	50
Pb	653	193

Offsite Comparison. In September of 2003, the LTU and an offsite non-contaminated location (in Union, KY) were sampled for comparison of cover, richness and the incidence of invasive species. The offsite location was approximately the same size (2.2 hectare) and contained areas in the first year of succession (to pair with the three plots that were hand-removed during the previous 3-year study) and another in the 4^{th} – 5^{th} year of succession (Figure 3.3). The site had been used to raise hay and tobacco, but was left fallow since a clearing for a nearby housing development began in 1999. Using satellite photos, sampling areas were selected to approximate the quadrat arrangement on the LTU paying attention to the direction of light exposure and the distance to the nearest edge vegetation. This was not intended to qualify the plots for use in a paired test, but to direct sampling to equally complex areas of the same size. Eleven 20 x 20 plots were chosen within the quadrats with the same spacing as found on the LTU plots. Triplicate random subplots were measured using the same procedure as before.

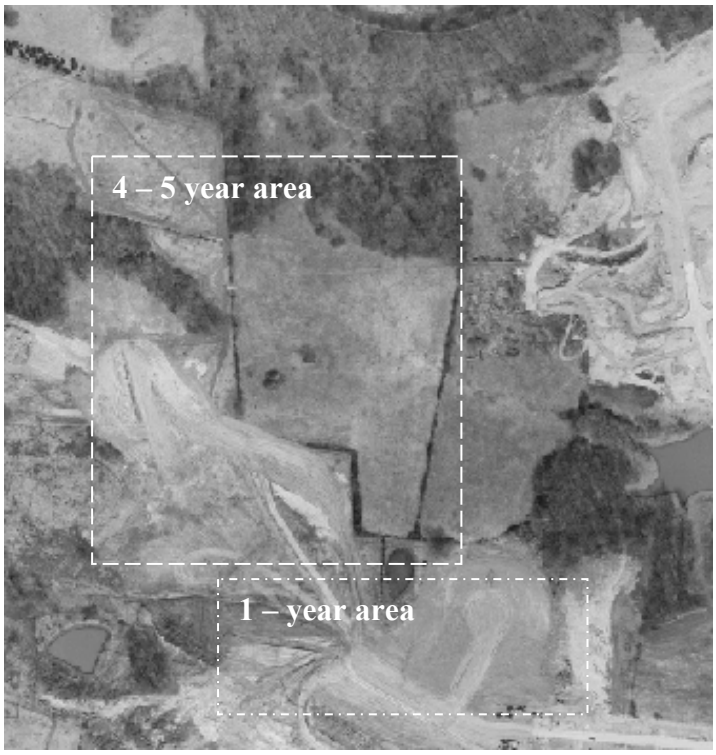


Figure 3.3. Aerial photo of offsite location. The lower right corner was cleared in preparation for a housing development in 1999 (the time of this photo) and again in 2002. The neighboring fields (center and upper left) had been left unmangaged since the development work began. Eleven LTU plots that had naturally revegetated since 1999 were compared to 11 oldfield plots (approximately 5 years of plant succession). The three LTU control plots from which vegetation was continually removed were compared to three plots set up in the lower right region of photo.

Statistical Analyses. Data from annual sampling were used to estimate (at the plot level) cover, abundance, diversity, and similarity (Table 3.2). Cover was defined as the total area of plant coverage within $1m^2$ and abundance was the number of individuals within the same area. At the subplot level, proportion cover and proportion abundance were determined for groups (e.g. by species, lifespan, invasive status). These values were then averaged first to the plot level, and then for all five months for a yearly value.

Generally, the term "species density" refers to the number of species per meter square, or in this study, the subplot. Alpha diversity is basically equivalent to richness, (S), or the number of species per unit of area. Alpha diversity was used in this study on a per-plot basis representing the total species count from the three subplots. Beta diversity (β) may be used to determine the number of distinct communities in multivariate space or for a large sample area (McCune & Grace, 2002). Hence, β will be calculated on a plot, per month and per year basis. The Shannon-Weiner (H') index is useful in discriminating subtle differences in diversity between sites (Barbour et al., 1999; Magurran, 1988). The $\log_2$ (as opposed to base 10 or e calculation) provides the most variation across years and within subplots of this study site, as would be expected from a study with the comparatively small sample size ($1m^2$). EcoStat 3 (Exeter Software) was used to calculate diversity measures on the plot, month and year scale. NTSYSpc 2.11a (Applied Biosystems) was used to calculate distance matrices (Dice Dissimilarity, Manhattan Distance) and the TPFGA Mantel (Miller, 1997) test was used to test for significant correlation between them. This index was also used to determine co-occurrences among plant species based on the plots in which they were found.

Table 3.2. Calculations and definitions of plant measurements.

Variable	Term	Indication	Formula
Cover	Percent Cover	Total area of plant coverage within $1m^2$ with minimum value of 1% to 100%.	1% cover = 0.01 m^2
Abundance	Number	Total number of individuals of all species within a subplot.	Abundance = $\Sigma_{individuals}$
Proportion	Proportionate Cover	The total cover of one group (e.g. invasives) relative to total. Determined at subplot level.	Prop. $Cover_{invasive}$ = $Cover_{inasive}/Cover_{total}$
	Proportionate Abundance	The total number of individuals in one group (e.g. invasives) relative to total number individuals. Determined at subplot level.	Prop. $Abund._{invasive}$ = $Total_{inasive}/Total_{all}$
Diversity	Species Density	Number of species per $1m^2$.	Density = $S_{subplot}$
	Alpha Diversity	Basically equivalent to richness, (S). Determined at plot level.	α = # $species_{plot}$

	Beta Diversity	Number of distinct communities in entire sample area (LTU) based on differences in species composition between plots.	$\beta = (S_c/S) - 1$
			S_c = # spp in composite sample
			S = average # spp in sample unit

	Gamma Diversity	Cumulative richness of an entire sampling area.	$\gamma = S_{LTU}$
	Shannon Wiener (H')	Both species richness and evenness (equitability) are taken into account for this index.	$H' = -\sum_{i=1}^{s} (p_i)(\log_2 p_i)$ p_i = proportion of species *i* s = number of species
Productivity	Biomass	Plant dry weight within a $0.33m^2$ subplot along perimeter of plots taken at end of growing season.	Biomass = gDW
Similarity	Dice Coefficient	Presence-absence between two communities used to determine percentage of species in common.	Dice = $2*S_{both} / S_{total}$ where: S_{both} = # spp in common S_{total} = total # spp

Succession is, technically, *yearly* rather than seasonal (monthly) changes. Therefore, plot richness, abundance, and diversity (a function of richness and abundance) were summed over all months to account for appearance and turnover of species throughout the season. Total cover, on the other hand, generally increases throughout a normal growing season since most plants exhibit indeterminant growth. Therefore, the yearly cover value (which in terms of analysis, will be compared to yearly abundance, number, diversity, etc.) will be an average of the five months. This will reduce the effects of season to more appropriately describe succession.

Statistical analyses were conducted using SYSTAT 10 (SYSTAT Software, Inc.). Repeated measures of plant growth data were tested for relationships to environmental, spatial and soil contamination variables with average values calculated per plot ($n = 11$) with year as the repeated variable ($n = 3$). Distance to edge was determined at the subplot level. Regression analysis assessed the relationship of edge distance to richness, diversity, cover at the subplot level within each sampling month.

Yearly changes in light and moisture regimes were assessed using factor analysis. Light (at 1 m from ground) and soil moisture from the June 2000 (initial), August (2001) and August (2002) samplings were reduced using factor analysis in order to assess whether or not these variables changed throughout the study period. Light and moisture were assessed separately. For use in repeated measures (above, to model the affect of environment on plant growth), variables were combined within sampling year and reduced to two factor scores (per year) separating the plots on the basis of light/moisture/temperature characteristics.

RESULTS

Table 3.3 summarizes the overall yearly changes in vegetative community diversity, cover and biomass. Figures 3.4a – 3.4d show changes within plots through the three year study period.

Richness and Diversity. Richness and diversity increased during the study period, appearing to increase overall in yearly increments and within each plot (Figures 3.4 and 3.5).

Table 3.3. Whole Site Plant Community Characteristics. Diversity and Dominance calculated from sums of all plots within year. Average plot richness is the monthly average of within-sampling of cumulative subplots (n = 5). Notation (*a,b,c*) indicates significant difference at $p \leq 0.05$ level, Bonferroni Hypothesis Test.

	Year 1	Year 2	Year 3
Diversity (H')	0.15	0.36	1.10
Site Richness (γ)	28	44	57
Average Plot Richness (α)	14.4 ± 4.2^{a}	19.4 ± 5.5^{b}	25.1 ± 4.0^{c}
Species Turnover (β)	1.95	2.27	2.27
Average Cover	$32.6\% \pm 12.1^{a}$	$63.1\% \pm 14.0^{ab}$	$79.0\% \pm 12.1^{b}$
Average Biomass (DW per 0.3m)	$38g \pm 17.4^{a}$	$50g \pm 25.1^{a}$	$129g \pm 59.0^{b}$

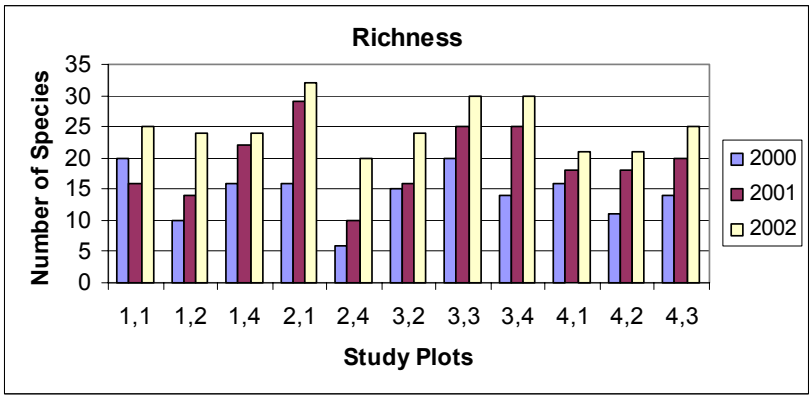


Figure 3.4a. Richness per plot for the study period. Richness is the total species that occurred in plot throughout the study year (e.g. a total species count for all 5 summer months).

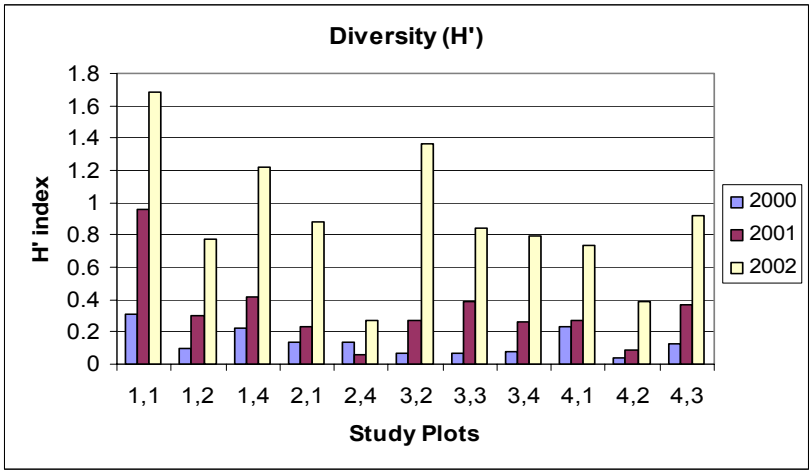


Figure 3.4b. Diversity per plot for the three year study period. Shannon-Weiner (H') index measures diversity as a function of species number and evenness.

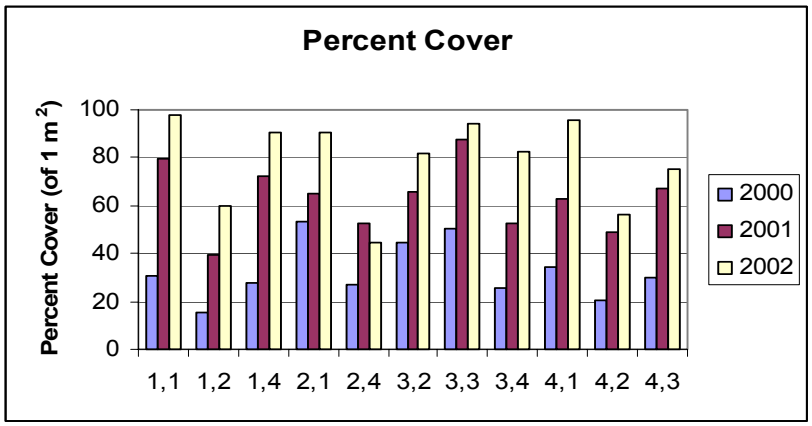


Figure 3.4c. Cover per study plot for the three year study period. Within plot percent cover was determined by averaging the five-month sampling.

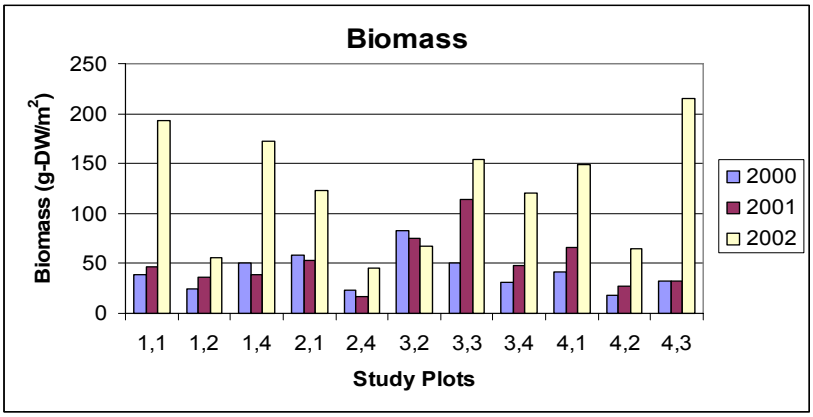


Figure 3.4d. Biomass per study plot for the three year study period. Biomass was calculated as the average of three subplots taken at the end of the growing season.

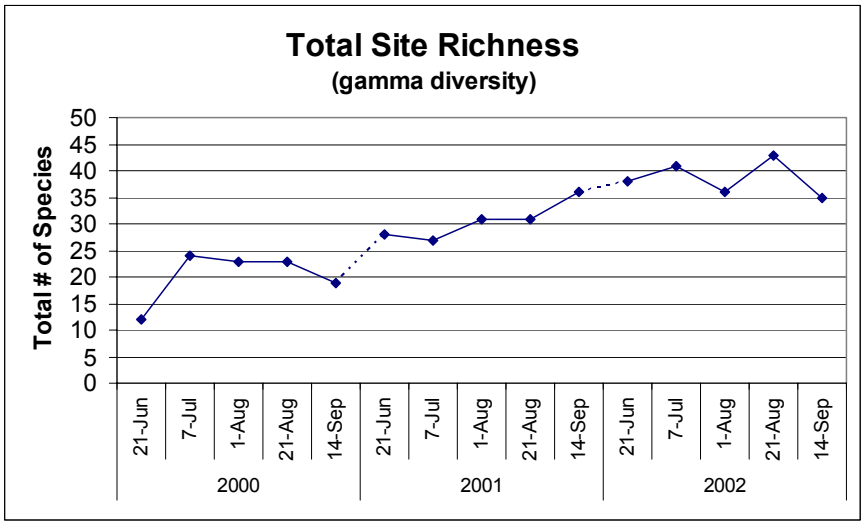


Figure 3.5. Species richness (gamma diversity) on the study site over the three year study period.

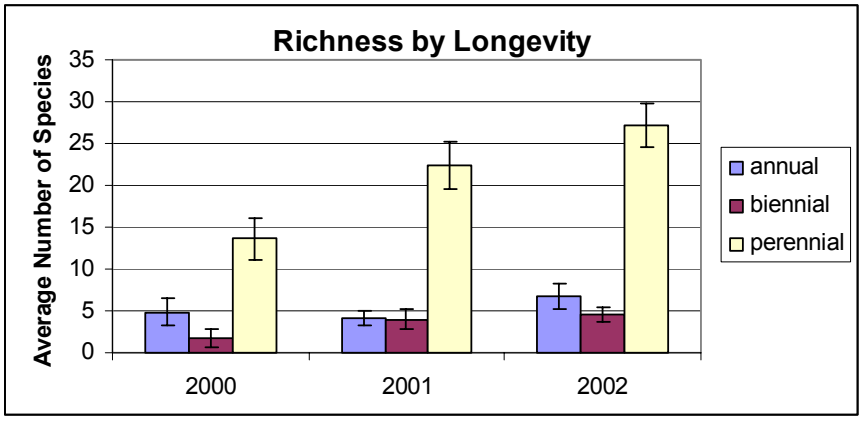


Figure 3.6. Average species richness within plant lifespan categories. Values are calculated from average number of species from each month (n = 5) within groups.

The number of annual and biennial species remained steady throughout the study period; however, the number of perennial species gradually increased over the study months. By the third year, the number of perennial species remained at or above 25 (Figure 3.6). Further, the fact that beta (species turnover) for whole site was the same in year 2 and 3 indicates the community is stabilized (Table 3.3), though new species recruitment is

occurring. It was also determined that the species composition (similarity between plots) was independent from the distance between plots (Table 3.4).

Table 3.4. Results of Mantel Tests for Multivariate Matrix Correlation (999 iterations). Matrix of geographical distances between plots was modeled for correlation to dissimilarity between species found within the plots.

Test Matrices	r statistic	p value (upper, lower tail)	Interpretation
Species Dissimilarity (Dice)*Geographical Distance (Manhattan Block)	-0.0284	0.566, 0.435	Plot communities not spatially correlated.

Another finding was that as distance to the edge increased, richness decreased (Table 3.5). This relationship was significant ($p < 0.05$) at the subplot level for all sampling dates until the final two samplings. Species density (# species/m^2) increased with time and decreased with distance to the edge. The negative relationship between distance to the edge and species density was significant ($p < 0.05$) for all sampling dates except the final two sampling. This suggests that after approximately 2.5 years of succession, the encroachment of species coming from the edge became equalized.

Table 3.5. Line equations and statistical significance of distance to edge (m) in determining species richness (# spp/m^2).

Sampling Date	Richness = Coeff(m) + Constant	p (2 Tail)	r^2
June 2000	-0.07D + 3.54	0.001	0.316
July 2000	-0.11D + 6.88	0.011	0.190
AugA 2000	-0.09D + 7.11	0.001	0.281
AugB 2000	-0.07D + 6.10	0.021	0.160
Sept 2000	-0.13D + 6.49	< 0.0005	0.411
June 2001	-0.08D + 6.88	0.005	0.224
July 2001	-0.09D + 7.61	0.021	0.161
AugA 2001	-0.10D + 8.49	0.003	0.246
AugB 2001	-0.11D + 8.59	0.001	0.291
Sept 2001	-0.10D + 8.92	0.013	0.185
June 2002	-0.09D + 9.93	0.013	0.182
July 2002	-0.10D + 11.08	0.014	0.179

AugA 2002	-0.11D + 9.58	0.001	0.307
AugB 2002	-0.04D + 8.98	0.446	0.019
Sept 2002	-0.032 + 7.96	0.405	0.022

Biomass and Cover. Though perennial ryegrass had been planted prior to the final tilling and was dominant at the start of the study, the majority of biomass (productivity) in subsequent years was due to other species (Figure 3.7). There was an increase in overall cover within each plot over the study period from 2000 to 2002 (Figure 3.8). In many plots, the growth of ryegrass was erratic, jumping in the second year, and falling back by the third year. This may be weather related as rye has a rather high water need relative to the colonizers from the natural community and the last year of the study was, on average a hotter, drier growing season than the previous two years, as shown in Table 3.6 (USDA, 2002). The increase in cover was steady throughout the three year study except for one plot (Figure 3.8). In plot 2,4 loss of cover appeared to be largely due to the response of ryegrass to the dry conditions in the third year. This is supported by the large amount of cover from non-ryegrass species.

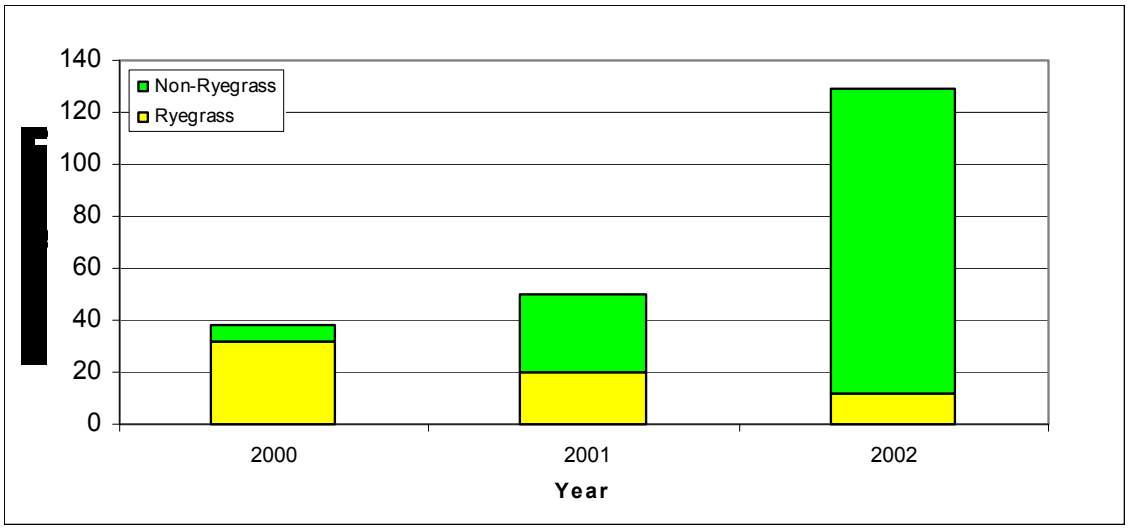


Figure 3.7. Biomass increased significantly in the three years of natural revegetation. The contribution of the naturally revegetated species is responsible for the significant increase. Y-axis shows biomass (g DW per m^2).

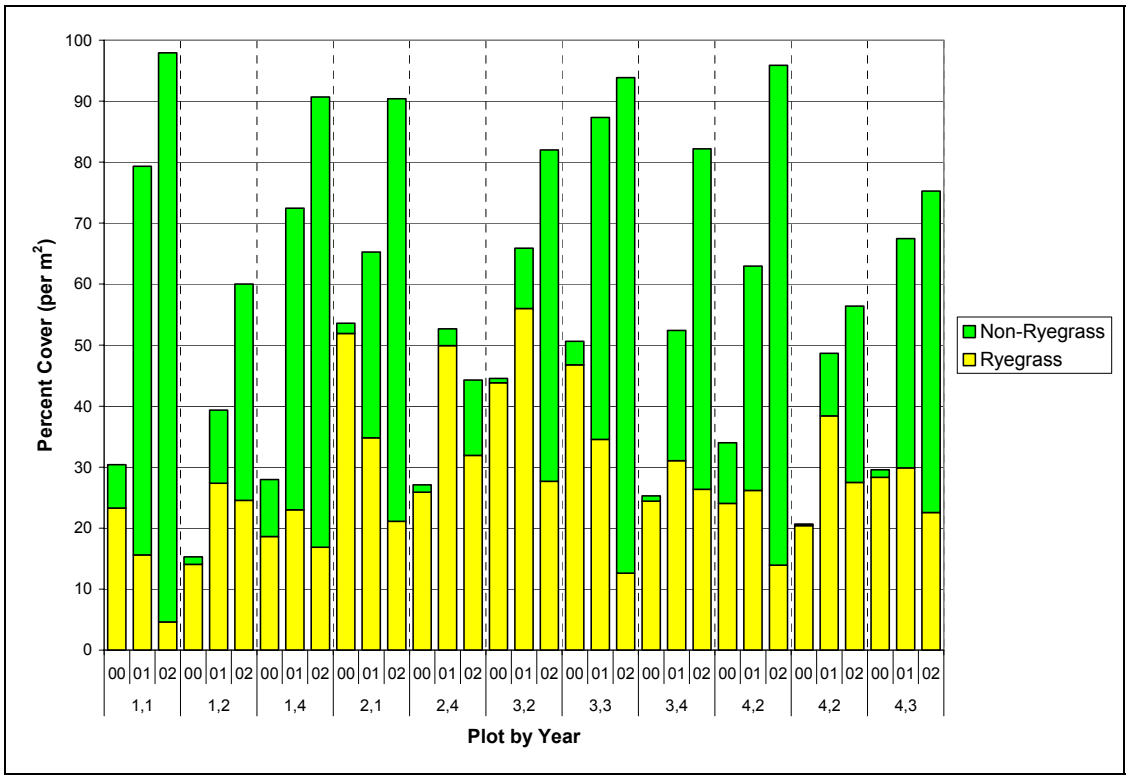


Figure 3.8. Average annual total cover per plot by ryegrass (light grey) and other species (dark grey).

Table 3.6. Average reported monthly precipitation and temperature for Cincinnati area. Averages from June to September. During the first year of study, precipitation and temperature were normal. The second year was wetter than normal. The final year received less rain and had higher temperatures than normal.

	Year		
	2000	2001	2002
Precipitation			
Total (cm)	10.2	13.5	7.1
Departure from Normal (cm)	1.0	4.3	-2.3
Temperature			
Average (°C)	21.3	21.6	23.9
Departure from Normal (°C)	-0.9	-0.5	1.4

Table 3.7. Statistics for 1-way ANOVA testing the significance of year (2000, 2001, 2002). Bonferroni tests with p-value less than 0.05 are listed below.

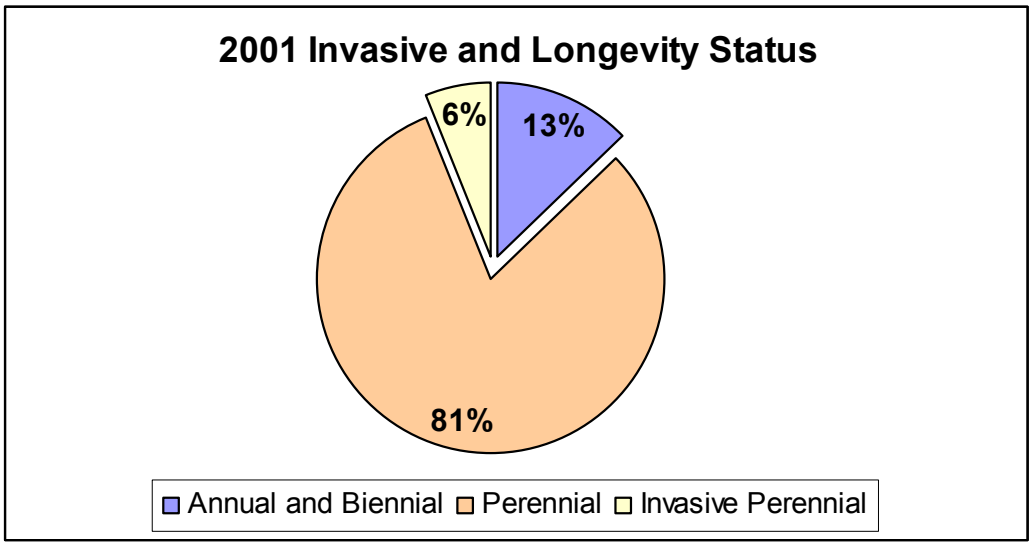
Dependent Variable	p-value	F-Ratio	Bonferroni Hypothesis Test (significant if < 0.05)
Average Cover	<0005	27.4	2000 < 2002
Average Cover Non-Rye	<0005	23.6	2000 < 2001 < 2002
Average Cover Rye	0.035	3.8	2001 > 2002
Average Biomass**	< 0005	23.3	2000 = 2001 < 2002
Richness (γ Plot, Year)	< 0005	14.9	2000 < 2001 < 2002
Abundance	0.107	2.41	
Proportion Annual & Biennial	0.102	2.47	
Proportion Perennial (less ryegrass) to Total	0.005	6.411	2002 > 2000
Proportion Solidago Canadensis	< 0.0005	16.225	2002 > 2000
Proportion Invasive Cover*	0.331	0.993	

* Data available for years 2001 and 2002 only.

** Perimeter of control plots also included for overall succession.

Invasive Species. Six invasive species colonized the LTU: *Lonicera maackii, Lonicera japonica, Dipsacus fullonum, Melilotus alba, Vinca minor* and *Ailanthus altissima*. In three years, the proportion abundance of the invasive species (to total abundance) did not significantly change (Table 3.7) nor did the proportion of invasive species cover increase significantly (Figure 3.9). However, in certain plots, invasive species cover is increasing rapidly (Figure 3.10). Invasive species did not appear to prevent establishment of later succession species. There was no correlation between the number of invasives to the number of native and native perennial species.

Figure 3.9. Proportion of Species Cover Categorized by Species Lifespan and Invasive Status (LTU invasive species are perennial). Graphs represent the average proportionate cover from revegetated plot (n = 11).



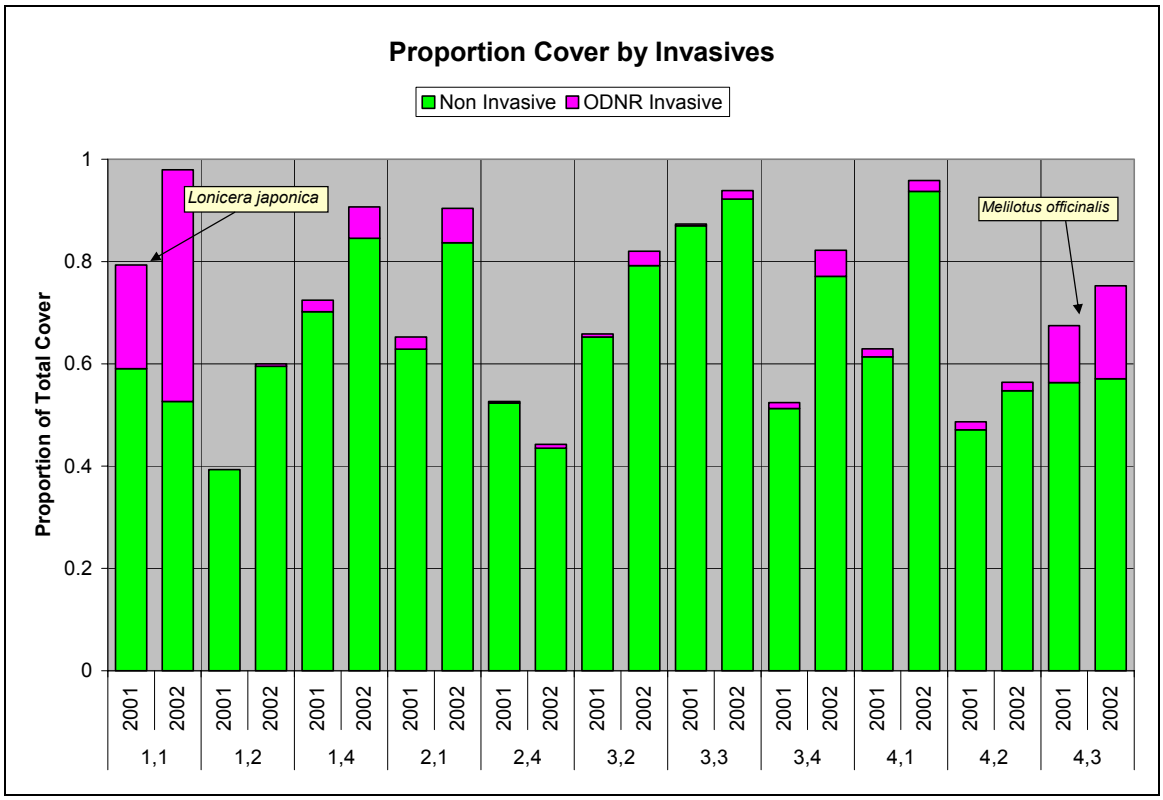


Figure 3.10. Incidence of Invasive Species. Proportion of cover by invasive species by plot. The two plots with the highest invasive cover are dominated by different species, as indicated: 1,1 with *L. japonica* and 4,3 with *M. officinalis*.

Edaphic, Contaminant, and Environment Analysis. Soil grab samples indicated plant nutrients were within optimal range for agricultural species, and therefore, probably sufficient for naturally revegetating species (it is likely these wild species can tolerate levels of nutrients that would otherwise be moderate to low for agricultural species). In two years, the overall organic matter and CEC increased significantly (in a paired t-test) (Table 3.8). These properties are expected to further stabilize metal contaminants. In the control plots, nitrogen had significantly dropped in the study period (0.65 to 0.37, $p = 0.05$), but remained the same (0.41 to 0.37, $p = 0.175$) in revegetated plots. Contamination data were averaged from the first two years' soil core analysis (Table 3.1) and were reported in Chapter 4. Briefly, the metal loading is highly correlated within

fraction such that where one metal is high, all metals are high. Labile and total metals tended to sort together; whereas, bioavailable (mobile) metals had a slightly different loading pattern.

Table 3.8. Characteristics of LTU Soil. Each value is the mean of 14 replicates from each plot around the LTU. For all plots, calcium levels are elevated above the agronomical optimal level as recommended by Spectrum analytical. Magnesium levels were high only for plots 2,4 and 3,1 – especially the latter. Potassium levels are rated moderate to good except for plot 4,1. Phosphorus levels were moderate to low.

Year	*pH*	*% OM*	*C:N*	*TOC (mg.g)*	*CEC*	*lbs / Acre*			
						P	K	Ca	Mg
2001	7.57	4.86*	27.1	0.20	17.86*	27	221	12603	619
2002	7.56	5.83**	28.6	0.21	18.86**	27	233	13410	691

Environmental data (light, temperature, sun exposure) were obtained for initial (June 2000) and later successional (August 2001, August 2002) conditions to account for plant-induced changes in light and temperature. All light variables sorted together on the first axis (explains 78% of the total variance) with the second axis (16% of total variance) separating initial light variation from the later two samplings (Figure 3.11). Based on the distribution of plots by their factor scores, the vegetated plots tended to have high loadings on the second axis indicating relative changes in light during the study period. Moisture had a much stronger separation from initial to final conditions. The first two years sort together on the first axis, representing 54% of the variation. However, the August 2002 (drought year) moisture has a strong loading on the second axis accounting for 32% of the moisture variation. Factor scores indicated control plots were moister than the vegetated plots, especially during the drought year. For inclusion in repeated

measures, each sampling years' environmental variables were reduced using factor analysis. For all of the years, light (and temperature) and moisture separated from each other on the first and second axes, respectively. For all years, the reduced factors represented at least 76% of the site variation.

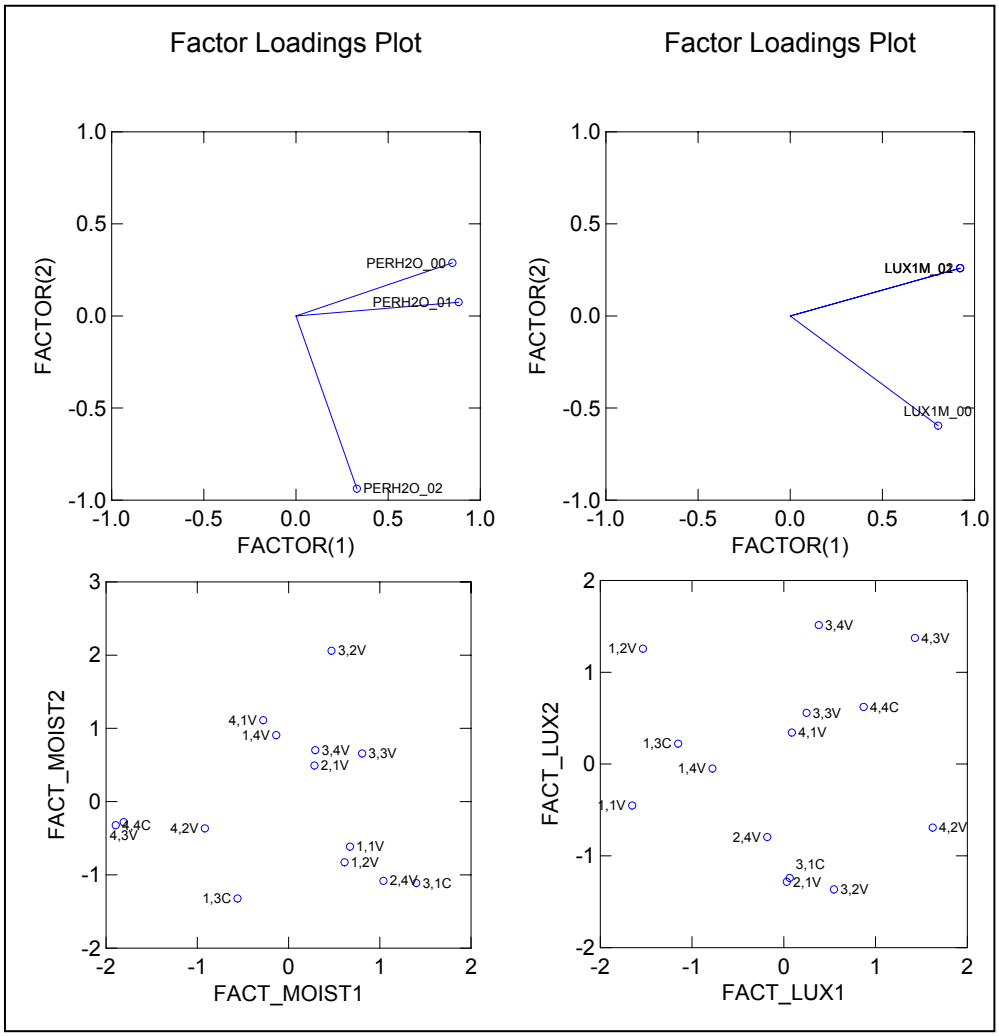


Figure 3.11. Factor analysis of moisture and light data for initial conditions (June 2000), and later conditions in August of 2001 and 2002. Control plots are denoted with the plot number followed by "C", all others are revegetated and the plot number is followed by a "V".

Relationship of Plant Succession to Other Variables. There was no relationship between metal loading and plant cover, biomass, diversity or richness within year nor repeated over the three year period. However, there were significant relationships between environmental factors and plant growth. Cover and abundance increases were related to the reduced factors representing light and moisture variation around the site. Species density (or richness at the subplot level), as mentioned above, decreased with distance to the edge. It was also found that biomass had a significant negative correlation with distance to the edge ($p = 0.013$) in the third season. Diversity (a combination of richness and evenness) was not correlated to any of the environmental variables.

Offsite Comparison. The offsite location had the same relative percent cover and richness as the LTU (Figures 3.12 and 3.13). At the time of sampling, only the five more frequent invasive species were present on the LTU: *M. alba, L. japonica, L. maackii, D. fullonum* and *A. altissima*. The same species were present offsite with the addition of *Daucus carota, Melilotus officinale, Circium arvense,* and *Rosa multiflora*. All offsite plots had invasive species, whereas only 8 of the 4-yr and 2 of the 1-yr onsite plots contained invasives. Because of the absence of invasive species in some plots, the variation of proportion invasive abundance is high, but still, significant differences exist. For the 4 year succession plots, the invasive abundance is significantly higher offsite. Onsite, the incidence of invasives was significantly higher in the 1yr plots than the 4 year plots. The proportion cover of invasives was not significant for year or location (Table 3.9).

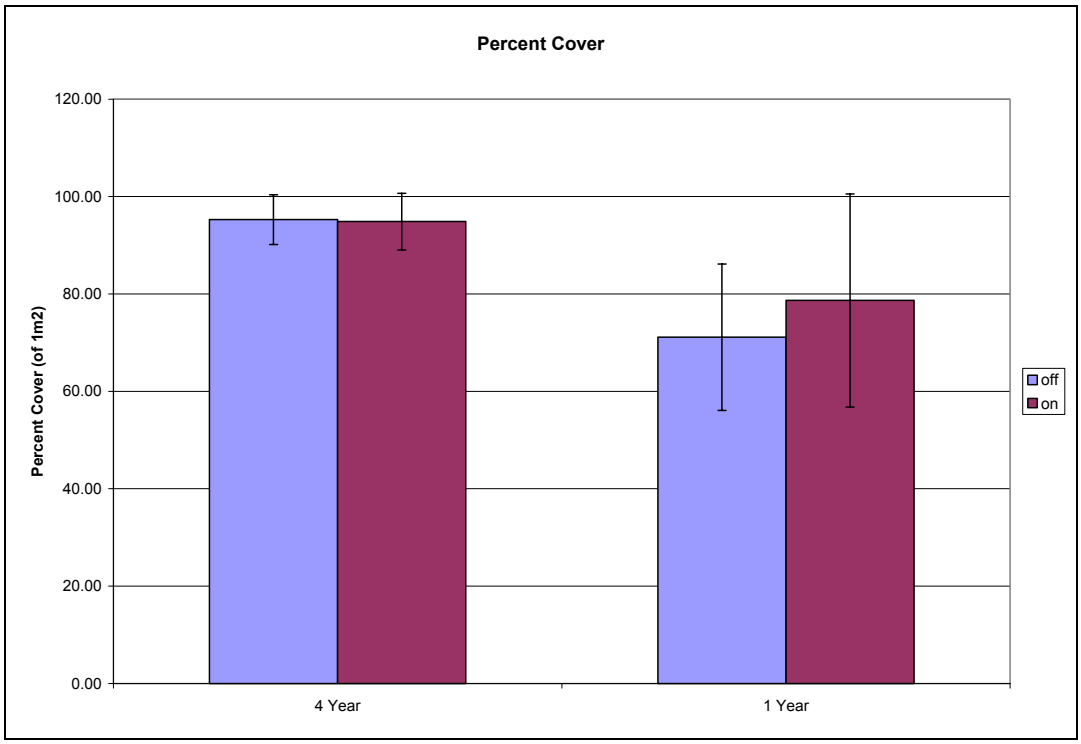


Figure 3.12. Cover on the LTU relative to an unpolluted offsite location. Two-way ANOVA indicated the year of succession was significant, but not location.

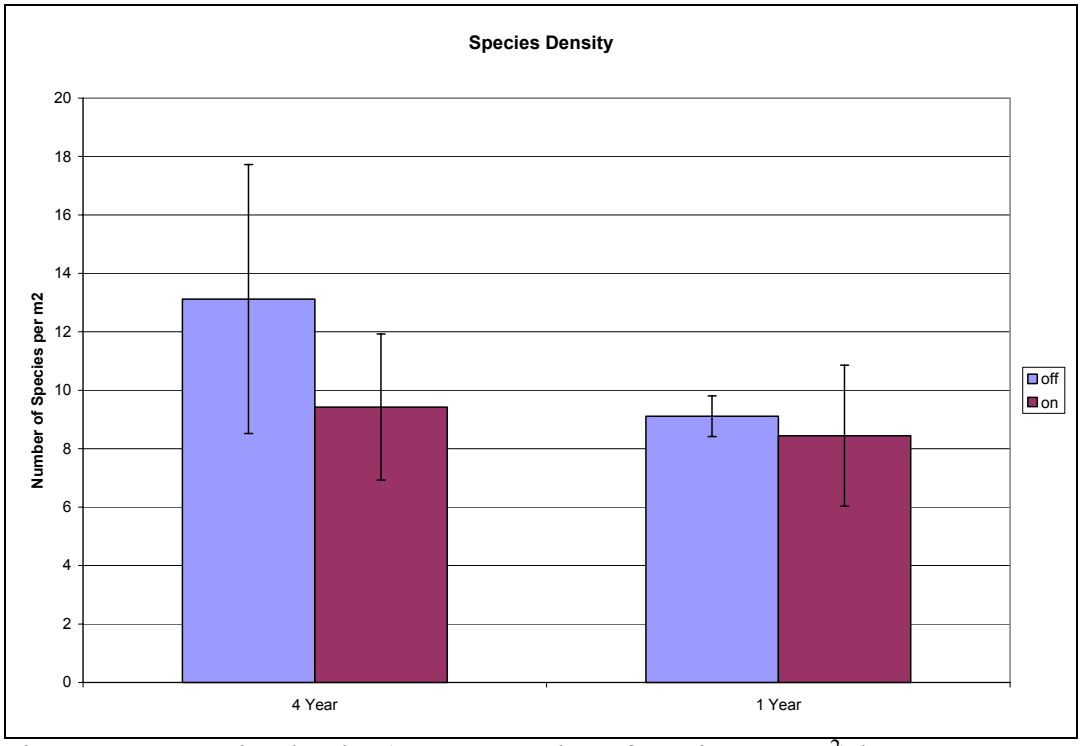


Figure 3.13. Species density (average number of species per $1m^2$) between years and location. Though, on average, the offsite oldfield had higher species density, two-way ANOVA indicated density is not significantly different between years or location.

Table 3.9. Statistical results of Two Way ANOVA comparing LTU and non-polluted community (Location) and years fallow (Year). The average of the three subplots was determined for each measured variable, then these values were modeled for ANOVA.

Measured Value	Test	p - value	Interaction Tests
Average Cover	Year	< 0.0005	
	Location	0.406	
	Year*Location	0.353	
Plot Species Density	Year	0.130	
	Location	0.183	
	Year*Location	0.351	
Proportion Invasive Cover	Year	0.724	
	Location	0.494	
	Year*Location	0.117	
Proportion Invasive Abundance	Year	0.081	
	Location	0.265	
	Year*Location	0.012	
		Year	Year 4: on < off Year 1: N.S.
		Location	Onsite: 4 yr < 1 yr Offsite: N.S.

DISCUSSION

The revegetation of the natural community on this contaminated site appears to be progressing in a manner expected for normal secondary succession. Species richness, in just two seasons, nearly tripled. Cover, reaching an average 79%, is well beyond that needed to prevent erosion (EPA, 1993). As would be expected in a non-contaminated system, annuals were replaced by perennials. Important increases seen in biomass in the second and third years were associated with the natural vegetation and not seeded species (*L. perenne*). Despite less than optimal climatic patterns in the third year, this significant increase in the productivity and diversity of vegetation indicates community resilience, and is evidence of a healthy, functioning community (Rapport et al., 1998). The progress of succession (cover, richness) on this contaminated site is not unlike that of a

comparable uncontaminated site. The incidence of invasive species was actually less than the offsite area and typical of a plant community recovering from agricultural management.

Though the previous study (Chapter 2) found slight phytotoxicity of site soil, large scale effects of contamination were not evident. Metal and petroleum soil levels are much higher than would be found in a clean site; however, there was no relationship between the contaminants and plant growth. As far as this study could assess, normal succession was occurring without regard to these elevated exogenous materials. Given the very low bioavailability of the metals (under 1 μg/g in the mobile fraction for non-essential metals), plants likely were not exposed toxic levels. The significant increase of organic matter during this three year period of natural revegetation is likely to contribute to stabilization of remaining contaminants, thereby further reducing the phytotoxicity of metals or petroleum compounds.

The results of this study indicate that environmental variables were much more influential to plant succession patterns than the contaminants. Not surprisingly, plant productivity (cover and biomass) responded to light and moisture regimes. However, the fact that productivity and diversity increased in all plots regardless of the plot-specific microclimate supports the advantages of allowing natural revegetation over managed cover systems. Through natural selection, species that specialize in harsher conditions thrive without the competition of less tolerant species. Another notable trend in the LTU community was that higher richness and productivity occurred in areas closest to established edge vegetation. This "edge effect" was more dominant for richness in the beginning; however, in the third season, biomass was negatively affected by distance

from the edge. This finding has management implications. Since greater plant productivity and richness occur first in areas closest to established edge vegetation, landfarms that leave undisturbed patches of vegetation may experience a faster rate of natural revegetation. Similarly, newly constructed sites might design for maximizing the perimeter edge length relative to the interior area.

Like any other disturbed area, there is potential for invasive species to dominate on contaminated soil. Because invasive species tend to out-compete native species, their abundance may negatively impact the ecological value of natural revegetation (DiTomaso, 2000). The consequence of invasive species presence is site specific and, in some cases, may have no negative effect on the non-invasive community (D'Antonio & Meyerson, 2002). On the LTU, the invasive species seemed to be restricted to two plots: 1,1 and 4,3. As is characteristic of invasive species, these species established themselves and became increasingly dominant within these two plots. *Melilotus albus* is a nitrogen fixer, and therefore, may have benefits to soil quality that outweigh its invasive status. *Lonicera japonica* is typically one of the more aggressive invasive species in the region (ODNR, 2000). On this site, the frequency of *L. japonica* is decreasing (Appendix 3A) indicating it is confined to only a few plots.

The observations of this study might have been complicated by the early establishment of perennial ryegrass (Figure 3.1) of the managed cover system used between the years of 1992 and 1998. However, the plots that had been continually weeded throughout the study (control plots) became established with the same pioneer species as seen in the other 11 revegetated plots, despite the early ryegrass establishment

in the latter (Tables 3.10a,b). This suggests even without the early establishment of ryegrass (i.e. on barren soil), a similar community may have occurred.

Table 3.10a. Dominant plant species (greater than 10% cover) in control plots removed in June prior summer season of 2002. The area occupied by species is listed in decreasing plot coverage (m^2 or equivalent to 100% subplot cover).

Control Plot 1,3	m^2	Control Plot 3,1	m^2	Control 4,4	m^2
Erigeron annuus	*7.8*	*Solidago canadensis*	*4.1*	*Cyperus strigosus*	*1.7*
Lonicera japonica	*4.3*	*Polygonum persicaria*	*1.8*	*Solidago canadensis*	*0.8*
Lolium perenne	*1.1*	*Cyperus strigosus*	*0.9*	*Conyza canadensis*	*0.4*
Conyza canadensis	*1.0*	*Bidens frondosa*	*0.3*	*Echinocloa crus-galli*	*0.3*
Solidago canadensis	*0.7*	*Echinocloa crus-galli*	*0.3*	*Lolium perenne*	*0.2*
Ambrosia artemisiifolia	*0.7*	*Lolium perenne*	*0.2*	*Polygonum persicaria*	*0.2*
Polygonum persicaria	*0.2*	*Agrostis perennans*	*0.1*	*Carduus nutans*	*0.2*
Cyperus strigosus	*0.2*				
Setaria glauca	*0.2*				
Echinocloa crus-galli	*0.1*				

Table 3.10b. Frequency of above species in revegetated plots part of larger study (n = 11).

Species	Frequency		
	2000	2001	2002
Ambrosia artemisiifolia	0.09	0.18	0.18
Bidens frondaos	0.00	0.09	0.55
Carduus nutans	0.00	0.09	0.36
Conyza canadensis	0.73	0.82	0.91
Cyperus strigosus	0.00	0.73	0.36
Echinocloa crus-galli	0.82	0.73	0.73
Erigeron annuus	0.82	0.45	0.64
Lolium perenne	1.00	1.00	1.00
Lonicera japonica	1.00	0.27	0.36
Polygonum persicaria	0.45	0.45	0.36
Setaria glauca	0.00	0.27	0.09
Solidago canadensis	0.73	1.00	1.00

Other contaminated sites slated for ecological restoration may benefit from utilizing (seeding or planting) species from this study. The species on this site demonstrate tolerance to contaminants and initial early succession conditions. In particular, *Solidago canadensis* (Canadian goldenrod) became established in all test plots, perhaps owing to its high vegetative spread rate. This species also achieved the highest total cover of all establishing vegetation, surpassing the ryegrass in 2002. This increase in cover was significant (Table 3.7). In addition to species with high frequency and cover, ecological restoration also focus on establishing of N-fixing species (Bradshaw, 1997). On this site, naturally occurring N-fixers were *Robinia pseudoacacia* (black locust) and *Melilotus albus* (white clover). The white clover, mentioned above, is considered invasive in Ohio. Black locust is a native tree species that was also present in the surrounding community.

This study showed that in the absence of cover management, a diverse vegetative community formed directly on contaminated soil. From an ecological perspective, letting the natural community self-establish is more sustainable than managing sites by human intervention (Bradshaw, 1997). A self establishing plant community, through the process of natural selection, has already optimized plant growth for microclimatic niches and tolerance to remaining contamination (Ewel, 1999). For other sites with contaminant levels low enough or unavailable to plants, rigorous seeded cover management may not be necessary. In this study, the *in situ* plant community gradually replaced the seeded vegetation. Significant degradation of organic contaminants was found in the planting depth in the study plots (see Chapter 4 of this dissertation). Metal uptake was assessed for the most common species presented in this study (Chapter 5 of this dissertation). The

range of metal concentration in shoot tissue is within a normal range for plants growing on uncontaminated soil. On similar aged contaminated sites where there is low risk initially, allowing the natural community to form may yield a more diverse, sustainable community than managing vegetation.

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APPENDIX 3A. Latin nomenclature, common names and characteristics of plant species naturally colonizing the Land Treatment Unit. Frequency indicates the number of plots (out of 11) in which species was found during sampling year 2000 (1), 2001 (2), and 2002 (3). Rank is the rank in proportion final cover (2002) within each group (Forbs, Grasses, Trees/Shrubs).

FORBS

Family	Genus species	Common Name	Habit	Duration	U.S. Nativity & Invasive Status	Frequency			Rank
						'00	'01	'02	Forbs
Apocynaceae	*Apocynum cannabinum* L.	Indian hemp	F	P	NAT	0	0	9	17
	Vinca minor L.	common periwinkle	S, V	P	INT, INV	0	9	9	25
Asteraceae	*Ageratina altissima* (L.) King & H.E. Robins. var. *altissima*	white snakeroot	F	P	NAT	36	73	73	11
	Ambrosia artemisiifolia L.	ragweed	F	A	NAT	9	18	18	5
	Bidens bipinnata L.	Spanish needles	F	A	NAT	0	0	9	25
	Bidens frondosa L.	devil's beggartick	F	A	NAT	0	9	55	10
	Carduus nutans L.	musk thistle	F	B, P	INT	0	9	36	19
	Conyza canadensis (L.) Cronq. var. *canadensis*	Canadian horseweed	F	A, B	NAT	73	82	91	6
	Erigeron annuus (L.) Pers.	eastern daisy fleabane	F	A	NAT	82	45	64	4
	Eupatorium serotinum Michx.	late-flowering thoroughwort	F	P	NAT	0	18	55	12
	Euthamia graminifolia (L.) Nutt. var. *graminifolia*	flat-top goldenrod	F	P	NAT	0	73	64	8
	Lactuca biennis var. *integrifolia* (Moench) Fernald	tall blue lettuce	F	B	NAT	0	0	9	20
	Lactuca serriola L.	prickly lettuce	F	B	INT	36	55	55	14
	Rudbeckia hirta L. var. *pulcherrima* Farw.	blackeyed Susan	F	A, B, P	NAT	0	18	9	22
	Solidago canadensis L.	Canada goldenrod	F	P	NAT	73	100	100	1

	Symphotrichum pilosum (Willd.) Nesom var. *pilosum*	Hairy white oldfield aster	F	P	NAT	9	18	82	2
	Taraxacum officinale G.H. Weber ex Wiggers	common dandelion	F	P	INT	18	55	73	13
	Vernonia gigantea (Walt.) Trel. spp. *gigantea*	giant ironweed	F	P	NAT	0	18	55	9
Dipsicaceae	*Dipsacus fullonum* L. ssp. *sylvestris* (Huds.) Clapham	Fuller's teasel	F	B	INT, INV	0	18	36	18
Euphorbiaceae	*Euphorbia maculata* (L.)	spotted sandmat	F	A	NAT	18	27	9	22
Fabaceae	*Melilotus officinalis* (L.) Lam.	white (yellow) sweetclover	F	A, B, P	INT, INV	45	36	36	3
Labiteae	*Mentha spp.*		F	P		0	0	18	16
Onagraceae	*Epilobium coloratum* Biehler	purpleleaf willowherb	F	P	NAT	0	9	0	25
	Oenothera biennis L.	common evening primrose	F	B	NAT	9	45	64	7
Polygonaceae	*Polygonum convolvulus* L.	black bindweed	V, F	A	INT	0	9	18	22
	Polygonum persicaria L.	spotted ladysthumb	F	A, P	INT	45	45	36	15
Rosaceae	*Fragaria vesca L.*	wild strawberry	F	P	NAT	0	18	0	27

GRASSES AND SEDGES

Family	Genus species	Common Name	Habit[i]	Duration	U.S. Nativity & Invasive Status	Frequency			Rank
						'00	'01	'02	Grass
Cyperaceae	*Carex sartwellii* Dewey	Sartwell's sedge	G	P	NAT	0	0	18	12
	Cyperus strigosus L.	strawcolored flatsedge	G	P	NAT	0	73	36	6
Poaceae	*Agrostis perennans* (Walt.) Tuckerman	upland bentgrass	G	P	NAT	9	9	27	7
	Agrostis stolonifera L.	creeping bentgrass	G	P	NAT	0	0	9	9

	Dactylis glomerata L.	orchard grass	G	P	INT	9	18	18	8
	Echinochloa crus-galli (L.) Beauv.	barnyard grass	G	A	INT	82	73	73	4
	Lolium arundinaceum (Schreb.) S.J. Darbyshire	tall fescue	G	P	INT, Seeded	91	100	100	2
	Lolium perenne L.	perennial rye (planted)	G	P	NAT, Seeded	100	100	100	1
	Schizachyrium scoparium (Michx.) Nash.	little blue stem	G	P	NAT	0	0	64	5
	Setaria glauca (L.) P. Beauv.	yellow foxtail grass	G	A, P	INT	0	27	9	11
	Sporobolus vaginiflorus (Torr. Ex Gray) Wood	poverty drop-seed	G	A	NAT	0	0	9	3
	Vulpia bromoides (L.) S.F. Gray	brome fescue	G	A	INT	55	0	18	10

TREES, SHRUBS AND WOODY VINES

Family	Genus species	Common Name	Habit	Duration	U.S. Nativity & Invasive Status	Frequency			Rank
						'00	'01	'02	Trees
Aceraceae	*Acer negundo* L.	boxelder	T	P	NAT	55	45	73	7
	Acer rubrum L.	red maple	T	P	NAT	36	45	55	12.5
Anacardiaceae	*Toxicodendron radicans* (L.) Kuntze spp. *radicans*	eastern poison ivy	S, V	P	NAT	0	0	9	16
Caprifoliaceae	*Lonicera japonica* Thunb.	Japanese honeysuckle	V	P	INT, INV	100	27	36	1
	Lonicera maackii (Rupr.) Herder	Amur honeysuckle	S	P	INT, INV	0	91	100	2
Cupressaceae	*Juniperus virginiana L.*	eastern redcedar	T	P	NAT	0	0	18	12.5
Fabaceae	*Robinia pseudoacacia* L.	black locust	T	P	NAT	0	0	09	16
Moraceae	*Morus alba* L.	white mulberry	T, S	P	INT	0	0	09	16
Oleaceae	*Fraxinus americana* L.	white ash	T	P	NAT	55	45	100	2

Plantanaceae	*Platanus occidentalis* L.	American sycamore	T	P	NAT	91	100	91	5
Rosaceae	*Prunus serotina* Ehrh.	black cherry	T, S	P	NAT	0	9	18	12.5
Salicaceae	*Populus deltoides* Bartr. ex Marsh.	eastern cottonwood	T	P	NAT	27	73	64	8
	Salix interior Rowlee	sandbar willow	T, S	P	NAT	0	9	27	4
Simbaroubaceae	*Ailanthus altissima* (P. Mill.) Swingle	tree of heaven	T	P	INT, INV	91	73	73	12.5
Ulmaceae	*Ulmus americana* L.	American elm	T	P	NAT	100	100	100	6
Vitaceae	*Parthenocissus quinquefolia* (L.) Planch.	Virginia creeper	V	P	NAT	18	36	82	9
	Vitis vulpina L.	Frost Grape	V	P	NAT	64	64	55	10

F = forb, G = graminoid, T = tree, S = shrub, V = vine

P = perennial, A = annual, B = biennial

NAT = U.S. native, INT = introduced. Source: USDA Plants Database.

INV = invasive. Source: Ohio Department of Natural Resources "Ohio's most invasive species".

APPENDIX 3B. Offsite Species List.

Abutilon theophrasti Medik.
Acer negundo L.
Acer rubrum L.
Achillea milefolium L.
Acylpha virginica L.
Agrostis perenans (Walt.) Tuckerman
Ambrosia artimisiifolia L.
Asclepias syriaca Walt.
Barbarea vulgaris R., Br.
Bidens frondosa L.
Chenopodium album L.
Circium arvense (L.) Scop.
Conyza canadensis (L.)
Cretagus spp.
Cynodon dactylon (L.) Pers.
Cyperus strigosus L.
Dactylis glomerata L.
Daucus carota L.
Dipsacus fullonum L.
Echinocloa crus-galli L. (Beauv.)
Erigeron annuus (L.) Pers.
Eupatorium rugosum Houtt.
Euphorbia maculate L.
Festuca arundinaceae
Fragaria vesca L. Porter
Fraxinus americana L.
Gleditsia triacanthos L.
Lactuca serriola L.
Lolium perenne L.
Lonicera japonica Thunb.
Lonicera maackii (Rupr.) Herder
Lychnis alba
Melilotus alba L.
Melilotus officinalis L. (Lam)
Oenthera biennis L.
Oxalis stricta L.
Panicum dichotomiflorum Michx.
Phleum pratense L.
Phytolacca americana L.
Plantago lanceolata L.
Plantago major L.
Polygonum hydropiperoides Michx.
Polygonum pensylvanicum L.
Potentilla recta L. *Prunella vulgaris* L.
Pyrus calleryana L.

Robinia pseudoacacia L.
Rosa multiflora Thunb.
Ruellia strepens L.
Rumex acetosella L.
Rumex crispus L.
Salix spp.
Sanicula canadensis L.
Schizachyrium scoparium (Michx.) Nash.
Secale cereale L.
Setaria verda
Sida spinosa L.
Solidago canadensis L.
Stellaria media (L.) Cyrillo
Symphoricarpos orbiculatus Moench
Symphyotrichum novae-angliae (L.) Nesom
Symphyotrichum pilosum Willd.
Taraxacum officinale G.H. Weber ex Wiggers
Toxicodendron radicans (L.) Kuntze spp. *radicans*
Tridens flavus (L.) Hitchc.
Trifolium agronium
Trifolium pratense L.
Trifolium repens L.
Ulmus Americana L.
Vernonia gigantea (Walt.) Trel.
Viola papilionaceae

CHAPTER 4

STABILITY OF CONTAMINANTS ON AN AGED PETROLEUM LAND TREATMENT UNIT UNDERGOING NATURAL REVEGETATION

HEATHER F. HENRY, ANNE M. VONDERHEIDE, AND JODI R. SHANN

Department of Biological Sciences ML6, University of Cincinnati, Cincinnati, OH 45221-0006

ABSTRACT

Theoretically, the dual processes of soil aging and ecological restoration should lead to increased stability of both soil contaminants and plant communities. In the absence of phytotoxicity, the vegetative community further enhances sequestration, prevents erosion, and contributes to the gradual mineralization of organic contaminants. However, the inherent heterogeneity associated with field-scale soils and contaminant deposition makes it difficult to assess the success of phytoremediation (as opposed to controlled greenhouse studies). One of the greatest challenges in a field scale study is taking into account (for sampling and analysis) the problems associated with inherent site variability. This study assessed changes in soil contaminant concentration on an aged petroleum landfarm left to revegetate naturally. Fourteen plots (20' x 20') were arranged throughout a 5.5 acre site: eleven of the plots were allowed to revegetate naturally, the other three were cleared of vegetation periodically to act as controls. Soil cores were extracted from these plots annually and were analyzed for total and available organic and metal contaminants as well as other edaphic factors. Stratification in soil cores was observed revealing two distinct layers: an upper layer of granular soil was distinctly overlying a lower layer which more closely resembled the original waste material (petroleum refinery sludges and emulsion oils). These layers, though variable in depth, were analyzed separately. The upper layer had a lower hydrocarbon concentration and higher metal contamination than the lower layer. Over three years, further degradation of total petroleum hydrocarbons (TPHs) and polycyclic aromatic hydrocarbons (PAHs) was observed in both layers. The reduction was significant in the upper layer, especially with the 4 and 5-ring PAHs. Degradation occurred in both naturally revegetated plots as well

as control plots where vegetation had been removed. Reduction in TPH and PAHs was not significant in the lower layer. There were no significant changes in the metal fractions from initial (2000) to final (2002), with the exception of a significant reduction of the lead associated with the organic fraction of upper layer soil. Metal concentration was not correlated to TPH and PAH loss. Two important points emerged from this study. Because the sampling design here was site specific, inherent variability was reduced enough that significant losses of organic contaminants was seen. Changes were seen in spite of the previous aging that had occurred – and the edaphic characteristics indicate continued contaminant sequestration. This study provides evidence that processes associated with phytoremediation by natural revegetation may be a slow but effective means of remediating and restoring aged contaminated sites.

INTRODUCTION

The past century of industrial activities generated pollutant byproducts that compromised human health and undermined ecological integrity. Only in the last thirty years have regulations existed to make illegal the release of contaminants into the air, water and on land illegal. One of the earliest clean-up strategies for cleaning sites with industrial byproducts was to deposit them in fenced-off terrestrial sites with variable degrees of containment. The petroleum refinery industry, especially, created numerous hazardous waste landfills on their properties called “landfarms”. By 1983, one-third of all refineries in the U.S. operated landfarms. New regulations in the later 1980s required industry to demonstrate stabilization or degradation of contaminants on these facilities. It was at this point that the possibility of contaminant degradation by “passive” means was

first explored. The term "natural attenuation" refers to the reduction (in mass, mobility or toxicity) of contaminants through physical, chemical and biological processes (Hejazi et al., 2003).

Landfarming, reconsidered in light of natural attenuation, could be considered a viable treatment option to clean these sites. Microbial degradation of petroleum hydrocarbons was well known (Hejazi et al., 2003); however, it was soon realized that the presence of plants (initially for erosion prevention) actually aided natural attenuation (Aprill & Sims, 1990; Reilley et al., 1996; Schwab et al., 1995). This phenomenon has been coined "the rhizosphere effect" whereby plant roots provide a soil environment for microorganisms that enhanced biodegradation (Reilley et al., 1996; Shann & Boyle, 1994). The use of plants to aid in the degradation and stabilization of contaminants became known as phytoremediation (Cunningham & Berti, 1993). Since then, phytoremediation has been used to remediate all classes of contaminants including organic, inorganic, radioactive, and mixtures (McCutcheon & Schnoor, 2003).

In contaminated soils that have aged for decades, the mobility of metals and the degradation of organics may be very slow (Bogan & Sullivan, 2003; Nam & Alexander, 2001). Metals become more stabilized as organic matter increases (Ahumada et al., 2004). With roots scavenging for other essential nutrients, sequestration at the root surface or within the rhizosphere is very common (Meager, 2000). Microbial degradation of organic contamination may be facilitated by root turnover, and/or emulsifiers released by some strains of bacteria (Hutchinson et al., 2003).

Though many greenhouse studies have shown increased contaminant degradation in the presence of plants, this has been a challenge to demonstrate in a field setting. The

first challenge for field scale assessment of phytoremediation is the establishment of a baseline for the site that accurately captures the preexisting variation of contaminant distributions. There is inherent heterogeneity of contaminants in most land treatment units because the hazardous wastes are deposited in truckloads of varying composition (Condit & Doherty, 2000). Though vertical homogeneity of contaminants is likely within the tilling depth, the soil below this depth may be less homogenous. Given such variability, coring efforts require a trade-off between limiting statistical power or sampling at close (e.g. 1 m) intervals (Wenzel & Blum, 1995). Intensive sampling would not only be expensive, but potentially destructive to the microbe-plant-soil structural integrity necessary in phytoremediation.

Closed landfarms provide an opportunity for field-scale trials to assess the potential of phytoremediation on sites with aged contamination. This study focuses on one such closed landfarm approved for "natural revegetation" as a means of establishing the vegetative cover. The closure plan did not require a clean soil "cap", thus allowing the opportunity to study the degradative potential of natural revegetation growing directly on the landfilled material. This study first characterized patterns of contamination on the site and then monitored for any changes in soil contaminants during the early period of natural revegetation plant establishment through full cover. The primary contaminants of concern on the site were heavy metals and polycyclic aromatic hydrocarbons (PAHs).

This study was conducted in parallel to a nationwide phytoremediation field trial, the Remediation Technology Development Forum (RTDF). RTDF is a consortium of government agencies, academic and industrial participants created for the purpose of testing the effectiveness of phytoremediation for various applications. While the parallel

RTDF study focused on the effects of managed planting treatments, this study assessed the degradation under passively established early successional vegetation. Though the results for the RTDF have not been released yet, their preliminary findings will be discussed in light of the discoveries made here.

MATERIALS AND METHODS

Study Site and Experimental Design. The area selected for this study is the Chevron Corporation Land Treatment Unit (LTU) in Hooven, Ohio. The LTU was opened in 1981 and received 10.6 million liters of petroleum refinery wastes from a nearby refinery, now out of service for approximately fifteen years. These wastes were disked into the limestone-based silty loam soil. Below the top soil is a clay layer which extends several feet to the Fairview limestone/shale formation. During the period of active landfarming (1990 to 1998), the LTU was tilled to a depth of 12 inches in the summer and as a winter vegetation cap, perennial rye (*Lolium perenne*) was seeded (Condit & Doherty, 2000). In 1998, Chevron began participation in the Research Technology Development Forum (RTDF) feasibility study of site cleanup using phytoremediation (EPA, 1999a).

The entire site was tilled for the last time in spring of 1998. Sixteen RTDF plots were arranged in random block design in four sections with contaminants above detection limit. Under the RTDF protocol, a variety of vegetative treatments (mixed grasses, hackberry, willow, and no vegetation) were established in an effort to compare degradation rates (EPA, 1999b). At this time, Chevron ceased maintenance on the site

(except for the RTDF study plots) and allowed the rest of the LTU to go fallow (Condit & Doherty, 2000).

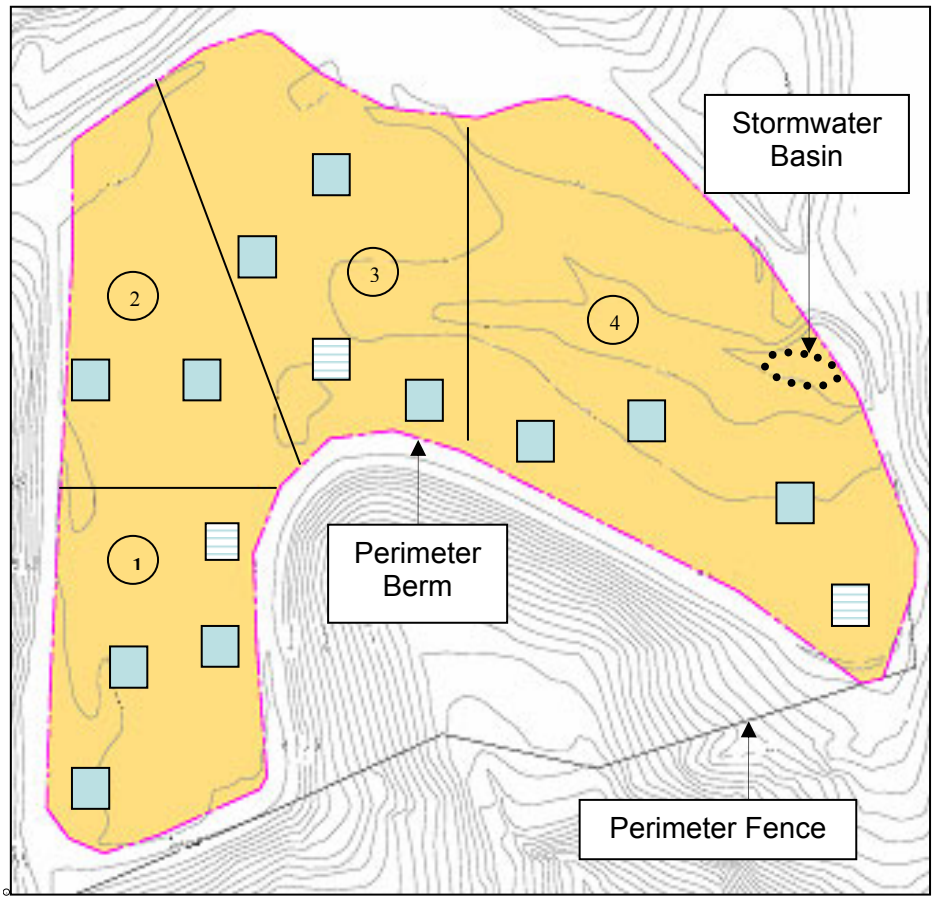


Figure 4.1 Study plot arrangement throughout the LTU site. The filled boxes represent natural revegetation, the striped boxes are control plots where vegetation was removed. The wedge shaped shaded area was leveled and filled with truckloads of petroleum refinery sludges. The perimeter berm is raised 1 m above the rest of the LTU to prevent runoff of contaminants. The LTU was constructed to slope towards the stormwater basin where water is collected and pumped back to the nearby refinery. The perimeter fence (the south portion shown below) circles around the entire site, restricting access to the LTU. Circles denote the quadrat number.

This natural revegetation study constructed parallel plots located within each RTDF quadrat arranged in a random block design (Figure 4.1). The sixteen 6.1 m x 6.1

m plots were delineated using flagging tape. Four of the 16 plots (one in each quadrat) were designated as controls in which hand-removal of vegetation was employed bi-monthly. The other plots received no additional maintenance from 1998 on. GPS (Global Positioning System) was used to locate latitude, longitude, elevation of plots and the site periphery (the berm).

In each year of the three year study, two replicate 75 cm deep cores were removed from each plot in October. Replicate cores were taken 50 cm apart. Cores (2.4 cm diameter) were taken to the side of plants, recording the overlaying vegetation. These cores were placed in a plastic liner, sealed, and stored in a -14.4° C freezer. Cores were analyzed according to visible rooting depth, texture and color characteristics. Duplicate cores within plots were analyzed separately as opposed to compositing in order to characterize variation within each plot.

Soil Metal Extraction and Analysis. Sequential extraction was used to assess the degree of bioavailablity of soil metals (Tessier et al., 1979). Approximately 2 g of sifted (2 mm mesh), oven dried soil was extracted with 25 mls of 0.5M KNO_3 for 16 hrs on a shaker (100-150 cycles per minute) and then centrifuged for 15 minutes at 5100 rpm. The supernatant was poured through a Whatman #42 filter paper and brought up to 25 mls with extractant solution. The process was repeated with DDI water (2 hr), 0.5M NaOH (16 hr), 0.05M Na_2EDTA (6 hr) and finally, 4M HNO_3 (16 hr at 70°C). The mobile (readily bioavailable) extracts (KNO_3 and H_2O) were combined and concentrated to 25 ml to overcome detection limitations (Sposito et al., 1982). Three replicates of each layer per core were extracted and then analyzed for metals using Atomic Absorption Spectrometry (Perkin Elmer 3110), and Inductively Coupled Plasma Atomic Emission

Spectroscopy (Thermo Jarrel Ash Corporation ICAP 61E Plasma Emission Spectrometer, Leeman) and Inductively Coupled Plasma Mass Spectrometer (Manufacturers: Agilent and Elan).

Soil Petroleum Component Extraction and Analysis. An accelerated solvent extraction system (Dionex ASE 200; Sunnyvale, CA) was used to extract samples from each layer of each soil core (Richter, 2000). ASE extraction cells (22 ml) were filled with 4g of soil mixed with 2g diatomaceous earth. The extraction solvent was methylene chloride-acetone (1:1, v/v). Heating time was 8 min, to reach 175°C. Extraction pressure was 1500 psi; static time was 5 min, flush volume was 70%, and the purge time 60 sec with 150 psi. Extracts were dried to 10 ml and then centrifuged for 10 min (5100 rpm), and the supernatant was dried to 4 ml and the bottle was rinsed with 1 ml MeCl. Standards were purchased for identification of 13 priority PAHs [EPA 525A, Ultra Scientific]: acenaphthylene, anthracene, benz[a]anthracene, benzo[k]fluoranthene, benzo[b]fluoranthene, benzo[ghi]perylene, benzo[a]pyrene, chrysene, dibenz[a,h]anthracene, fluorene, indeno[1,2,3-cd]pyrene, phenanthrene, and pyrene. Duplicates were run every 20 samples to determine relative percent deviation according to EPA Method 3500B (EPA, 1996a).

PAHs were separated on a gas chromatograph (GC 14A; Shimadzu, Columbia, MD) in the split mode (2:1), with a flame ionization detector (FID). The column was a DB-XLB (JW Scientific) proprietary phase of low polarity (60m x 0.25mm internal diameter x 0.25µm film thickness). The injection port temperature was 300ºC, column temperature was static at 95ºC for 0.5 min then increased by 5°C per min to 340ºC, and held for 7 min. All solvents and chemicals were reagent grade. Randomized duplicates

determined instrument reproducibility was 10%. Randomized duplicate of extraction procedure indicated relative percent duplicate was 30% for total PAHs (EPA, 1996a).

Integration of the nonspecific hydrocarbon peaks between 18 and 55 minutes (roughly the retention range of diesel) was calculated for each chromatogram (EPA, 1996b). The RF, based on average used for 13 PAH standards for each day of analysis to account for daily variation in instrument response. EZChrom Elite Software (Scientific Software, Inc.) was used to calculate areas of PAHS and TPH. PAHs were measured peak to peak, whereas the TPH was measured from baseline to baseline, following modification of EPA Method 3560. Verification of PAHs using GC-MS (Hewlett-Paccard 5890 Series II) was done for select samples.

Statistical Analysis. PAH and metal concentrations were log transformed, after assignment of a minimal number to account for samples that read below the detection limit (non-zero substitution: 0.05µg/g for metals and 0.04µg/g for PAHs). Replicate samples were averaged and a plot average was calculated using the replicate cores. SYSTAT 10 (Applied Biostatistics) was used to test plot averages for significant differences over three years (2000, 2001, 2002), across treatments (vegetated, unvegetated), and by strata (layers upper and lower). GIS Imaging (ArcView GIS 3.2, ESRI) was used to create exploratory maps of vegetation and contaminants. Point data from each plot were used as the z-value for grid interpolation using IDW method, nearest neighbor (12), and power (2).

RESULTS

Soil Core Physical Characterization. Physical examination of the cores revealed layering within the top 75 cm of soil, overlying the natural clay barrier (Figure 4.2). The top 30 cm of soil had a friable texture, and was light brown. Below this was a layer of dark, sticky material with an intense phenolic odor. These layers, though variable by depth, were present across the site. Given the fairly consistent nature of the original waste and the absence of a clean soil cap, the distinct features of the upper layer likely resulted from the two decades of tilling and the cover of ryegrass added in 1996. The underlying layer, generally below the tilling depth, is assumed to be more indicative of the original material. These layers were determined in each core, and analyzed separately. The "upper" layer was characteristically tan to brown, and the "lower" oily layer was a black color. Hence, color (Munsell Color Chart: 5Y 3/2) was used to separate soil layers (Bohm, 1979). They are referred to here as the upper and lower layers.

For each core, the depth of each layer was measured as was the depth of root penetration. There was no significant change in the thickness of the layers over three years. However, in vegetated treatments, the densely-rooted depth increased significantly by the third year ($p = 0.005$, Bonferroni between years 2000 and 2002) despite the variation in precipitation throughout the years. Starting with 12.3 cm (± 12.0 cm) in 2000, the average rooting depth increased to 24.1 cm (± 11.5 cm) in 2002. There was no statistical difference between years in densely-rooted depth of the control treatment. Despite continual removal of establishing vegetation in control plots, the presence of roots persisted (average of 8.5 cm in 2000 to 14.9 cm in 2002). This is likely due to plant

roots from surrounding vegetation scavenging for water in control plots (Taiz & Zeiger, 1991).

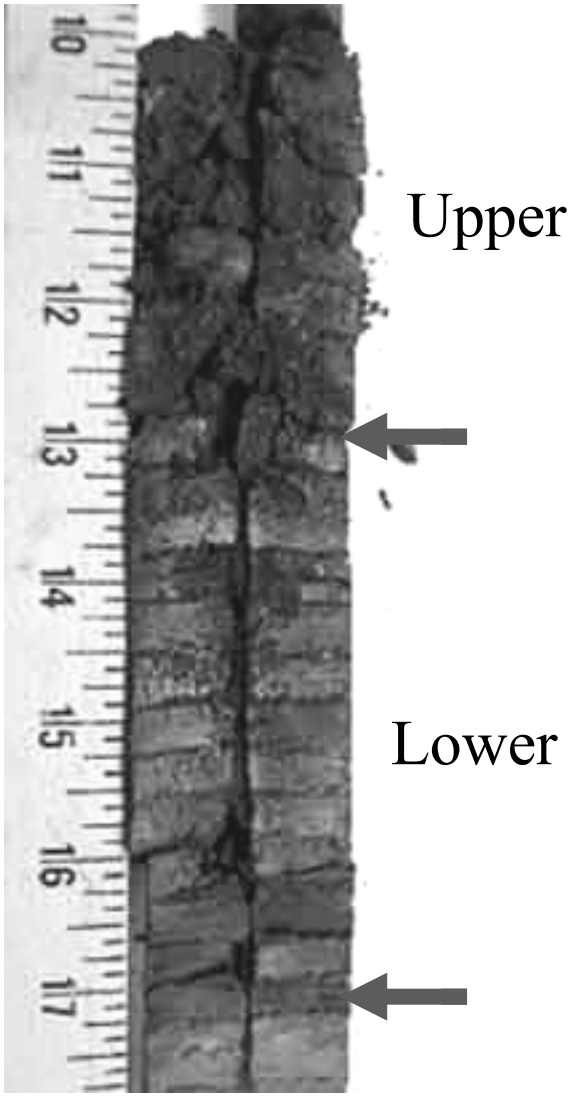
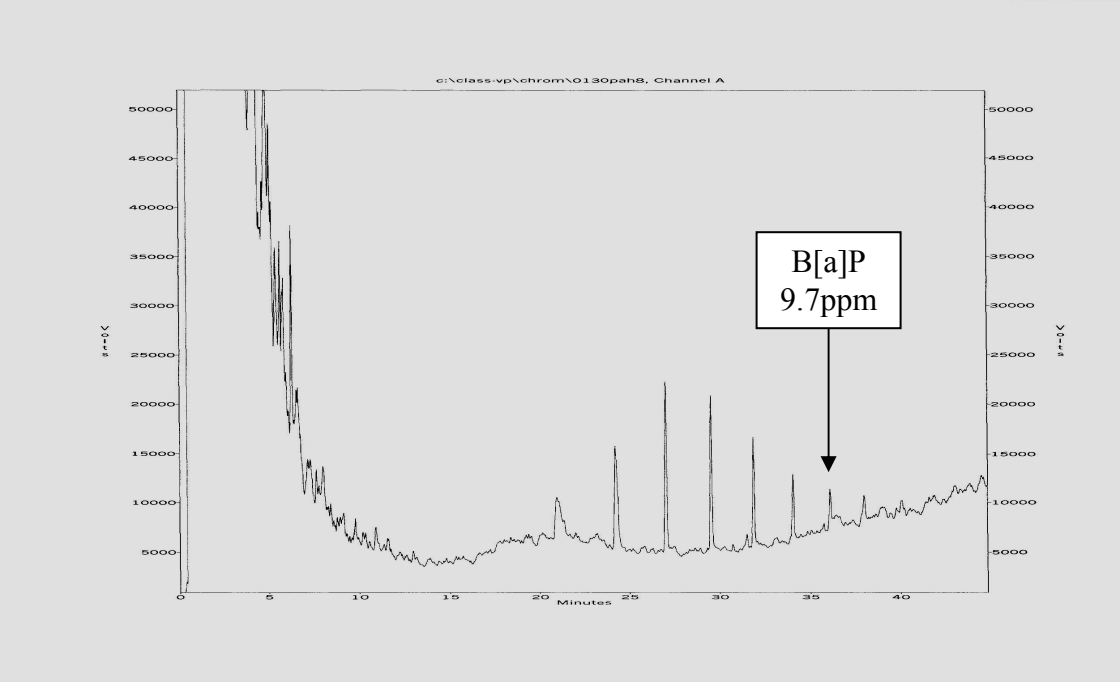


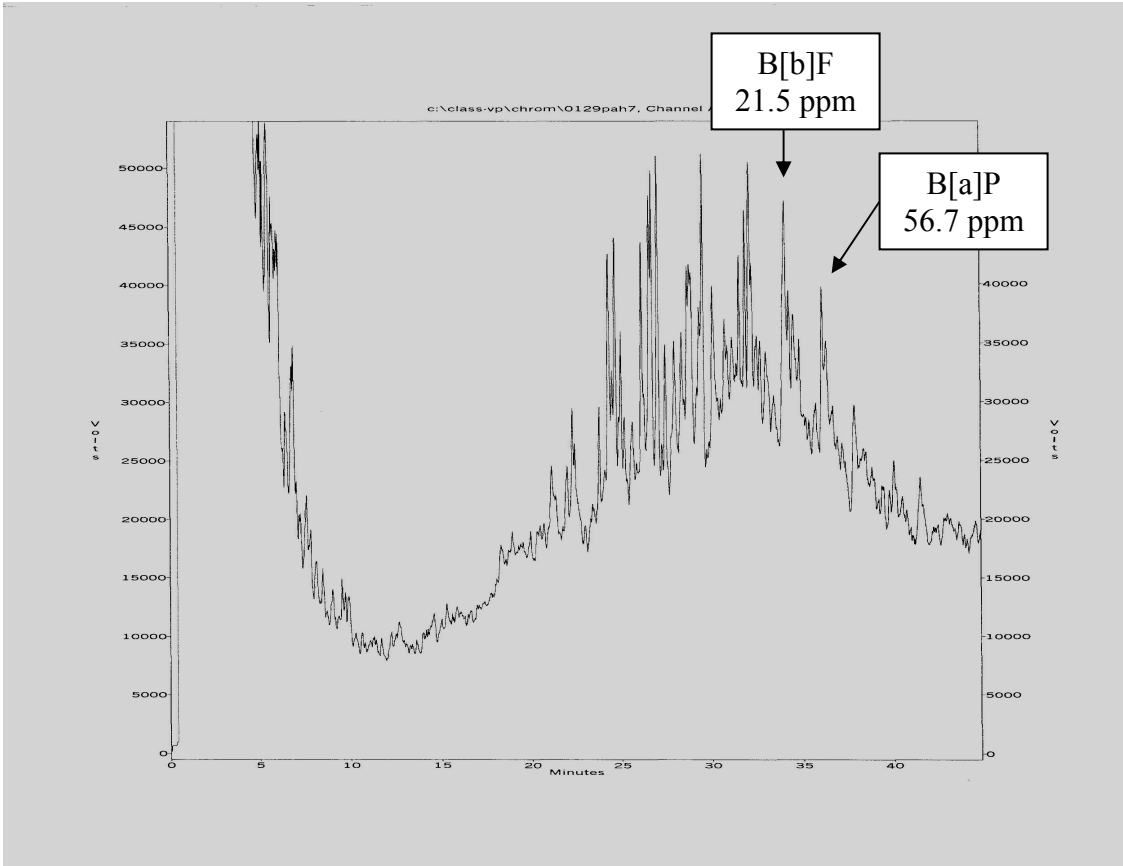
Figure 4.2. Soil stratification in LTU soils: the upper, planted layer and lower, presumably similar to the original materials (petroleum sludges from refinery processes). Depth is shown in inches. The upper layer has a loose to friable consistency similar loam soil, though initially sludge material disked into soil. The lower layer remains a sticky consistency. The depth of the "upper" and "lower" layers varies throughout the site. Therefore, this study separated the layers by the stratification as noted by the arrows. The parallel study followed the RTDF protocol specifying separation of cores at a fixed depth (at 30 cm or 11¾").

Figure 4.3. Chromatograms (GC-FID) of representative upper and lower soil layers, shown at the same response scale (0 - 50,000 volts). The PAHs with the highest concentrations are labeled (verified on GC-MS). The upper layer (a), under the influence of planted vegetation shows the recalcitrant PAHs (noted below) and very little remaining unresolved petroleum hydrocarbon. The lower layer (b), underlying the tilled/planted depth, shows a large proportion of the response was due to the unresolved petroleum hydrocarbons. The presence of peaks at the retention time of PAHs is noted; however, given the large TPH area, accurate quantification of individual PAHs is compromised using FID detection.

a.



b.


B[b]F
21.5 ppm
B[a]P
56.7 ppm
c:\class-vp\chrom\0129pah7, Channel
Volts
Minutes
0
5
10
15
20
25
30
35
40
5000
10000
15000
20000
25000
30000
35000
40000
45000
50000

PAH Assessment: Upper vs. Lower. Figures 4.3a and 4.3b show typical chromatographs obtained for each soil layer. The lower layer had a high concentration of unresolved total petroleum hydrocarbons making it more difficult to distinguish between PAHs and hydrocarbons that happened to share the same retention time. Hence, integration of PAHs peaks was done using peak to peak area (as opposed to baseline to baseline) as a conservative approach. Coefficient of variation within plot and on a site level (from plot averages) are listed in Table 4.1.

The results of ANOVA applied on soil PAH and TPH concentrations across the site (12 plots) for years 2000 and 2002 are listed in Table 4.2 for both control and revegetated plots. The total PAHs in the lower layer were significantly higher than the upper layer, as was TPH. Over the three year period, there was an overall reduction in TPH and total PAHs (TPAH). This reduction was significant ($p < 0.0005$) for the upper layer, but not for the lower layer (Table 4.2a and Figure 4.4). In the upper layer, TPH and TPAH were significantly reduced from initial to final within each of the treatments (Table 4.2b). There was no statistical difference between treatments in the reduction of TPH. On the other hand, the interaction between year and treatment indicates that TPAH reduction in the unvegetated treatment was actually greater than that of the unvegetated treatment. Overall, the per-plot loss of TPH was positively correlated to the loss of TPAH ($p < 0.0005$) within layer (Table 4.2c). The concentration of TPH and TPAH in did not significantly change during the study period regardless of treatment.

Table 4.1. Coefficient of variation for both layers within plot and across the site for 2000 and 2002.

Layer	Year	Average Coefficient of Variation Within Plots		Coefficient of Variation Between Plots	
		TPH	TPAH	TPH	TPAH
Upper	2000	0.43	0.40	0.61	0.37
	2002	0.36	0.42	1.23	0.61
Lower	2000	0.70	0.72	1.39	0.85
	2002	0.60	0.57	0.64	0.78

Table 4.2a. ANOVA by layer using TPH and TPAH as dependent variables against two levels of year (2000 and 2002).

Layer	1-Way ANOVA	F-ratio	p value
Upper	TPH*Year	37.224	< 0.0005
	TPAH*Year	21.184	< 0.0005
Lower	TPH*Year	0.045	0.832
	TPAH*Year	0.523	0.473

Table 4.2b. ANOVA in TPH and TPAH of upper layer. Two levels of year (2000, 2002) and two treatments (cleared, revegetated).

Dependent Variable	Independent Variables	F - Ratio	p - value
TPH	Year	37.356	< 0.0005
	Treatment	2.137	0.150
	Year*Treatment	2.868	0.096
TPAH	Year	29.500	< 0.0005
	Treatment	2.759	0.103
	Year*Treatment	6.169	0.016
	Interaction Test		
	2000: TPAH*Treatment	0.517	0.478
	2002: TPAH*Treatment	6.383	0.018

Figure 4.2c. Correlation of petroleum hydrocarbon loss in soil. Loss calculated as the difference between years (within plot) as a proportion of the initial concentration.

Correlation	Layer	Coefficient	F- ratio	p – value
Loss of TPAH * Loss of TPH	Upper	0.803	48.845	< 0.0005
Loss of TPAH * Loss of TPH	Lower	0.768	39.820	< 0.0005

Individual PAHs were investigated for site-wide reduction of PAHs in the upper layer (Figure 4.5). Significant reduction in the upper layer was only seen in benzo[k]fluoranthene (SMR = 0.107, p = 0.014), benzo[a]pyrene (SMR = 0.077, p = 0.038) and benzo[b]fluoranthene (SMR = 0.126, p = 0.007). PAHs are commonly grouped by ring-number since this affects the degree of sequestration and degradation. When grouped by ring number, there were significant reductions in the 4-ring as well as the 5-ring PAHs but no significant reduction in 3-ring and 6-ring PAHs (Figure 4.6).

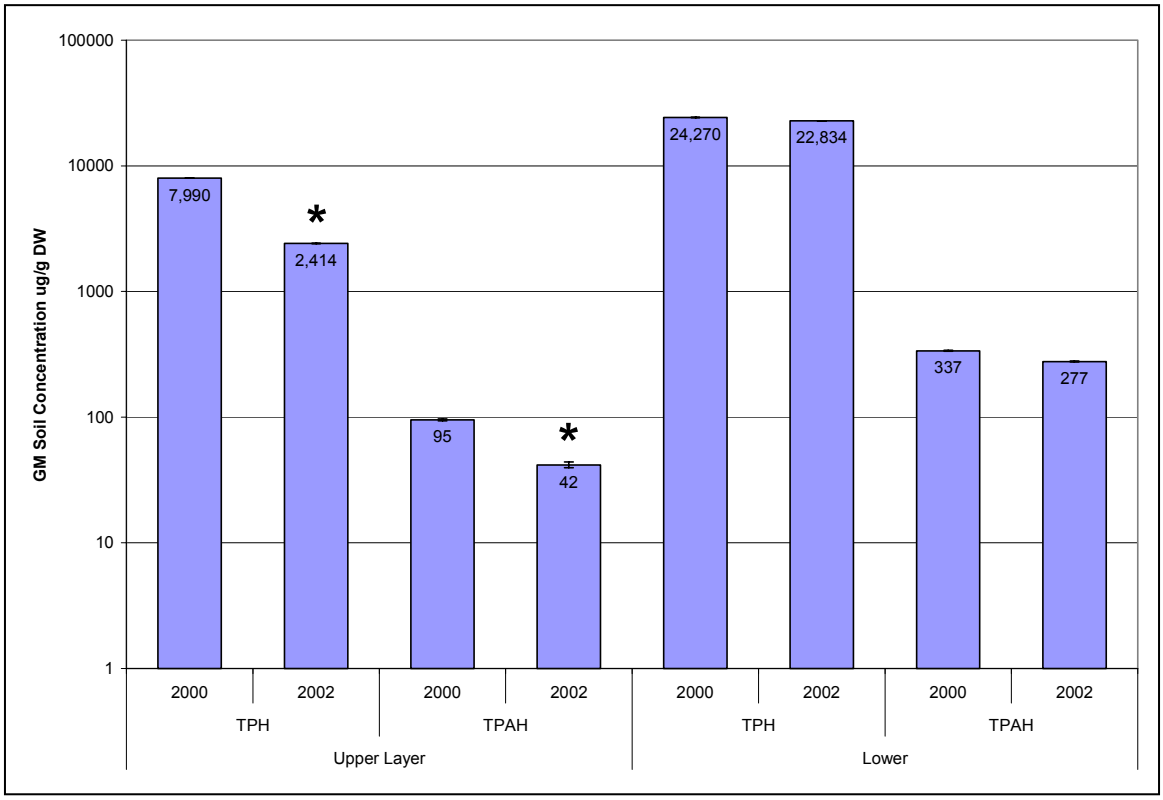


Figure 4.4. Geometric mean of Total Petroleum Hydrocarbons (TPH) and Total Polycyclic Aromatic Hydrocarbons TPAH). Reduction in the upper layer TPH and PAHs were significant ($p < 0.0005$).

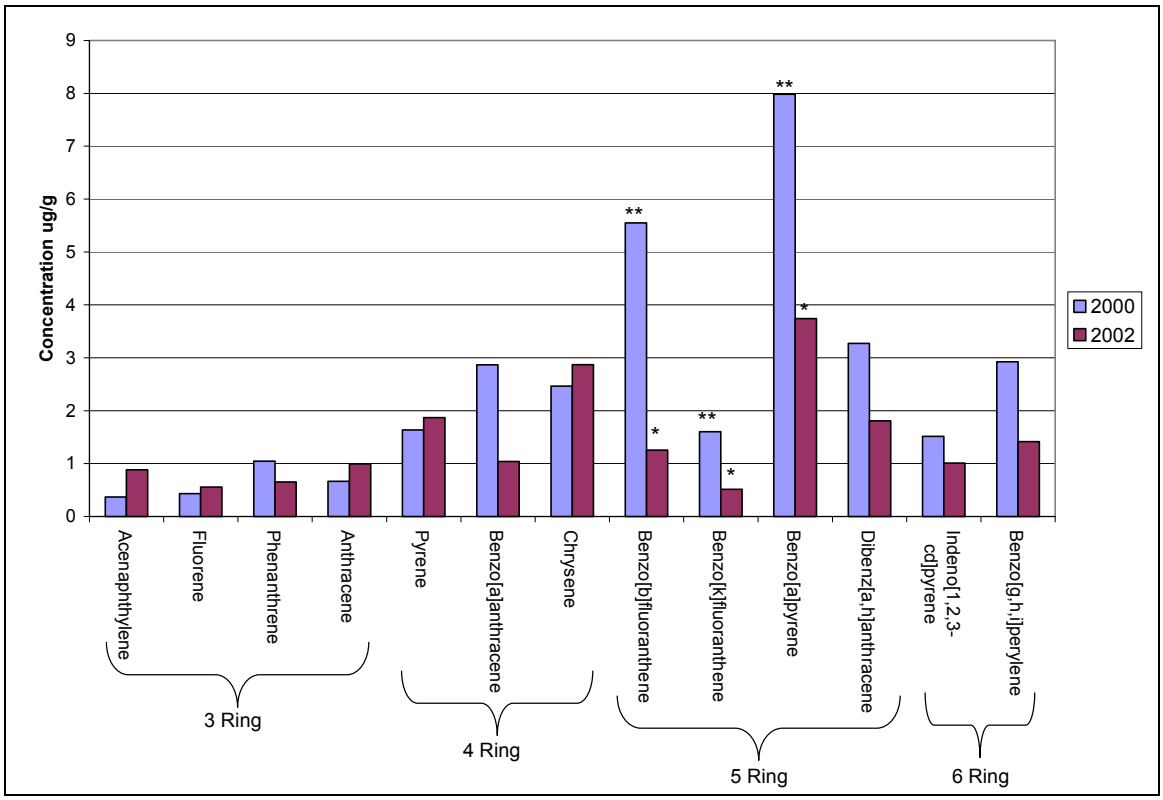


Figure 4.5. Individual PAHs in the upper layer soil. Significant reduction between years is noted by the asterisk for benzo[b]fluoranthene and benzo[k]fluoranthene and benzo[a]pyrene.

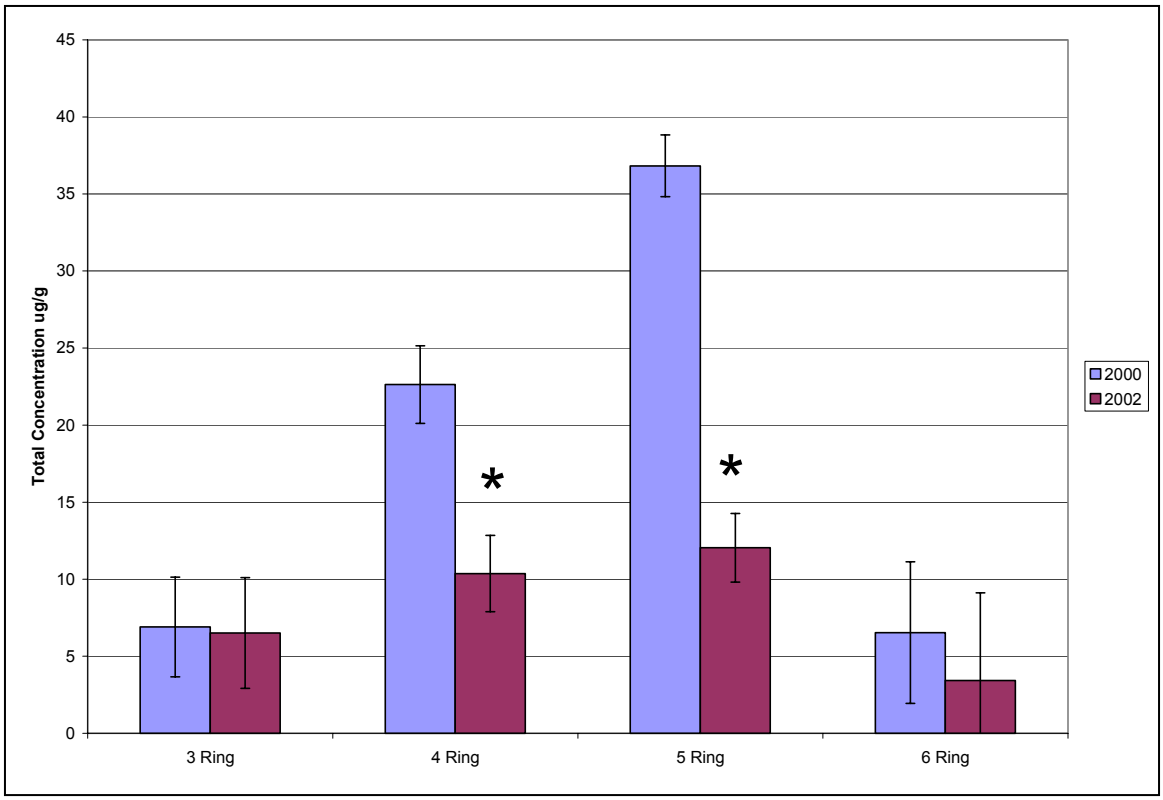


Figure 4.6. Upper layer soil PAHs by ring number. Loss of PAHs by Ring Number in upper layer soil. Concentration values are reported in µg/g. Significant loss occurred in 4-ring PAHs (SMR = 0.144, $p = 0.004$) and 5-ring (0.320, $p < 0.0005$) PAHs.

Change in Metal Mobility, Lability. For all metals, the upper layer had significantly ($p < 0.0005$) higher metals than the lower layer for total metal loading (Figure 4.7). Table 4.3 shows the concentration of metals within each sequential fraction. There had been no reduction in metal concentrations during the study period in any fraction obtained from sequential extraction. Of the other fractions, only organic lead significantly decreased from initial measurement to final (Figure 4.8).

Table 4.3. Average concentration (μg/g) of metals within the sequential fractions. For each metal, the total metal is significantly higher in the upper level ($p < 0.0005$).
4.3a. Upper Layer Soil.

	Bioavailable		Organic		Clay-Oxide		Residual		Total	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Cr	0.7	0.1	6.2	0.8	16	5	470	1	493	4
Ni	0.6	0.1	1.5	0.1	6	3	65	2	74	3
Cu	2.6	0.3	18.1	1.2	15	4	54	2	90	7
Zn	3.4	0.4	11.6	0.8	51	11	313	26	378	18
Pb	0.5	0.2	80.3	22.9	155	19	393	21	630	34

4.3b. Lower Layer Soil.

	Bioavailable		Organic		Clay-Oxide		Residual		Total	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Cr	0.3	0.0	3.4	0.8	16	5	347	29	367	28
Ni	0.7	0.1	1.4	0.1	6	2	53	4	74	6
Cu	2.0	0.3	6.6	1.0	7	2	58	8	61	5
Zn	3.9	0.8	13.2	0.9	50	7	248	43	318	13
Pb	0.3	0.1	40.3	8.3	63	11	215	31	315	39

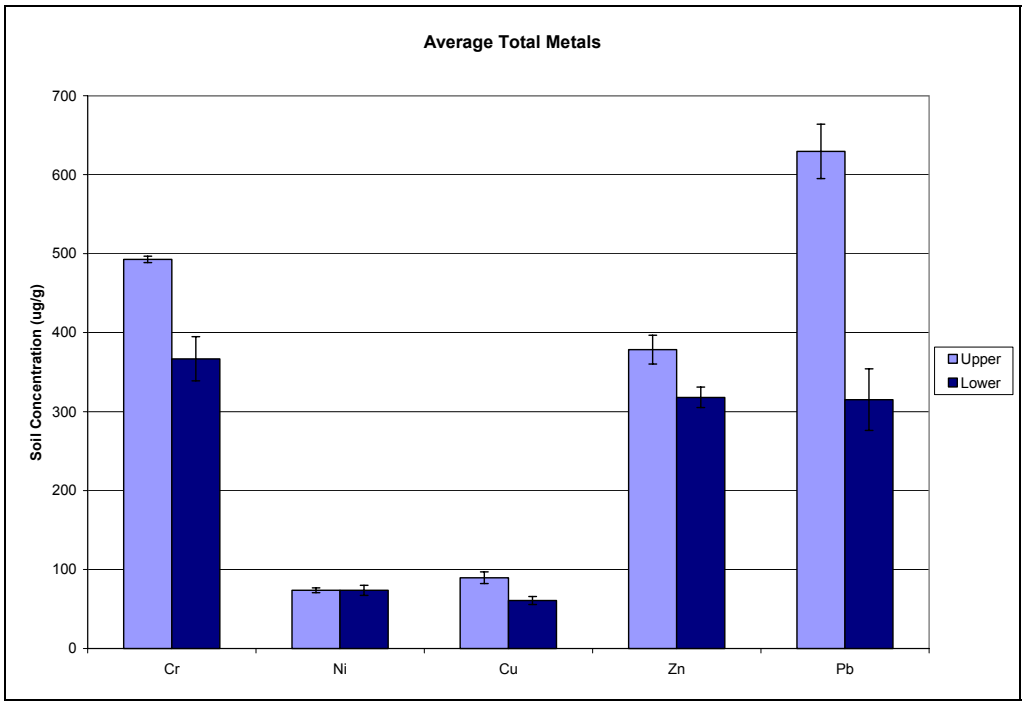


Figure 4.7. Average total metal concentration in upper and lower layer. Error bars indicate the differences between the average of the three years sampled. The upper layer is significantly higher than lower layer for all metals ($p < 0.0005$).

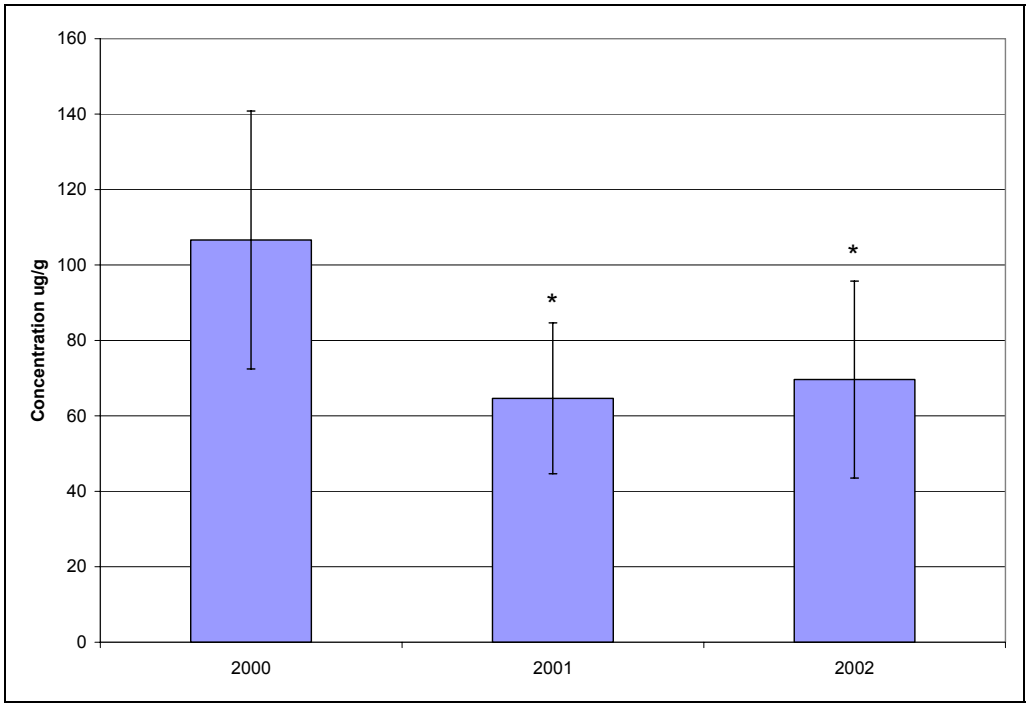


Figure 4.8. Average organic lead fraction from sampling years 2000, 2001 and 2002. The second two years were significantly lower than the first year (2001, $p = 0.001$; 2002, $p = 0.003$).

Total metals were mapped to compare to TPH, PAH and the percentage loss of the organic contaminants (Figures 4.9). Regression analysis indicated that the percent loss of TPH and PAHs are highly correlated ($y = 0.99x - 0.13$; $R^2 = 0.806$; $p < 0.0005$). However, the loss of organic contaminants was not correlated to initial levels nor to metal loading. The two plots with the highest total metals had higher than average loss of TPH and PAHs (Figures 4.9 and 4.10).

Figure 4.9. GIS imaging of total metal (a), total PAHs (b), and TPHs (c) averaged over the three years.

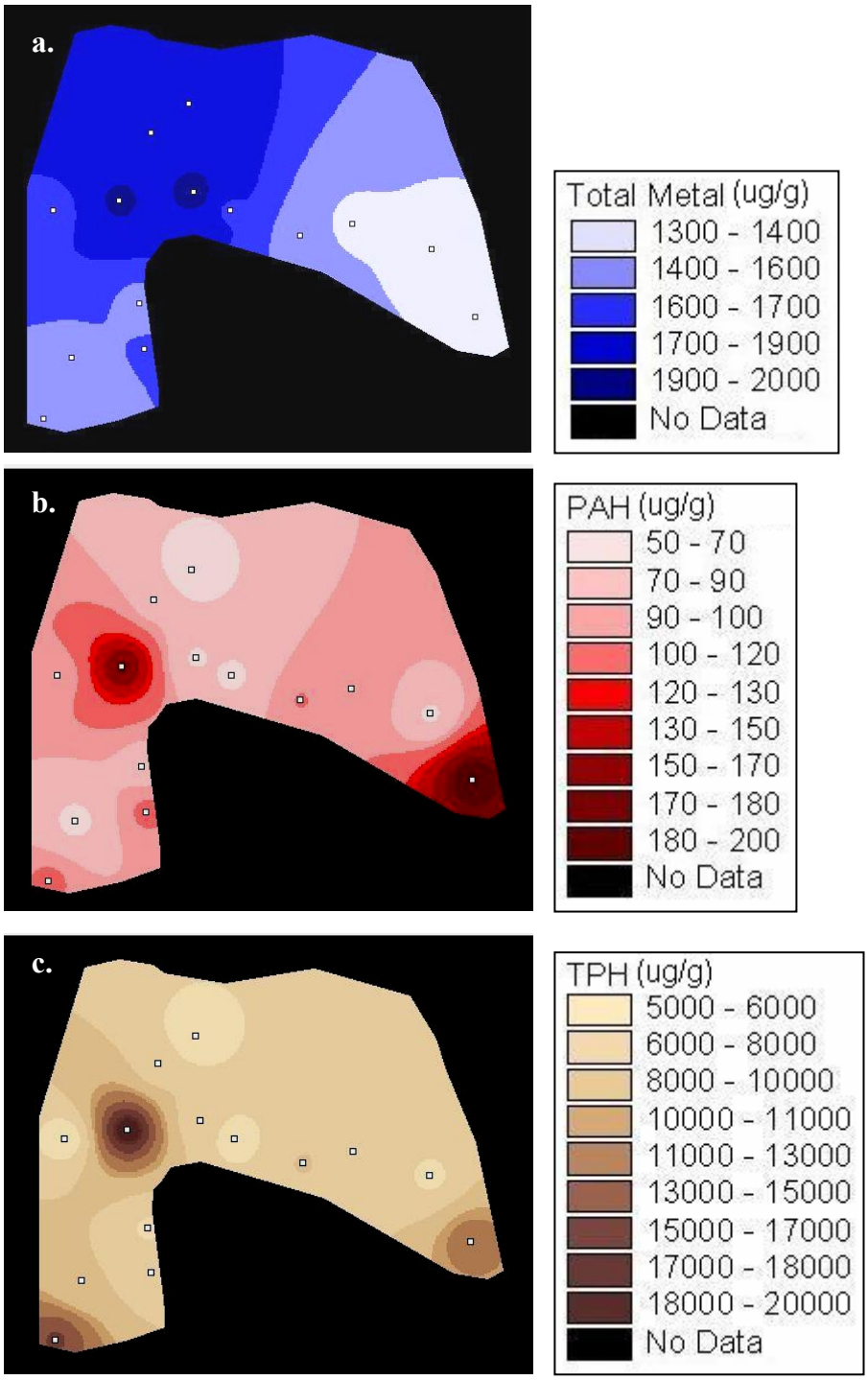
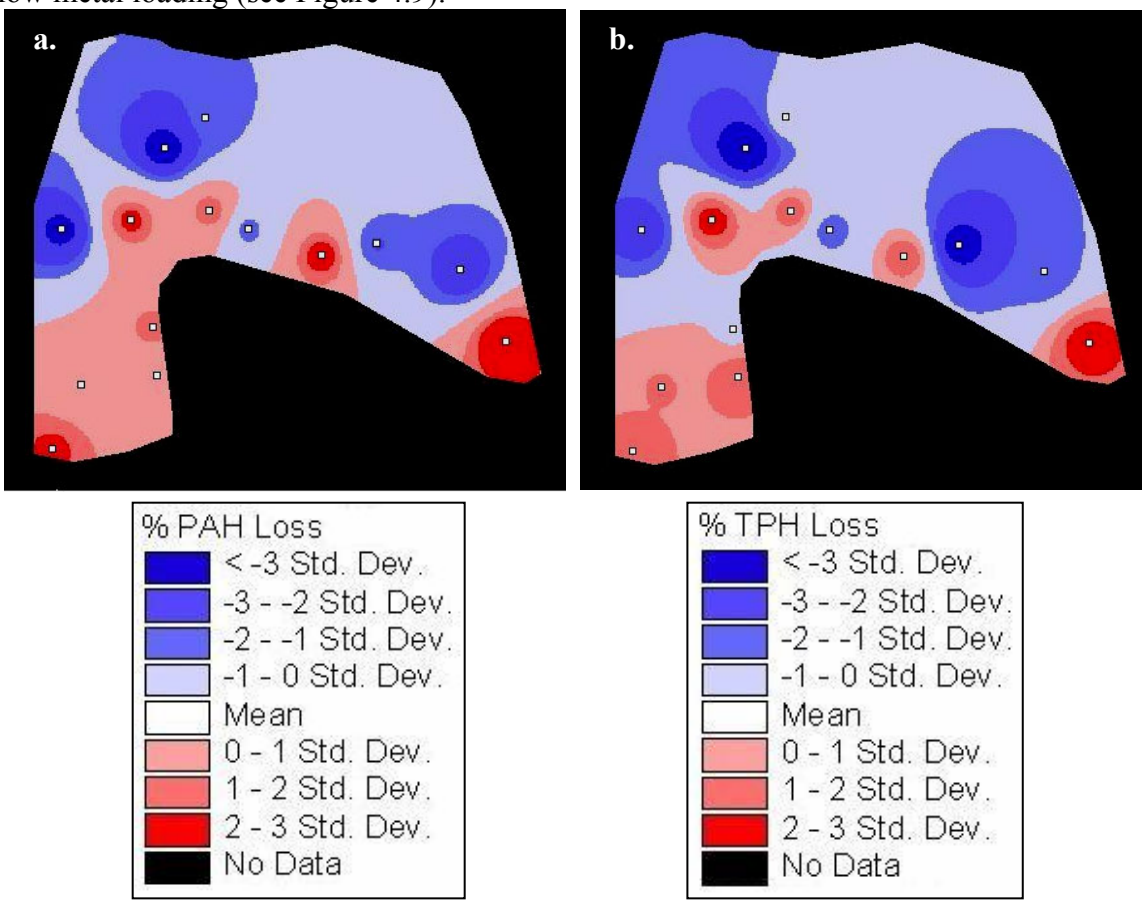


Figure 4.10. Loss of PAHs (a) and TPH (b) are highly correlated. However, the areas of greatest reduction (red) are not related to initial TPH/PAH concentration or to areas of low metal loading (see Figure 4.9).



DISCUSSION

Historical Assessment. On the site used in this study, the sludge material applied would have been uniform by depth, initially. Prior to the start of this study, management on the landfarm included seeding of ryegrass and monthly tilling. The influence of that early management likely caused the layering seen in the vertical profile of the LTU soil. Petroleum refinery compounds are commonly degradable under the aerobic conditions facilitated by tilling and therefore would result in the reduced hydrocarbons in the upper layer, as seen on this site (Downey et al., 1999). Planting may have been responsible for the enrichment of metals in the upper layer. In the earlier years of ryegrass planting (from 1992 to 1998), available metals may have been drawn toward the upper layer by a concentration gradient created by active roots. This would result in the significantly higher metals now seen in the top 20 to 30 cm depth. Through time, it would be expected that the sequestration of metals into soil, as well as the addition of organic matter from plant growth, would slow the upward migration of metals. For all metals, between 60 and 95% were not readily extractable. Nonetheless, there were no significant differences in the pools of available (mobile or labile) metals over three years, with the exception of lead in the organic fraction. Though root-mediated diffusion of metals toward the planted region may have occurred in the past, it did not occur over this three year study (or at least was not detected).

Overcoming Variability in Field-Scale Projects. One of the significant challenges in a field scale study is taking into account the problems associated with inherent site variability. The measured variability is due to the originally heterogeneity in soil sludge application, layering, and sampling method (Nedunuri et al., 2000). For this reason a prior greenhouse treatability study was conducted using the site soil as an

indication of the potential for phytoremediation. The study indicated significant reduction in the labile pool of PAHs after a few weeks of planting with various grasses and leguminous plants (Gomez, 2001). It was concluded that further degradation of these aged contaminants would be possible.

This presumption was verified by the field sampling done here. There was significant degradation in mean concentrations of TPH and PAHs in the upper layer of the site investigated over three years. The most apparent reduction was in the 5-ring PAHs, unlike the prior treatability study where the greatest reduction was in the 4-ring PAHs. It is possible degradation moves through stages beginning with more easily metabolized 3- and 4- ring PAHs that are degraded to the point where any remaining PAHs are fully sequestered and therefore, physically unavailable. At this point, more recalcitrant PAHs (5 and 6 ring) would start to degrade. This has been demonstrated in other largescale projects with chlorinated compounds. The site-wide progress or "stage" of degradation can be ascertained by recognizing the compound actively being degraded (Templeton et al., 2002). Further PAH reduction would continue at the pace of root penetration of sequestered PAHs (Olson et al., 2003).

Comparison to the RTDF Study. The degradation of soil hydrocarbons, as predicted in preliminary treatability studies and as found by this study, was not easily detected in the field by the RTDF sampling. Of all RTDF sites (12 in the U.S.), the Cincinnati Chevron site showed significant variability in TPH and PAH concentrations (Kulakow & Feng, 2003). In the present study, layers differed significantly in original composition and characteristics; however, RTDF protocols specified analysis of soil by a fixed depth (0 – 30 cm) rather than by layer. This sampling strategy was intended to minimize the measured variability of plot contamination. However, combining the

highly concentrated lower layer with the upper layer made it difficult to evaluate the response of phytoremediation in the field. To overcome some of the variability encountered in the RTDF study, PAH concentration was normalized by the concentration of hopane, a highly stable aliphatic petroleum hydrocarbon, as an indication of change from "initial" petroleum contamination. Hopane normalization reveals a degradation trend seen in Figure 4.11, but the sampling strategy may have prevented identifying statistical reduction overall and between treatments (statistical variability not shown).

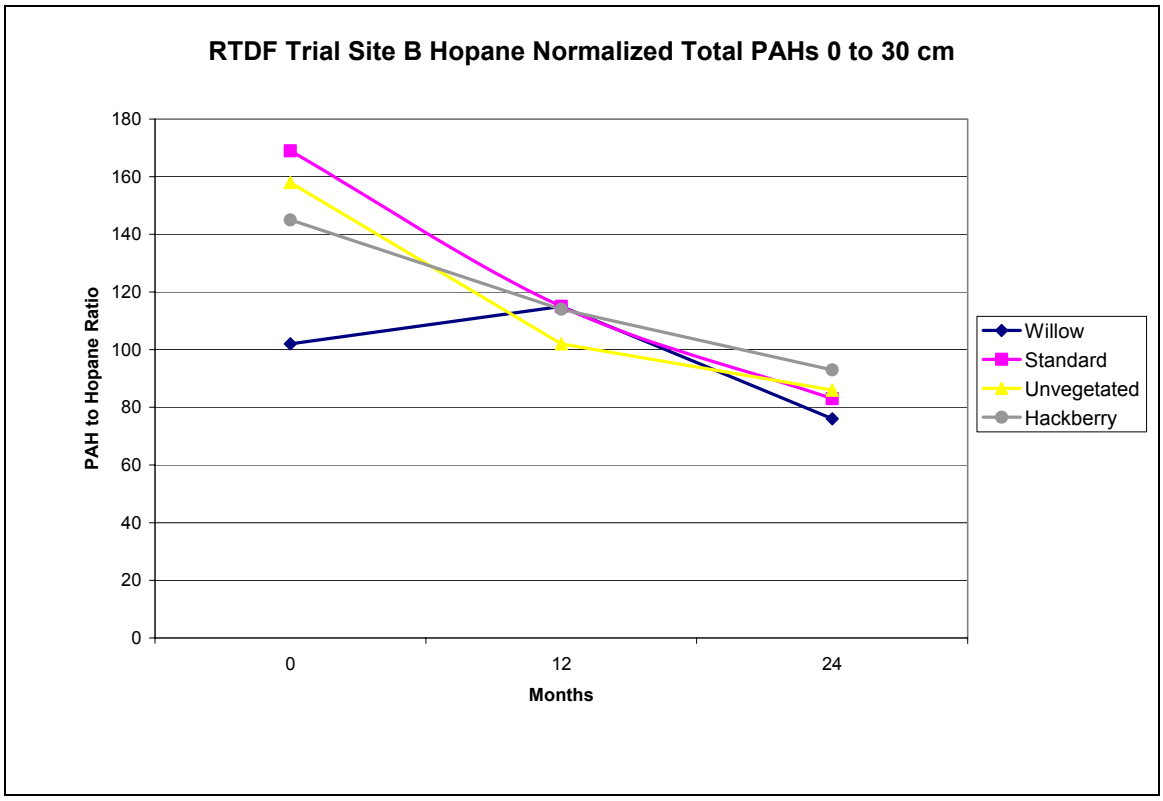


Figure 4.11. Preliminary results of the parallel RTDF study. The reduction in variability of PAH:Hopane ratio in year 1 (month 12) and year 2 (month 24) may be associated with changes in sampling procedures or analysis, but this has not been confirmed (Kulakow & Feng, 2003).

Therefore, despite different management (tilling control plots, urea addition, irrigation) and sampling (fixed depth as opposed to by strata) the degradation trends in the RTDF study were similar to that of this study. These results suggest that the additional cost and

effort adopted by the RTDF study was not necessary to accomplish the same endpoint (25 to 50% reduction in PAHs).

Contaminant Reduction in Controls. In this study, the reduction in TPH and PAHs was significant for both the vegetated and unvegetated control – this was also observed in the prior greenhouse treatability study (Gomez, 2001). In this study, control plots were managed by removal of colonizing plant species at the start of the summer season and on a bi-weekly basis throughout the growing season (Table 4.4). Despite continuous plant removal, root material remained in the control plot cores. The density of root material was not significantly different that those found in the revegetated plots. Degradation of contaminants in the control plots may be a function of biological impacts. The management of controls in this study, created conditions of forced root turnover. Forced root turnover, both in this study and other RTDF studies, may have caused succession in microbial community from those capable of simple carbon degradation (as is released in large quantities by living plants) to communities that degrade more complex, resilient carbon compounds, such as lignins or phenolics (Lipson et al., 2002). Such benzene-metabolizing microbial communities would be more likely to degrade PAH compounds in soil. There is also evidence that recalcitrant compounds experience the greatest degradation during plant scenescence (Hedge & Fletcher, 1996).

It is also possible that living plant communities release compounds that inhibit the growth of lignin or phenolic degrading bacteria or fungi. Though this mechanism has not previously been discovered *in situ*, it follows that such microorganisms may threaten plant growth by degrading protective phenolic secondary compounds. Hence, microorganisms in soil without living plants present may be released from inhibitition – resulting in an increased degradation.

Table 4.4. Dominant plant species above 10% cover in cleared control plots listed in order of decreasing cover. These were the plants removed in June of 2002 that had become established in the control plots since the end of the prior summer season.

Quad 1	Quad 2	Quad 3
Erigeron annuus	*Solidago canadensis*	*Cyperus strigosus*
Lonicera japonica	*Polygonum persicaria*	*Solidago canadensis*
Lolium perenne	*Cyperus strigosus*	*Conyza canadensis*
Conyza canadensis	*Bidens frondosa*	*Echinocloa crus-galli*
Solidago canadensis	*Echinocloa crus-galli*	*Lolium perenne*
Ambrosia artemisiifolia	*Lolium perenne*	*Polygonum persicaria*
Polygonum persicaria	*Agrostis perennans*	*Carduus nutans*
Cyperus strigosus		
Setaria glauca		
Echinocloa crus-galli		

Study Summary. The presence of a non-managed vegetation cover appeared to be effective in metal stabilization and organic degradation. At the start of this study, there were five contaminants above the residential standard necessary to obtain clean closure: benzo[a]pyrene, benzo[b]fluoranthene, dibenz[a,h]anthracene, chromium, and lead (Condit & Doherty, 2000). In the study period, the upper layer benzo[b]fluoranthene decreased significantly and is now within the residential standard. Despite significant overall reduction, benzo[a]pyrene (3.75 μg/g site average, upper layer) as well as dibenz[a,h]anthracene (1.8 μg/g site average, upper layer), both 5-ring PAHs of particular concern for carcinogenicity, remain above the residential standard of 0.465 μg/g soil. Because metals are immutable, reduction of these compounds to meet the goal is impossible in this natural revegetation scenario. The standard, however, does not distinguish between bioavailability of metals – hence a site with 100% soil metal availability would be kept to the same standard. Nonetheless, the mobile metal fraction of these two metals (Cr, 0.67 μg/g; Pb, 0.62 μg/g) is below the residential standard (Cr, 74 μg/g; Pb, 37 μg/g). Further, the labile fraction (the sum of mobile, organic acid-, and

clay oxide-bound fractions) of chromium was less than 25 μg/g on average and therefore below the target goal. With a higher proportion in the organic-acid and clay-oxide fractions than was seen with the other metals, the labile fraction of lead is not below the concentration goal. In summary, over three years, metals became more fixed, while PAHs and other hydrocarbons were degraded by 50%, albeit, slowly. Collectively, this suggests the most basic, most likely sustainable form of phytoremediation – natural revegetation – is a reasonably effective means of addressing contaminants on sites with aged pollutants.

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CHAPTER 5

UPTAKE OF METALS IN EARLY SUCCESSION PLANT SPECIES COLONIZING AN AGED PETROLEUM REFINERY LAND TREATMENT UNIT

HEATHER F. HENRY AND JODI R. SHANN

Department of Biological Sciences ML6, University of Cincinnati, Cincinnati, OH 45221-0006

ABSTRACT

Ecological restoration of metal contaminated sites, by passive (natural) revegetation or through cover management, has become a popular end-use strategy. One obvious concern for allowing natural revegetation of metal contaminated sites is the possibility of metal accumulation by plants and subsequent introduction into the food chain. In this study, metal uptake was determined for early succession plant species colonizing an aged petroleum landfarm. These plant species were in direct contact with metal-enriched soil (*i.e.* no clean soil cap). The total metal loading on this site contained levels of Cr, Zn, and Pb up to 100 times that of background level soil as well as elevated levels of Ni and Cu. Metal content in plant root and shoot tissue was highly variable, but shoot tissue still fell within the upper limit of background range found in normal plants. Though the soil metal concentrations also varied onsite, there was no relationship between total metal loading in soil and the root or shoot tissue metal content. Further investigation of the soil metals indicated that between 60 to 90% of the total metal loading was in a form unavailable to plants (i.e. moderately to highly sequestered). Root metal content was related to labile soil fractions of Zn ($p = 0.020$) and Cu ($p = 0.024$); shoot content to labile fractions of Pb (< 0.0005) and Cr ($p = 0.040$). A much better predictor of metal uptake was the species involved. Patterns in life history strategy also emerged as a determinant. Metal accumulation was significantly higher in monocots than in dicots. Within the dicot species, perennials tended to have lower metal uptake than annuals. Given the impracticality of testing all early succession species growing on metal contaminated soil, identifying uptake patterns based on plant lifespan and type may help predict transfer into natural populations.

INTRODUCTION

Ecological restoration of contaminated sites has become a popular end-use strategy in the United States and around the world (Bradshaw, 1997). Brownfields, otherwise a detriment to a community, can be converted to greenspace, increasing wildlife habitat and recreational areas, and boosting the local economy (Kearney et al., 1999). In efforts to reclaim land contaminated by mining-related practices, native species revegetation has become the focus for restoration efforts (Brown & Amacher, 1999). Some cases of restoration of contaminated sites require the addition of soil amendments to minimize phytotoxicity and encourage growth (Munshower, 1994). On many aged sites, where contaminants are sequestered, no restorative action is necessary as the natural plant community becomes established through the process of secondary succession (Tordoff et al., 2000).

One obvious concern for allowing natural revegetation of metal contaminated sites is the possibility of metal accumulation by plants and subsequent introduction into the food chain (Dousset et al., 2001; Marmiroli & McCutcheon, 2003; Piechalak et al., 2003; Wong, 2003). When revegetation is accomplished through seeding or planting, metal-tolerant excluder species are chosen. This is not the case when natural communities of plants are allowed to establish and grow on metal contaminated soil. Regardless of the means used to revegetate, metal uptake by plants is an obligatory part of risk estimation of these sites. Modeling may be used in the absence of real field data, but may oversimplify the complexity of metal uptake in natural populations.

To a certain extent, if secondary succession is occurring despite high phytotoxic metal contamination, bioavailability is likely low enough to allow establishment of a broad array of plant species (Vangronsveld et al., 1996). In aged soils, metal mobility is

often reduced, as is plant exposure, uptake and transfer into the food chain. Soil properties are a key determinant of the amount of metal available to plants. High cation exchange capacity (CEC), organic matter, and clay content decrease metal mobility in soil and uptake by plants by providing binding sites that attract and stabilize cations in soil.

Nonetheless, even in aged soils with low metal mobility, plants growing directly on metal rich soil have been reported to contain metal concentrations in leaf tissue higher than plants growing in background soils (Barazani et al., 2004). This may be explained by the plants' ability to alter soil chemistry to increase cation mobility in the soil, a phenomenon it uses to extract essential elements from soil. Hence, the amount of metal in plant tissue does not always correlate to estimates of metal mobility (Brandt & Rickard, 1996). Sequential extraction methods can be used to assess the degree of metal associated with various soil fractions (exchangeable, soluble, organic-acid bound, clay-oxide bound and residual). Readily mobile metals are found in the exchangeable and soluble fractions, whereas, the organic and oxide bound metals are considered labile, or capable of being mobilized by natural processes (Sposito et al., 1982; Stover et al., 1976). The residual fraction is not normally available in a natural setting.

The mechanisms of mobilizing soil cations vary between plant species, as do differences in metal uptake. Metals vary in their affinities to soil sinks and their plant-induced mobility. Plant essential cations (Zn and Cu) may be accumulated at much higher rates than non-essential metals without disturbing (to a point) plant metabolism. On the other hand, non-essential metals such as lead are likely excluded from plant uptake or are quickly immobilized in the plant (Fodor et al., 1998). Another confounding factor in the presence of multiple metals is competition for root uptake sites, making it

even more difficult to predict metal transfer into vegetation (Brandt & Rickard, 1996).

This study monitored metal accumulation by the most abundant plant species established naturally on a post-closure petroleum waste land treatment unit. Metal accumulation was compared between fourteen of the most abundant species. The same species were collected from an unpolluted offsite location for growth and metal content comparison.

MATERIALS AND METHODS

Primary Study Site. The area selected for this study is a petroleum refinery waste landfarm in Hooven, Ohio. The site, though un-operational since 1980, contains levels of heavy metals (i.e., lead, chromium, and zinc) higher than that of background soil (Table 5.1). From 1990 until 1998, periodic landfarming (tilling) was practiced in the summer months and a cover crop of perennial ryegrass *(L. perenne)* was established for winter cover. The risk-based, post-closure plan specified natural revegetation as the cover management strategy to stabilize metals and slowly degrade remaining PAHs (Condit & Doherty, 2000). Based on preliminary data, closure was approved without the addition of a clean soil cap.

Plant Sampling. The site was sampled in 2003, four years after closure. The species richness and abundance was previously determined for this site (Chapter 3). The 2.2 hectare site was divided into 4 quadrats for sampling for plant material. Fourteen of the most common forb and grass species were collected from each quadrat (see Table 5.2 for listing and general characteristics). Within in each quadrat, a pair of individuals of each species was harvested during the growing season just prior to peak bloom, one representing the largest size and the other the smallest size in flower. In the field, percent

cover (vertical projection) of each species was recorded, as well as height and root extent (Mueller-Dombois & Ellenberg, 1974). For each plant, mass was determined separately for plant shoot and root: after soaking, plants were dried to a constant weight at 40°C and analyzed separately for metals.

Offsite Location for RegionalBackground Metal Levels. The seven most common species (in terms of abundance and total coverage) were also collected on an uncontaminated rural site in its fourth year of secondary succession. The site was a former agricultural area in Union, Kentucky that had been purchased by a developer in 1999 and, since then, was left undisturbed. The area of collection was approximately one acre. These species were as follows: *Ambrosia artemisiifolia*, *Lolium arundinaceum*, *Melilotus officinalis*, *Solidago canadensis*, *Symphotrichum pilosum*, *Conyza canadensis,* and *Echinochloa crus-galli*. Sampling criteria were as above.

Plant Tissue Analysis. Metal content in plant tissue was determined by acid digestion of plant material followed by ICP-MS analysis. Prior to digestion, plants were soaked in distilled water for approximately 3 hours (Keane et al., 2001). Plant tissue was then rinsed 10 times with distilled water, oven dried at 60°C for 48 h and ground in a mill. Powdered leaf tissue (0.5 – 2 g) was ashed in a muffle furnace at 550°C for 5 hr (Azcue & Mudroch, 1994). The ash was dissolved in 2 ml concentrated HCl and heated to boiling on a hot plate to extract total leaf metals. Samples were filtered (Whatman 41), brought to 20 ml with 4 M HNO_3 and stored in plastic bottles at 4°C until analysis. Leaf digests were analyzed for Cr, Cu, Zn, Pb and Ni by inductively coupled plasma mass spectrometry (ICP-AES). Each instrument was standardized on a curve using stock metal concentrations in a 4 M HNO_3 solution (Azcue & Mudroch, 1994).

Soil Analysis. Soil characteristics and metal content were determined from within quadrats and for the offsite location. Three 15 cm-depth soil samples were collected in air-tight grab bags (~ 300 g total) from four locations within the four quadrats. These samples were composited and paired (by proximity) with plant sampling. Field soil moisture was determined by calculating the dry weight of soil (after 4d in 40°C oven) as a proportion of initial wet weight (approximately 40g). The remaining composite soils were sent to an agricultural station for analysis of pH, organic matter, CEC, P, K, and Mg. To best estimate the soil metal level (which was assumed to vary across the site), two 75 cm cores were removed from the same area. These replicate cores were analyzed separately for mobile, labile, and total metals (Sposito et al., 1982; Tessier et al., 1979). Total carbon and nitrogen were also determined in cores using a Perkin Elmer Series II Elemental Analyzer 2400 with combustion oven at 640ºC and reduction at 925ºC, according to specifications. Soil characterization and metal content were determined for the offsite location using only composite sampling.

Statistical Analysis. Metal soil concentration was assessed on a plot basis by averaging the plot-replicate core metal concentrations taken periodically the previous three years. Bioconcentration factor (BCF) (ratio of tissue metal concentration to soil metal concentration) and metal translocation (ratio of shoot metal concentration to root metal concentration) was determined per plant (Kim et al., 2003). Plant cover, biomass, height and tissue metal concentration were log transformed to attain normality. Averages of each metal concentration in root and shoot were calculated onsite for all species (n = 14), and for the species involved in the onsite/offsite comparison (n = 7). Initially one-way ANOVA tested the dependent variable metal concentration (Cr Root, Cr Shoot, Ni Root, Ni Shoot, etc.) by species. Bonferroni post hoc tests determined the pair-wise

matrix determining significance between species. Next, metal tissue content was modeled for the effect of species with log transformed plant growth characteristics (cover, root length, plant height, shoot dry weight, root dry weight). This was done to eliminate any species/grouping effects that might be attributable simply to inherent differences in growth biomass. Then ANCOVA tested soil metal loadings crossed with species to assess which metal fraction, if any, further explained differences in metal concentration. Based on initial inspection of data, a pattern in metal uptake appeared to depend on whether the species was a monocot, a perennial dicot, or annual/biennial dicot. Hence, metal uptake was analyzed by such groupings. For comparison with the offsite unpolluted location, two-way ANOVA assessed interactions between plant species/grouping and location (on or off site) for each of the metal (Cr, Ni, Cu, Zn, Pb) and tissue (root, shoot) combinations. When interactions were present, the locations were analyzed separately to identify differences between species/groups.

RESULTS

Soil Analysis. Average soil metal concentrations are reported in Table 5.3. Lead, zinc, and chromium had the highest metal loading at 50 to 100 times that of the comparison site. Average soil characteristics are reported in Table 5.4. There were no significant differences between onsite quadrats for total carbon, organic carbon, nitrogen, CEC or pH.

Plant Tissue Metal Analysis. The mean concentration of metal in plant tissue is reported in Table 5.5. The average root tissue in onsite species was highest for Zn (40 μg/g) followed by Cr (28 μg/g), Cu (26 μg/g), Pb (23 μg/g) and Ni (7 μg/g). The trend in shoot shoot tissue was similar with Zn the highest (27 μg/g) followed by Cu (5 μg/g), Cr

(2.9 μg/g), Pb (1.7 μg/g) and Ni (1.3 μg/g). These shoot values fall within the range of normal metal concentrations reported in literature (Table 5.6).

A comparison was made between the seven onsite species that were collected from an uncontaminated site. One way ANOVA determined that the concentration of metals in root tissue of onsite plants was significantly higher for Cr ($p < 0.0005$), Pb ($p < 0.0005$), Cu (0.002), and Zn ($p = 0.029$), and lower for nickel ($p = 0.003$) (Table 5.7). As for shoot metals, only lead was significantly higher onsite ($p = 0.021$; 1.3 μg/g onsite, 0.8 μg/g offsite); however, there was considerable variation among species. Onsite plant tissue metal concentration was tested for correlation to soil total metal; however, none of the soil to tissue concentrations were significant.

Species and Tissue Metal Content. One-way ANOVA indicated the effect of species is significant for all combinations of metal and plant tissue. For all metals, the relationship between root to shoot metal concentration was not significant when species were taken into account, except for the essential element zinc ($p = 0.003$). Bonferroni hypothesis tests of the pair-wise comparisons revealed trends suggesting the monocot species, as a group, tended to have higher metal levels, especially for non-essential elements. Though differences exist between species with regard to shoot/root ratio, cover, dry weight, etc., these factors were not significant covariates in determining metal tissue content. Species differences in metal bioconcentration factor (BCF) and translocation are shown in Appendix 5C.

Species and Soil Metal Concentrations. To assess the relationship between soil metal and plant tissue metal, the effect of species was included in the ANCOVA model. For root metal concentrations, the bioavailable Cu ($p = 0.024$) and labile Zn ($p = 0.020$) concentrations were significantly correlated (Table 5.8). Labile Cr ($p = 0.040$) and Pb (p

< 0.0005) are correlated to shoot concentrations. Nickel was not related to any of the metal fractions. Total soil metals did not explain the plant tissue concentration, with the exception of the significant correlation to lead shoot tissue. However, this relationship may be due to the high correlation between soil labile and total fractions of lead ($p < 0.0005$). This would be true for Pb and not the other metals since the labile fraction of lead is proportionately greater (~ 40 %) than for the other metals (~ 15%).

Plant Type and Lifespan Groupings. Table 5.9 shows the absolute metal concentrations between groups, metals, and plant part. For all metal root concentrations, monocots were significantly higher than the dicots (Figure 5.2). For nonessential elements (Ni, Pb, Cr), the annual dicot root concentrations were significantly higher than perennial dicots. In shoot tissue, monocots were significantly higher for nonessential metals than the dicots. Annual dicots had higher lead and copper than the perennial dicots. Biological concentration factor follows a similar trend: monocots concentrated the metals more than the dicots. Translocation of metals by monocots was much lower than that of the dicots, and the perennial dicots had significantly higher translocation of Cr than annual dicots (Table 5.11).

Variation in metal accumulation between plant groupings was further investigated to see if the patterns observed onsite held for offsite plants. Each combination of the log-transformed metal concentration (Cr, Ni, Cu, Zn, and Pb) and plant part (root, shoot) dependent variables were modeled against the independent variable plant type (monocot, annual dicot, perennial dicot). Where an interaction between species and location was present (when $p < 0.01$), one-way ANOVA was used on a per-species basis to test the significance of location on metal uptake. The same pattern appeared offsite as onsite: grasses had significantly higher metal concentrations than the forbs for nonessential

metals Cr and Ni in both root and shoot tissue (Table 5.11). Though the root lead concentration was also higher for onsite monocots, the shoot tissue concentration was not different between groups. The perennial species tended to have a lower metal accumulation than the annual/biennial species.

Plant Growth onsite vs. offsite. Growth onsite was compared to offsite by calculating the relationships between plant dry weight (both shoot and root) and cover. Not surprisingly, shoot and root biomass are significantly correlated (GLM: MSR = 0.648, $p < 0.0005$); however, species and/or location are not significant as covariates. A similar result was observed for the relationship between shoot biomass to percent cover: location does not play a significant role in this relationship, but in this case, species is a significant covariate (GLM: MSR = 0.687, $p < 0.0005$). Also, a negative correlation between stem chromium and shoot biomass was observed when looking at all onsite and offsite species (GLM: MSR = 0.227, $p < 0.0005$).

DISCUSSION

The soil metal level on the contaminated site was significantly higher than local background levels. However, the metal levels in plant tissue did not reflect this difference. Although root metal concentration was significantly higher in onsite roots than offsite roots, metals were not necessarily taken up into the shoot. Accordingly, the average shoot metal concentration fell within the range reported for normal plant species (Table 5.6) and was not substantially different than the offsite plants for Cr (1.5x offsite), Cu (0.8x offsite), Ni (0.3x offsite), Zn (1.0x offsite) and Pb (1.6x offsite). These findings support the well-founded notion that root uptake and translocation are separate processes (Meager, 2000; Piechalak et al., 2003). The root is compartmentalized into two zones

(the cortex and the stele) separated by highly selective tissue, the endodermis with its Casparian strip (Taiz & Zeiger, 1991). Hence, metals cations that penetrate the root cortex usually become bound to cell walls or accumulated in the intercellular space, greatly limiting further uptake.

Sequential extraction of soil metals indicated the majority of metals were in an unavailable (residual) form. As opposed to the total level, metals found in root tissue fell within the range estimated for the labile fractions (Table 5.13). For the lighter-weight metals in this study (Cr, Ni), the average root tissue metal concentration was equivalent to the average labile fraction (the sum of bioavailable, organic acid and clay-oxide bound). Copper and zinc concentrations in root tissue were two-thirds to one-half that in the labile fraction. These two metals are essential and the plant would have mechanisms for their uptake and transport. Lead, much heavier (and larger) than the other metals in this study, was one-tenth as concentrated in the root as was found in the labile fraction. This apparent exclusion of lead was also seen in studies by Raskin (Raskin, 2000). Hence, even though potentially available (based on soil chemistry), root accumulation of Pb is apparently hindered to a greater extent than the other metals. This suggests that plant root uptake is not only a function of labile fractions, but also a function of plant immobilization mechanisms and, possibly, metal molecular weight.

The variation of metal in soil was weakly associated with the variation in plant tissue metal but only as a covariate for species. Though root metal and labile metals were within comparable ranges, the variation in soil metal concentration only explains the variation found in root metal concentrations for essential elements copper and zinc. Lead and chromium shoot concentrations were correlated to the labile fraction (i.e. metals that may become mobile by plant-induced mechanisms). Nonetheless, chromium and lead

had the lowest translocation of all the metals. Total metal variation only "explained" variation in lead shoot tissue; however, this relationship may be due to the high correlation between soil labile and total fractions of lead ($p < 0.0005$). This would be true for lead and not the other metals since the labile fraction of lead is proportionately greater (~ 40 %) than for the other metals (~ 15%).

Previous studies of metal uptake in natural populations have focused on monocots or dicots: side-by-side comparisons, as was done in this study, are rare. Nonetheless, these groups have different survival strategies that may impact metal uptake patterns. Monocots exhibit a growth and physiology unlike that of dicots. Though not directly measured in this study, the roots of monocots tend to be fibrous with a high surface area for nutrient acquisition. Dicots have a more pronounced vertical root axis, forming a conical-shaped mass known as the taproot. This may result in a lower root to bulk-soil contact area (nutrient transport, instead, would occur through fine root projections emerging laterally from the taproot) (Taiz & Zeiger, 1991).

Management Implications. The landfarm studied here was approved for passive (natural) revegetation despite elevated metal levels. When compared to a local, non-contaminated site, the metal content in the shoot tissue of the same species was not substantially different between sites. The lower pH and organic matter on the offsite location may have contributed to increased availability of any metals present (Forstner, 1995). However, a better explanation is that landfarm metal contaminants, having aged for 20 years, are highly sequestered (unavailable) and immobilized due to the relatively higher pH and organic matter.

The risk to wildlife that natural revegetation may pose appears to be limited. Of the metals that are of concern for bioaccumulation (Cr and Pb) only lead was

significantly higher in onsite plant tissue, although at that low level, it is unlikely the difference is of biological significance (Condit & Doherty, 2000). For metals of particular concern for food chain bioaccumulation (Cr and Pb), the BCF from soil to shoot ranged from 0.1% to 2.7% for chromium, and 0.1% to 1.3% for lead (Appendix 5C). These results indicate some of these naturally revegetating species may be candidates for "phytostabilization" – particularly the perennial dicot species. Phytostabilization is a remediation technique that utilizes plants that are poor translocators of metal contaminants to aboveground plant tissues that could be consumed by humans or animals.

Above all, this study has demonstrated that species is a significant determinant of metal uptake in aged soils. Therefore, identifying uptake patterns based on plant lifespan and habit may help predict transfer into natural populations. For other contaminated sites undergoing natural revegetation, the earliest stage of succession (in temperate climates) is dominated annual forbs (dicots) and grasses (monocots). Gradually, these species are replaced by perennial dicots, though this process takes decades to complete (Odum, 1963). In a separate study (Chapter 3 of this dissertation) the shift from annual species to perennial species was observed in just three years. Though replacement of some grass species was seen in the three year study, monocots remain an important part of the forming community. Coupled to the results in this chapter, as the course of succession continues and the grass/annual sere is replaced by dicot perennial species, the risk of metal uptake into plant tissue may slowly decrease. The associated increase in organic matter (also seen in Chapter 3) and stabilization of metals at the plant-root interface provides further evidence that allowing natural succession may be a sustainable and low risk end-use strategy for contaminated sites with low metal mobility.

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Table 5.1. Study site metals, the typical background-level soil metal reported in the literature and the geometric mean and range of total heavy metal concentrations found on landfarm.

Element	Clean Silty-Loam Soil, (µg/g)	Upper Layer (0 – 30 cm) GM (µg/g)	Lower Layer (30 – 45 cm) GM (µg/g)
Cr	51	493 (299 – 649)	326 (199 – 471)
Ni	26	71 (85 – 58)	57 (34 – 95)
Cu	23	89 (59 – 105)	69 (47 – 91)
Zn	60	362 (243 – 439)	274 (190 – 360)
Pb	28	644 (406 – 1045)	292 (126 – 455)

From *(Kabata-Pendias & Pendias, 2001).

Table 5.2. Latin nomenclature, common names and characteristics of plant species selected for this study. For dicot grouping, annual and biennial species were combined.

Genus species	**Common Name**	**Plant Type and Lifespan**	**Family**
Ambrosia artemisiifolia L.	ragweed	D, A	Asteraceae
Conyza canadensis (L.) Cronq. var. *canadensis*	Canadian horseweed	D, B	Asteraceae
Cyperus strigosus L.	strawcolored flatsedge	M, P	Cyperaceae
Dactylis glomerata L.	orchard grass	M, P	Poaceae
Echinochloa crus-galli (L.) Beauv.	barnyard grass	M, A	Poaceae
Erigeron annuus (L.) Pers.	eastern daisy fleabane	D, A	Asteraceae
Euthamia graminifolia (L.) Nutt. var. *graminifolia*	flat-top goldenrod	D, P	Asteraceae
Lactuca serriola L.	prickly lettuce	D, B	Asteraceae
Lolium arundinaceum (Schreb.) S.J. Darbyshire	tall fescue	M, P	Poaceae
Lolium perenne L.	perennial rye	M, P	Poaceae
Melilotus officinalis (L.) Lam.	yellow sweetclover	D, P	Fabaceae
Oenothera biennis L.	evening primrose	D, B	Onagraceae
Solidago canadensis L.	Canada goldenrod	D, P	Asteraceae
Symphotrichum pilosum (Willd.) Nesom var. *pilosum*	white oldfield aster	D, P	Asteraceae

Type: D = dicot, M = monocot
Duration: A = annual, B = biennial, P = perennial.

Table 5.3. Average metal concentration (μg/g DW) in extracts removed sequentially from soil. The mobile fraction is a combination of the KNO_3 (exchangeable) and water (soluble) fractions which represent readily available metals. Metal removed by NaOH (organic-acid bound) and Na_2EDTA (bound to clay oxides) represent the labile fraction, which may become available to plants through natural processes. Metals found in the residual fraction (HNO_3) are highly sequestered and likely unavailable to plants.

Availability:		Labile			Non-Labile	Total
Fraction:		Mobile	Organic	Clay-Oxide	Non-Mobile	
Extractant:		KNO_3, H_2O	NaOH	Na_2EDTA	HNO_3	
Onsite	Cr	0.67 ± 0.19	5.8 ± 2.0	18.3 ± 2.0	470.1 ± 86.1	495
	Ni	0.58 ± 0.14	1.5 ± 0.4	5.1 ± 0.9	64.8 ± 87.7	72
	Cu	2.57 ± 0.44	18.5 ± 62.9	16.7 ± 2.2	55.5 ± 7.2	93
	Zn	3.46 ± 0.93	12.1 ± 3.5	54.9 ± 9.4	298.0 ± 39.4	368
	Pb	0.62 ± 0.31	85.6 ± 24.0	162.0 ± 48.6	405.3 ± 128.6	653
Offsite	Cr	0.01 ± 0.01	0.4 ± 0.1	0.6 ± 0.1	7.1 ± 1.0	8
	Ni	0.75 ± 0.24	0.4 ± 0.1	3.5 ± 0.2	18.0 ± 0.8	23
	Cu	1.00 ± 0.62	1.6 ± 0.2	1.8 ± 0.0	1.3 ± 0.1	6
	Zn	3.01 ± 0.84	0.7 ± 0.1	4.2 ± 0.1	17.3 ± 1.7	25
	Pb	0.19 ± 0.11	0.1 ± 0.0	5.7 ± 0.3	0.8 ± 0.1	6

Table 5.4. Soil characteristics of LTU (onsite) and comparison site (offsite). Each value is the mean of 14 grab samples from around the LTU collected for a 2002 study. The offsite is a composite of three grab samples, with the assumption the variability of offsite edaphic factors would be lower.

Year	pH	% OM	C:N	TotalC (% by wt)	CEC	lbs / Acre			
						P	K	Ca	Mg
Onsite	7.6	5.8	28.6	9.6	18.86	27	233	13410	691
Offsite	6.3	2.6	3.8	1.51	17.00	27	202	4319	264

Table 5.5. Geometric mean metal concentration in onsite plants (average of each species average; n = 14).

	Root		Shoot	
Metal	Mean	Stdev	Mean	Stdev
Cr	27.9	2.6	2.8	2.1
Ni	6.9	3.0	1.3	2.7
Cu	25.7	2.0	4.7	1.7
Zn	41.2	1.9	27.6	2.0
Pb	22.9	3.1	1.6	2.0

Table 5.6. Metal concentrations considered background levels for plant leaf tissue on background level soil (Kabata-Pendias, 2001). Essential metals are indicated as well as the relative toxicity of the metals. The maximum shoot concentration of onsite plants is reported in μg/g DW with the species in parenthesis.

Metal	Essential to Plants	Relative Toxicity to Plants	Upper Limit Normal Metal Concentration (μg/g DW)	Maximum on LTU (μg/g DW)
Cr	N	Moderate-High	5-30	28.8 (*D. glomerata*)
Ni	Y/N	Moderate-High	10-100	61.3 (*D. glomerata*)
Cu	Y	Moderate-High	20-100	17.7 (*C. canadensis*)
Zn	Y	Low-Moderate	100-400	111.2 (*S. pilosum*)
Pb	N	Moderate	30-300	31.4 (*L. perenne*)

Table 5.7a. Average metal concentration in the species sampled both onsite and offsite (n = 7 species). These were a combination of monocot (n = 2), dicot annuals (n = 2) and dicot perennials (n = 3).

Metal	Root				Shoot			
	Onsite		Offsite		Onsite		Offsite	
	GM	SD	GM	SD	GM	SD	GM	SD
Cr	23.5	3.0	4.5	2.2	2.7	1.5	1.8	1.5
Ni	3.8	2.5	9.3	2.0	0.9	1.7	3.5	1.5
Cu	22.6	2.2	10.8	1.6	3.8	1.8	4.9	2.0
Zn	30.5	2.1	18.2	1.3	22.3	2.4	22.4	2.1
Pb	15.9	3.7	1.2	5.7	1.3	1.8	0.8	1.8

Table 5.7b. Statistical results for average onsite vs. offsite location. These values represent a one-way ANOVA combining all species (n = 7) tested for location (on or offsite).

On vs. Offsite	MSR	p - value	Interpretation
Cr Root	0.267	< 0.0005	on > off
Ni Root	0.139	0.003	off > on
Cu Root	0.148	0.002	on > off
Zn Root	0.080	0.029	on > off
Pb Root	0.314	< 0.0005	on > off
Cr Shoot	0.049	0.089	N.S.
Ni Shoot	0.501	< 0.0005	off > on
Cu Shoot	0.019	0.294	N.S.
Zn Shoot	0.000	0.972	N.S.
Pb Shoot	0.089	0.021	on > off

Table 5.8. Regression analysis of soil metal concentration to metal concentration in plant tissue for cases when there was a significant soil fraction relationship.

Dependent Variable	Independent Variables: Species & Covariates	Degrees of Freedom	F-Ratio	p - value
Cr Root	Species	13	19.888	< 0.0005
Ni Root	Species	13	23.160	< 0.0005
Cu Root	Species	13	12.425	< 0.0005
	Cu Mobile	1	5.305	0.024
	Cu Labile	1	0.200	0.656
	Cu Total	1	1.220	0.273
Zn Root	Species	13	10.912	< 0.0005
	Zn Mobile	1	0.000	0.995
	Zn Labile	1	5.634	0.020
	Zn Total	1	3.453	0.067
Pb Root	Species	13	8.264	< 0.0005
Cr Shoot	Species	13	8.391	< 0.0005
	Cr Mobile	1	1.214	0.274
	Cr Labile	1	4.393	0.040
	Cr Total	1	1.819	0.182
Ni Shoot	Species	13	18.070	< 0.0005
Cu Shoot	Species	13	11.825	< 0.0005
Zn Shoot	Species	13	11.743	< 0.0005
Pb Shoot	Species	13	7.599	< 0.0005
	Pb Mobile	1	0.215	0.644
	Pb Labile	1	20.082	< 0.0005
	Pb Total	1	20.745	< 0.0005

Table 5.9. Mean (M) metal uptake and standard deviation (SD) in onsite plants grouped as a combination of monocots, annual/biennial and perennial dicots.

	Perennial Dicots		Annual/Biennial Dicots		Monocots	
	M	SD	M	SD	M	SD
Root						
Cr	11	2	18	2	91	2
Ni	2	2	5	2	23	2
Cu	18	2	17	2	52	2
Zn	22	2	34	2	81	2
Pb	8	2	14	2	85	3
Shoot						
Cr	2	2	2	2	6	2
Ni	1	2	1	2	4	3
Cu	4	2	6	2	4	2
Zn	27	2	34	2	21	3
Pb	1	2	2	2	3	3

Table 5.10. Statistical results for metal concentration of onsite species. Plants were grouped as a combination of monocots (m), annual/biennial dicots (ad), and perennial dicots (pd).

	SMR	p - value	Hypothesis
Cr Root	0.663	< 0.0005	pd < ad < m
Ni Root	0.634	< 0.0005	pd < ad < m
Cu Root	0.422	< 0.0005	pd = ad < m
Zn Root	0.509	< 0.0005	pd = ad < m
Pb Root	0.583	< 0.0005	pd < ad < m
Cr Shoot	0.395	< 0.0005	pd = ad < m
Ni Shoot	0.467	< 0.0005	pd = ad < m
Cu Shoot	0.080	0.024	pd < ad
Zn Shoot	0.060	0.063	
Pb Shoot	0.300	< 0.0005	pd < ad < m

Table 5.11. Statistical results for biological concentration factor for onsite species. Plants were grouped as a combination of monocots (m), annual/biennial dicots (ad), and perennial dicots (pd).

	SMR	p - value	Hypothesis
Cr Root BCF	0.685	< 0.0005	pd < ad < m
Ni Root BCF	0.641	< 0.0005	pd < ad < m
Cu Root BCF	0.402	< 0.0005	ad = pd < m
Zn Root BCF	0.475	< 0.0005	ad < pd < m
Pb Root BCF	0.251	< 0.0005	pd < ad < m
Cr Shoot BCF	0.420	< 0.0005	ad = pd < m
Ni Shoot BCF	0.472	< 0.0005	ad = pd < m
Cu Shoot BCF	0.067	0.052	
Zn Shoot BCF	0.077	0.033	m < ad = pd
Pb Shoot BCF	0.256	< 0.0005	pd < ad < m
Cr Trans	0.110	0.006	m = ad < pd
Ni Trans	0.071	0.037	m < pd
Cu Trans	0.369	< 0.0005	m < ad = pd
Zn Trans	0.432	< 0.0005	m < ad = pd
Pb Trans	0.200	< 0.0005	m < ad = pd

Table 5.12. Test for interactions between location and habitat-duration. For metals without significant difference by location a one way ANOVA was used.

	Interaction	MSR	p - value	Habit-Duration
Cr Root	0.008			
	off	0.662	0.003	pd < ad = m
	on	0.736	< 0.0005	pd = ad < m
Ni Root	0.099	0.672	< 0.0005	pd < ad < m
Cu Root	0.019			
	off	0.362	0.084	N.S.
	on	0.514	< 0.0005	pd = ad < m
Zn Root	0.003			
	off	0.065	0.692	N.S.
	on	0.561	< 0.0005	pd = ad < m
Pb Root	0.443	0.671	< 0.0005	pd < ad < m
Cr Shoot	1-way	0.297	< 0.0005	pd = ad < m
Ni Shoot	0.264	0.655	0.002	pd = ad < m
Cu Shoot	1-way	0.570	< 0.0005	m < pd < ad
Zn Shoot	1-way	0.288	< 0.0005	m < pd = ad
Pb Shoot	< 0.0005			
	off	0.179	0.338	N.S.
	on	0.522	< 0.0005	pd < ad = m

Table 5.13. Metal in root tissue compared to metal fractions. These data are taken from Tables 5.3 and 5.4.

	MW	Site Average Root Metal Concentration	Site Average Labile Metal Concentration	Proportion Root to Labile
Cr	52.0	28	25	1.12
Ni	58.7	7	7	1.00
Cu	63.5	26	38	0.68
Zn	65.4	41	70	0.58
Pb	207.2	23	248	0.09

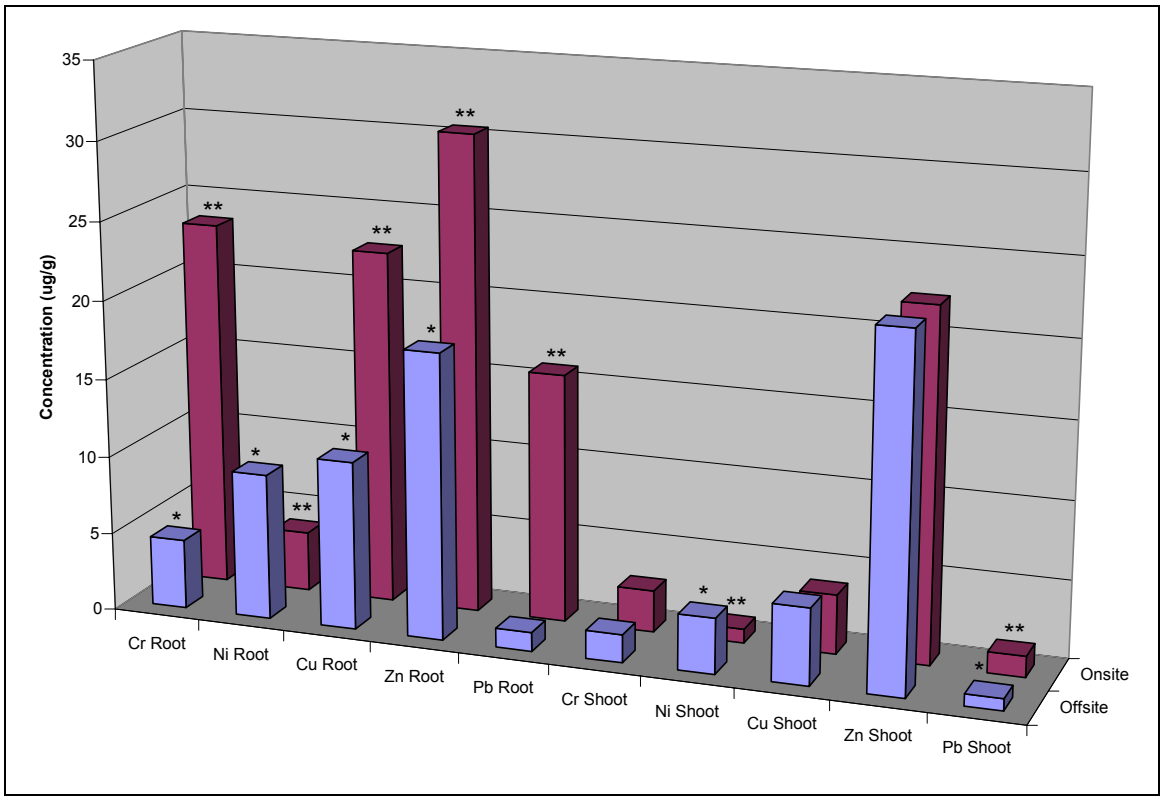

Figure 5.1. Metal Concentration in Onsite Species vs. Offsite Species (average of 7 species). Asterisk denote significant difference ($p < 0.0005$) between sites.

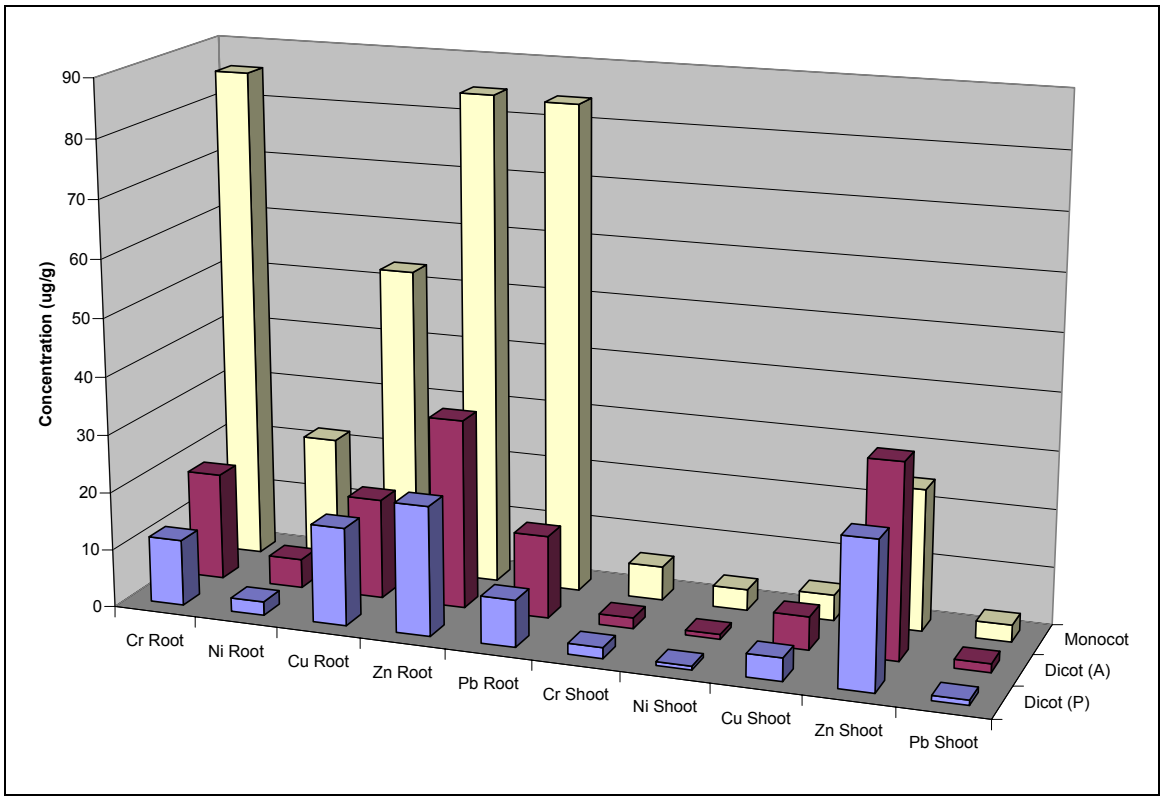

Figure 5.2. Metal Uptake by Species Type: Monocot, Dicot (Annual) and Dicot (Perennial). Refer to Table 5.11 for significant differences between groups.

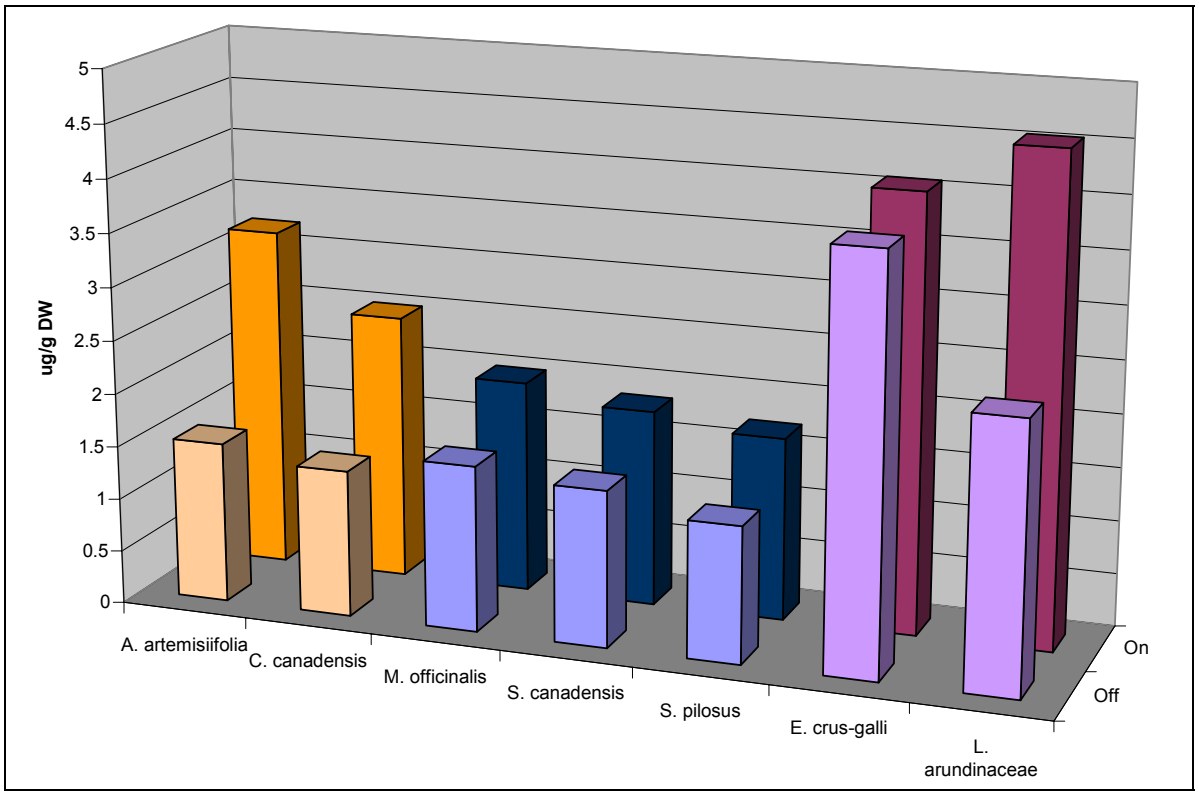

Figure 5.3. Chromium in offsite plants growing on background level soil, onsite plants came from the LTU. Annuals: *A. artemisiifolia* and *C. canadensis*. Perennials: *M. officinalis, S. canadensis* and *S. pilosus*. Monocots: *E. crus-galii,* and *L. arundinaceae*.

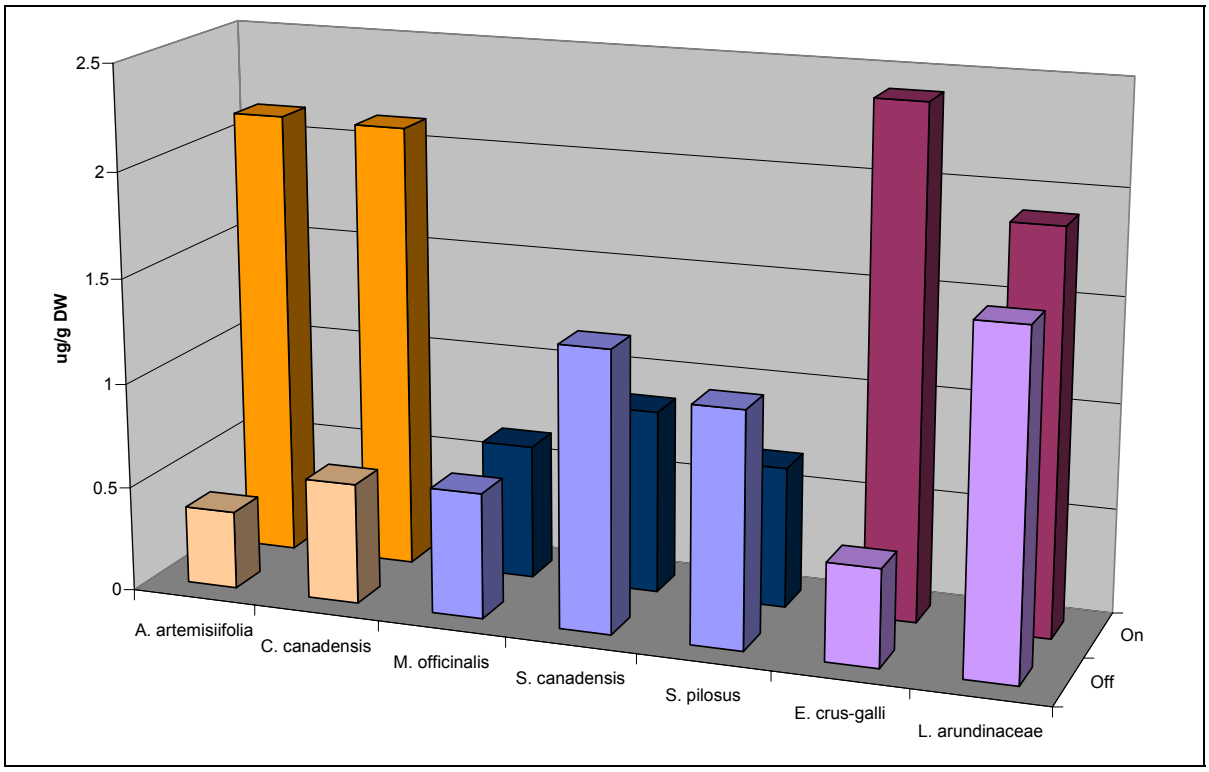

Figure 5.4. Lead in offsite plants growing on background level soil, onsite plants came from the LTU. Annuals: *A. artemisiifolia* and *C. canadensis*. Perennials: *M. officinalis, S. canadensis* and *S. pilosus*. Monocots: *E. crus-galii,* and *L. arundinaceae*.

APPENDIX 5A. Geometric mean and standard deviation of metal concentration in onsite plants (n = 8).

	Chromium		Nickel		Copper		Zinc		Lead	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
Annual Dicots										
A. artemisiifolia	23.6 ± 1.6	3.2 ± 1.8	3.9 ± 1.8	1.1 ± 2.2	11.7 ± 1.4	4.8 ± 1.8	15.1 ± 1.7	26.1 ± 2.1	8.8 ± 2.1	2.1 ± 1.2
C. canadensis	17.2 ± 2.5	2.5 ± 1.4	4.0 ± 3.1	0.8 ± 1.4	19.7 ± 1.7	12.9 ± 1.3	27.3 ± 1.9	57.7 ± 1.3	14.5 ± 2.4	2.1 ± 1.5
E. annuus	32.1 ± 1.5	0.6 ± 1.6	9.9 ± 1.6	0.4 ± 2.0	39.2 ± 1.3	5.2 ± 1.3	46.1 ± 1.4	18.3 ± 1.4	41.8 ± 1.7	0.8 ± 1.6
L. serriola	15.9 ± 1.3	1.9 ± 1.5	6.2 ± 1.5	0.7 ± 2.3	18.6 ± 1.8	4.5 ± 1.3	43.0 ± 1.4	49.9 ± 2.2	10.2 ± 1.6	1.1 ± 2.5
O. biennis	10.5 ± 1.9	2.3 ± 1.8	3.3 ± 2.2	1.6 ± 1.8	9.2 ± 1.6	4.4 ± 1.4	45.7 ± 1.8	30.6 ± 1.8	10.7 ± 1.4	2.2 ± 2.9
Perennial Dicots										
E. graminifolia	18.9 ± 1.3	1.9 ± 2.3	7.1 ± 1.3	1.0 ± 1.9	23.6 ± 1.2	7.7 ± 1.3	28.5 ± 1.4	52.7 ± 1.6	21.4 ± 1.5	1.6 ± 1.3
M. officinalis	9.0 ± 1.7	2.0 ± 1.9	1.4 ± 1.6	0.4 ± 1.4	7.7 ± 2.4	2.5 ± 1.2	20.1 ± 2.5	8.8 ± 1.4	5.2 ± 2.2	0.6 ± 1.5
S. canadensis	9.9 ± 1.4	1.8 ± 2.1	1.8 ± 1.4	0.8 ± 1.8	22.5 ± 1.3	3.7 ± 1.3	17.8 ± 1.3	31.0 ± 1.4	6.2 ± 1.6	0.9 ± 1.5
S. pilosum	10.1 ± 1.8	1.7 ± 2.0	1.8 ± 1.5	0.6 ± 1.5	20.1 ± 1.7	3.4 ± 1.5	24.6 ± 1.3	28.6 ± 1.8	6.2 ± 2.2	0.7 ± 1.9
Monocots										
C. strigosus	67.9 ± 2.0	3.6 ± 2.1	31.5 ± 1.4	3.7 ± 2.2	54.8 ± 1.3	11.6 ± 1.4	99.5 ± 1.4	37.4 ± 1.4	64.8 ± 1.1	1.6 ± 2.1
D. glomerata	103.5 ± 2.7	11.5 ± 1.8	44.4 ± 2.5	10.6 ±	79.6 ± 2.4	5.5 ± 2.4	101.0 ± 2.1	38.6 ± 1.8	85.4 ± 5.2	4.1 ± 2.7
E. crus-galli	61.5 ± 1.5	4.1 ± 3.7	11.0 ± 1.7	2.2 ± 1.4	55.8 ± 1.3	3.0 ± 1.5	110.1 ± 1.4	44.5 ± 1.3	104.3 ± 1.9	2.4 ± 2.4
L. arundinaceum	174.9 ± 1.9	4.6 ± 1.6	15.3 ± 1.4	1.1 ± 1.4	67.9 ± 1.8	1.9 ± 1.2	61.8 ± 1.3	5.3 ± 1.4	95.4 ± 2.6	1.9 ± 1.8
L. perenne	76.2 ± 1.4	8.9 ± 1.8	27.3 ± 1.7	5.8 ± 1.6	30.3 ± 1.6	4.9 ± 1.4	75.6 ± 1.9	23.3 ± 1.9	103.8 ± 2.7	6.3 ± 2.2

APPENDIX 5B. Geometric mean by grouping of monocots, perennial dicots and annual/biennial dicots.

	Chromium				Nickel				Copper				Zinc				Lead			
	Root		Shoot		Root		Shoot		Root		Shoot		Root		Shoot		Root		Shoot	
	GM	SD	GM	SD	GM	SD	GM	SD	GM	SD	GM	SD	GM	SD	GM	SD	GM	SD	GM	SD
Annual Dicots	18	2.0	1.9	2.0	4.8	2.3	0.9	2.2	17	2.0	6.02	1.7	33.5	1.9	34.3	1.9	14.4	2.2	1.61	2.2
Perennial Dicots	11	1.7	1.9	2.0	2.3	2.0	0.7	1.8	18	1.9	3.96	1.6	22.2	1.6	27	2.1	7.91	2.2	0.87	1.8
Monocots	91	2.0	6.3	2.3	23	1.9	3.7	2.8	52	1.9	4.27	2.2	80.8	1.6	21.4	2.6	84.7	2.7	3.11	2.6

APPENDIX 5C. Percent of metal in plant tissue relative to soil (BCF x 100%). Bioconcentration factor (BCF) was determined for root and Shoot and is defined here as the concentration of metal in the given part divided by the concentration in the soil (Kim et al., 2003). BCF was calculated on an individual plant basis prior to calculating average.

	Chromium		Nickel		Copper		Zinc		Lead	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
Annual Dicots										
A. artemisiifolia	5.7 ± 3.5	0.8 ± 0.6	5.9 ± 4.0	1.8 ± 1.6	13.5 ± 5.3	6.1 ± 4.7	4.8 ± 2.2	8.5 ± 4.6	2.1 ± 1.9	0.4 ± 0.1
C. canadensis	4.1 ± 2.5	0.5 ± 0.2	10.3 ± 12.1	1.2 ± 0.7	22.5 ± 10.3	13.7 ± 3.4	7.9 ± 4.7	15.5 ± 6.0	2.5 ± 1.5	0.3 ± 0.2
E. annuus	6.7 ± 3.2	0.1 ± 0.1	15.1 ± 6.9	0.6 ± 0.5	41.6 ± 14.7	5.4 ± 1.5	12.1 ± 4.3	4.8 ± 1.6	7.1 ± 4.9	0.2 ± 0.1
L. serriola	3.6 ± 1.3	0.4 ± 0.1	9.6 ± 4.2	1.3 ± 0.7	24.0 ± 18.7	5.0 ± 1.2	12.7 ± 5.4	17.2 ± 10.4	2.0 ± 1.1	0.2 ± 0.2
O. biennis	2.2 ± 1.0	0.5 ± 0.2	6.1 ± 5.0	2.6 ± 2.1	10.2 ± 3.9	4.7 ± 1.7	12.8 ± 5.6	8.6 ± 3.7	1.6 ± 0.5	0.4 ± 0.3
Perennial Dicots										
E. graminifolia	3.9 ± 1.3	0.5 ± 0.4	9.9 ± 1.8	1.6 ± 1.2	25.7 ± 6.8	8.3 ± 1.9	8.2 ± 3.3	15.8 ± 8.2	3.6 ± 1.9	0.3 ± 0.2
M. officinalis	1.7 ± 0.8	0.4 ± 0.3	2.2 ± 1.1	0.5 ± 0.2	10.0 ± 7.8	2.5 ± 0.4	6.9 ± 7.7	2.2 ± 0.7	0.9 ± 0.6	0.1 ± 0.0
S. canadensis	2.2 ± 0.8	0.5 ± 0.4	2.5 ± 0.6	1.3 ± 0.7	25.0 ± 6.6	4.1 ± 1.0	5.0 ± 1.0	8.8 ± 2.2	1.1 ± 0.5	0.2 ± 0.1
S. pilosum	2.4 ± 1.0	0.5 ± 0.4	2.6 ± 1.0	1.0 ± 0.5	23.9 ± 10.7	4.0 ± 1.6	7.1 ± 2.0	9.9 ± 9.4	1.2 ± 0.6	0.2 ± 0.2
Monocots										
C. strigosus	18.0 ± 9.8	0.9 ± 0.4	47.4 ± 20.5	7.3 ± 7.9	62.5 ± 12.7	14.0 ± 5.6	30.3 ± 12.5	11.5 ± 5.3	11.7 ± 4.4	0.3 ± 0.2
D. glomerata	29.1 ± 25.6	2.7 ± 1.7	82.6 ± 71.1	22.4 ± 29.6	108.1 ± 67.7	7.4 ± 4.7	31.8 ± 20.2	12.2 ± 8.2	30.2 ± 32.9	1.0 ± 1.0
E. crus-galli	12.9 ± 6.7	1.2 ± 0.9	16.0 ± 9.2	3.0 ± 1.3	57.4 ± 16.4	3.2 ± 1.3	29.9 ± 10.4	11.9 ± 4.0	16.2 ± 8.7	0.4 ± 0.2
L. arundinaceum	36.5 ± 17.0	0.9 ± 0.4	22.8 ± 7.1	1.7 ± 0.7	76.9 ± 34.9	2.0 ± 0.5	15.9 ± 4.7	1.4 ± 0.4	15.8 ± 9.8	0.3 ± 0.2
L. perenne	16.4 ± 7.1	2.2 ± 1.4	42.1 ± 28.5	8.3 ± 3.2	34.6 ± 17.3	5.5 ± 2.1	24.5 ± 14.8	7.5 ± 5.1	22.4 ± 23.2	1.3 ± 1.4
Averages										
Dicot (A) Average	4.3 ± 2.8	0.5 ± 0.3	9.4 ± 7.9	1.6 ± 1.4	21.8 ± 15.3	7.3 ± 4.5	10.2 ± 5.4	11.0 ± 7.1	3.0 ± 3.0	0.3 ± 0.2
Dicot (P) Average	2.5 ± 1.2	0.5 ± 0.3	4.1 ± 3.3	1.1 ± 0.8	22.0 ± 9.8	4.7 ± 2.4	6.7 ± 3.7	9.5 ± 7.6	1.7 ± 1.4	0.2 ± 0.1
Monocot Average	24.2 ± 17.7	1.7 ± 1.3	44.9 ± 44.0	9.3 ± 16.3	96.7 ± 45.8	6.1 ± 5.0	25.7 ± 14.5	8.3 ± 6.7	20.3 ± 20.7	0.7 ± 0.9

APPENDIX 5D. Average translocation from root to shoot for onsite species (n = 8). Translocation is calculated on a per plant basis by dividing the Shoot concentration of metal by the root concentration of metal.

		Chromium		Nickel		Copper		Zinc		Lead	
Habit	Plant	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Annual/ Biennial Dicot	*A. artemisiifolia*	0.18	0.15	0.40	0.33	0.57	0.57	2.33	1.82	0.29	0.18
	C. canadensis	0.18	0.15	0.33	0.36	0.74	0.45	2.56	1.73	0.24	0.25
	E. annuus	0.02	0.01	0.04	0.03	0.14	0.05	0.43	0.17	0.02	0.02
	L. serriola	0.13	0.05	0.17	0.09	0.28	0.13	1.38	0.63	0.15	0.10
	O. biennis	0.28	0.25	0.58	0.49	0.61	0.60	0.82	0.66	0.31	0.28
Perennial Dicot	*E. graminifolia*	0.15	0.17	0.18	0.16	0.33	0.09	1.92	0.62	0.08	0.04
	M. officinalis	0.25	0.14	0.27	0.08	0.42	0.30	0.57	0.40	0.17	0.14
	S. canadensis	0.28	0.28	0.58	0.37	0.18	0.06	1.84	0.67	0.16	0.09
	S. pilosum	0.29	0.38	0.38	0.19	0.21	0.18	1.33	0.90	0.22	0.35
Monocot	*C. strigosus*	0.08	0.10	0.15	0.15	0.22	0.09	0.38	0.04	0.03	0.03
	D. glomerata	0.20	0.26	0.37	0.32	0.12	0.14	0.50	0.33	0.13	0.17
	E. crus-galli	0.09	0.06	0.22	0.12	0.06	0.02	0.42	0.12	0.03	0.01
	L. arundinaceum	0.04	0.05	0.08	0.04	0.04	0.03	0.09	0.04	0.05	0.09
	L. perenne	0.15	0.11	0.27	0.19	0.20	0.14	0.35	0.15	0.09	0.08
Avg. Annual Dicot (n = 5)		0.16	0.10	0.30	0.21	0.47	0.25	1.50	0.93	0.20	0.12
Avg. Perennial Dicot (n = 4)		0.24	0.06	0.35	0.17	0.28	0.11	1.42	0.62	0.16	0.06
Avg. Monocot (n = 5)		0.12	0.07	0.23	0.13	0.13	0.08	0.36	0.17	0.07	0.05
Average All Species (n = 14)		0.17	0.09	0.29	0.16	0.29	0.22	1.07	0.82	0.14	0.10

DISSERTATION SUMMARY

The treatment of hazardous waste (both historical and newly created) will be an issue society must deal with for many decades to come. Phytoremediation has been demonstrated as an effective and economical process that uses plants to contain, sequester, and aid in degradation of contaminants. This dissertation investigated the phytoremediation potential of a natural plant community. The results of this study demonstrated the continued degradation of contaminants on an aged petroleum landfarm by passive revegetation. In the absence of cover crop maintenance, the average concentration of petroleum hydrocarbons and polycyclic aromatic hydrocarbons had reduced by 50% over a two year period. Metal mobility was not evident, nor was metal uptake into plants alarmingly higher than the content in an unpolluted, post agricultural old field. A diverse community of plant species became established and provided adequate cover necessary for erosion prevention. Further, the presence and increasing cover of later-successional tree species indicated that the establishing community was proceeding as would be expected, without regard to the contaminants.

The end-use strategy suggested here would be more cost efficient than labor-intensive landfarming and tremendously less expensive than dig and haul or other (engineering-based) *in situ* remediation strategies. Management decisions for aged hazardous waste facilities are made on a site by site basis with regard to regulations and with input from stakeholders: the company responsible for the wastes and, in many cases, the neighboring community. Understandably, communities are more concerned about risk of exposure to contaminants than the cost of treatment. On this particular site exposure is low because access is restricted, and both plant cover and site contouring ensures run-off is minimized or, at least, contained. Heavy metals were not readily available (mobile) and, as would be expected, uptake into plant tissue was minimal. However, in areas where plant populations may have been under constant metal selective pressures (for example, serpentine soils or areas of active ore mining) it is possible ecotypes exist that may accumulate metals aboveground as a tolerance mechanism. In such situations, allowing natural revegetation may pose additional metal uptake risks not seen in this study. Another consideration not addressed in this study was soil invertebrate loading, another potential source for metal/PAH introduction into the food chain. Although, the soil-to-invertebrate bioaccumulation link would be an issue for both managed and passive (natural) phytoremediation, this link *should* be addressed for a full assessment of risk to wildlife.

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APPENDIX A:

DETAILED METHODS SECTION

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Study Site: Chevron-Texaco Land Treatment Unit. The site used for this study was the Chevron Corporation Land Treatment Unit (LTU) located in Hooven, Ohio. The LTU was built in 1980 on a 2.2 hectare site by excavation, compaction of a natural clay liner, and construction of a peripheral berm. Six monitoring wells were constructed around the outside of this berm to monitor for any mobility into ground water. The site was opened in 1981 and received 10.6 million litres of petroleum refinery wastes from the nearby refinery, now out of service. These wastes were disked into the limestone-based soil to a depth of 40.64 cm. Below the top soil (60 – 90 cm) is a clay layer which extends for many feet to the Fairview limestone/shale formation. As a vegetation cap, perennial ryegrass (Lolium perenne) was planted and maintained from 1992 until 1998. During this time, the LTU was irrigated, tilled, and reseeded. In 1999, the site was tilled and was allowed to revegetate naturally as part of the post-closure cover strategy. Because this site is the only one in the United States with approval for natural revegetation, this study site provides the unique opportunity to follow the reestablishment of vegetation on petroleum waste-contaminated soil allowed to go fallow (Condit & Doherty, 2000).

The site still contains high levels of heavy metals (i.e., lead, chromium, and zinc) and PAHs . Stratification is evident in soil cores. The top layer (0 to 40.64 cm) has the consistency of commercial topsoil (loose to friable), below which is a black, sticky layer that extends into the clay layer. According to baseline data from the RTDF study, metals

are significantly higher in the upper layer of soil; whereas, PAHs are significantly higher in the lower level.

Plot Establishment. In 1999 the site was subdivided into 4 quadrats. Plots for this study were located within each quadrat arranged in a random block design. Sixteen plots 6.1 m x 6.1 m (37.21 m^2) were delineated using flagging tape. Four of the 16 plots (one in each quadrat) were designated as controls by continual removal of vegetation. Plots received no maintenance from 1998 on. GPS (Global Positioning System) was used to locate latitude, longitude, elevation of plots and the site periphery (the berm). In year 2000, a Chevron employee accidentally tilled one revegetated and one control plot. Therefore, those plots were removed from further analysis.

Characterization of LTU Contamination

Soil Cores. Each year of the three year study, two replicate 3' cores were removed from each plot. Replicate cores were taken 2' apart. Cores (1" diameter) were removed using an Environmental Soil Probe (ESP) designed to maintain soil horizon characteristics by minimizing compaction. Soil cores were taken between the 0,0 and 1,1 point of each plot. (2001: 10 and 17 or 3; 2002 10 and 15 or 5). Cores were taken to the side of plants, recording the overlaying vegetation. The ESP probed to the maximum depth possible, 36"**.** Hole depth was recorded as was the core length in the liner. From these parameters the percent compaction of the soil in the liner was estimated. These cores were placed in a plastic liner, sealed, and stored in a -14.4° C freezer.

Individual cores were removed from storage, placed in a hood and extended onto sterile paper. To minimize the mixing of layers, the region which appeared the most "black" was sliced through the middle starting first from outside of the dark region on the topsoil side and then cut lengthwise from the clay side into the black region. The core was split open and a digital photograph of the entire core taken. The cores were observed directly under a fluorescent lamp (equalizing light across specimen) and evaluated for the following soil characteristics: layer, structure, consistency, roots, and compaction (Table 1).

Table 1. Physical Characteristics of Soil Cores.

Layer	The LTU soil is stratified with a variable layer of original sludge material subtending the tilled, planted horizon soil. The "upper" layer was characteristically tan to brown, and the "lower" oily layer was a black color. Hence, color (Munsell Color Chart: 5Y 3/2) was used to separate soil layers (Bohm, 1979). This layering technique maximized evaluation of the within-layer status of contamination across the entire LTU (as opposed to standard depth analysis).
Structure	Granular: peds <0.5cm diameter, often where roots growing. Blocky: peds irregular, usually 1.5-5.0cm in diameter. Prismatic: peds several cm long, usually in lower horizons. Platy: thin, flat plates of soil usually found in compacted soil.
Consistency	Loose: stucture falls apart before handling. Friable: ped breaks with a small amount of pressure. Firm: ped breaks with a good amount of pressure. Sticky: ped squeezes between fingers in a tar-like (not clay like) way.
Compaction	The total core length as well as the depth of the hole from which the core was extracted was measured. The proportion of depth/length approximates the degree of compaction. Compaction is then used to correct for the depths of each layer.
% Moisture	Percent of water weight of total weight from sub-sample of soil core dried at 70°C for four days.
Roots	None: no visible roots at close examination using handlens. Few: roots visible only using handlens. Many: obvious presence of roots.

Soil Mass and Percent Moisture. Upper and Lower layers were weighed immediately after separation. A 10g portion of each layer was weighed and then dried to a constant weight to determine percent of water by weight. Because the black layer was sometimes very small, only a small bit of soil (4-5g) was available for this purpose. The total dry weight of each soil layer was calculated by subtracting the wet weight by the relative percentage of moisture of the sub sample. This dried subsample of soil was used to determine metal content. The remaining soil from both layer was sealed in air-tight bags and stored at -14.4° C.

Metals Extraction. To determine the location of metals within the soil fractions, sequential extraction procedures were used which selectively remove metals from the various components of soil. Sequential extractions use a series of progressively stronger solutions to break down the materials from each soil fraction that are likely to be affected by typical environmental conditions (Sposito et al., 1982; Tessier et al., 1979). The bioavailable metal fraction was defined here as a combination of exchangeable and sorbed fractions (Table 2). Approximately 2 g of sifted (2mm), oven dried soil was extracted with 25mls of the first extractant, 0.5M KNO3 for 16 hrs on a shaker (100-150 cycles per minute) and then centrifuged for 15 minutes at 5100rpm. The supernatant was poured through a Whatman #42 filter paper and brought up to 25 mls with extractant solution. The process was repeated except for the residual fraction was not shaken but placed in a 70°C oven for the reaction time of 16 hrs (Emmerich et al., 1982; Pierzynski & Schwab, 1993). The bioavailable extracts (KNO3 and H2O) were combined and concentrated to 25 ml to overcome detection limitations. Three replicates of each layer

per core were extracted and then analyzed for metals using Atomic Absorption Spectrometry (specific instrument), and Inductively Coupled Plasma Spectroscopy (Thermo Jarrel Ash Corporation ICAP 61E Plasma Emission Spectrometer, Agilent and Elan).

Table 2. Sequential Extraction of Metals based on Sposito (1982) and Tessier (1979).

Fraction	Extractant	*Portion of Soil*
Mobile, Bioavailable, Sorbed, Exchangeable	0.5 M KNO_3 (16 hr), H_2O (2 hr)	Adsorbed to the surface of clays, humic acids. Ionic composition affects the sorption-desorption process liberates these weakly adsorped metals (Sparks, 1995).
Organic Bound	0.5M NaOH (16 hr)	Metal complexation/peptization with humic and fulvic acids (organic polymers at the surface of soil particles). Oxidizing conditions (acidic root exudates) could reverse and release a soluble form of metals bound as such (Bogan & Sullivan, 2003).
Clay-Oxide Bound	0.05M NaEDTA (6 hr)	Associated with soil iron/manganese oxide bound metals. EDTA solubilizes metals by forming complexes that suit tri- and bivalent cations. This fraction may become availabile when plant exudates have chelating capabilities.
Residual	4M HNO_3 (16 hr at 70°C)	Representative of metals very tightly bound within the crystalline structure of primary and secondary minerals. These are not considered to be bioavailable in a reasonable span of time.

PAH Extraction. Fresh soil was removed from freezer (-14.4° C), sifted (2 mm) and extracted for determination of total PAHs. An accelerated solvent extraction system (Dionex ASE 200; Sunnyvale, CA) was used to extract samples from each layer of each soil core (Richter, 2000). One duplicate was run for every twenty samples to account for variability in the soil (EPA, 1996a). ASE extraction cells (22ml) were filled with 4g of soil mixed with 2g diatomaceous earth. The extraction solvent was methylene chloride-acetone (1:1, v/v). Heating time was 8 min, to reach 175°C. Extraction pressure was 1500psi; static time was 5 min, flush volume was 70%, and the purge time 60 sec with

150psi N. Extracts were dried to 10 ml and then centrifuged for 10 min (5100rpm), brought to 10 mls with acetone, and stored at 0ºC.

Prior to analysis, extracts were centrifuged for 30 min (5100rpm, 0ºC). Supernatant was passed through a glass wool plugged pipette, collected, and dried to 4 ml. One ml was transferred to a glass sample vial for analysis and the rest stored in a capped glass vial sealed with parafilm. To check for loss of PAH during sample processing, the test tube used for drying to 4ml was rinsed with MeCl (1 ml) and the pellet resuspended in MeCl. Both of these MeCl fractions were analyzed for PAHs. Standards were purchased (EPA 525A, Ultra Scientific) and used to identify the following PAHs in the extracts: acenaphthylene, anthracene, benz[a]anthracene, benzo[k]fluoranthene, benzo[b]fluoranthene, benzo[ghi]perylene, benzo[a]pyrene, chrysene, dibenz[a,h]anthracene, fluorene, indeno[1,2,3-cd]pyrene, phenanthrene, and pyrene.

PAHs were separated on a gas chromatograph (GC 14A; Shimadzu, Columbia, MD) in the split mode (2:1), with a flame ionization detector (FID). The column was a DB-XLB (JW Scientific) proprietary phase of low polarity (60m x 0.25mm internal diameter x 0.25µm film thickness). The injection port temperature was 300ºC, column temperature was static at 95ºC for 0.5 min then increased by 5°C per min to 340ºC, and held for 7 min. All solvents and chemicals were reagent grade. Randomized duplicates determined instrument reproducibility was 10%. Duplicate processes indicated RPD was 30% for total PAHs. Integration of nonspecific hydrocarbon peak between 18 and 55 minutes (roughly the retention range of diesel) was calculated for each chromatogram. The RF used based on average of 13 PAH standards for each day of analysis to account

for daily variation in instrument response. EZChrom Elite Software (Scientific Software, Inc.) was used to calculate areas of PAHS and TPH. PAHs were measured peak to peak, whereas the TPH was measured from baseline to baseline, following modification of EPA Method 3560 (EPA, 1996b). Verification of PAHs using GC-MS (Hewlett-Paccard 5890 Series II) was conducted on two samples, and upper and lower layer soil containing all of the PAHs.

Phytotoxicity of Soil. The lettuce (*Lactuca sativa* L.) bioassay allows the LTU soil to be rated for its relative toxicity compared to control soil and may provide the opportunity to compare to other contaminated soils with regard to its phytotoxicity (Greene et al., 1989; Knoke et al., 1999). Lettuce was chosen for its demonstrated sensitivity to metals (and somewhat sensitive to PAHs) compared to other plant bioassays (Keddy et al., 1995). Lettuce seeds (Black-Seeded Simpson from Gurney's) were soaked 20 min in 10% Clorox solution and then rinsed 5 times with distilled water, and placed in 9cm Whatman #41 filter paper in 10 cm Petri dish. Three replicates, as recommended, were made of a composite of the initial coring soils separated by layer. Sifted (2mm) soil was weighed out to 10 g (wet weight) into a centrifuge tube. (One gram of each wet weight soil was placed in a 40°C oven for 48 h to determine the relative dry weight). To the soil, 10 ml DDI H_2O (brought to pH 6.5) was added and agitated overnight (~18 h), then centrifuged 15 min at 5100rpm. Onto the filter paper, 5-7 ml of solution was poured. Ten 10 seeds per paper were placed in the Petri dish and spaced evenly, incubated in dark (at room temp) for 5 days. Dishes were briefly checked during incubation and pipetted with ~1 ml of supernatant solution if paper was dry.

After 5 days, for each dish, % seed germination was recorded and the individual root lengths to the nearest mm were measured using a hand caliper. If there is significant reduction in germination and or root elongation, then there may be reason to investigate it perhaps the vegetation thriving on site has undergone some sort of selection to better tolerate the soil conditions. On the other hand, if there is little reduction in germination and/or root elongation relative to control may indicate that the metals and PAHs are not bioavailable, allowing a suite of species to colonize without regard to plant toxicity.

Goldenrod Phytoxicity Study. The most common *in situ* species, *Solidago canadensis*, was studied to determine relative phytotoxicity of the upper and lower layer soil compared to topsoil. Seedlings (1mm) were removed from a sprouting goldenrod head growing on a landfarm contaminated with heavy metals and PAHs. These seedlings were transferred to pots containing sifted (2mm) soil brought up to field water capacity of 25% (using DDI water) in three treatment groups: LTU upper soil, LTU lower soil, topsoil (uncontaminated). The soil had been collected as part of the treatability study. The pots were placed into a metal pan and treatments were grouped together. Seedlings were grown in a controlled growth room (15/20 °C, 8h dark/16h light, 520 lux). Cellophane covered the treatments to preventing drying out. Plants grew for 2 months after which they were removed from their pots, and the longest 2 leaves were measured using a caliper. Also measured were the number of roots (defined as branching from the base of the leaves) the number of stolons (root-like projections emerging above the basal leaves) and the length of the two longest of each. The plants were rinsed in DDI water overnight,

and set out on paper towel to dry. Plants were separated by root and crown and weighed. The final biomass was insufficient for determining plant uptake.

Characterization of LTU Plant Community

Sampling. The analysis of vegetation began in 2000 and for each year of the study is based on five sampling events throughout the summer (in three week intervals). During each sampling event cover and count were measured in randomly located triplicate subplots (1 m^2) of each vegetated plot. Control (unvegetated) plots were checked bi-weekly for removal of seedlings. With this sampling strategy, roughly one third of the entire plot has been sampled by the end of the growing season.

Percent Cover. Cover is defined as the vertical projection of the crown or shoot area of a species to the ground surface expressed as a fraction or percent of a reference area (Mueller-Dombois & Ellenberg, 1974). Cover was measured here on a species basis within subplots. Digital photos taken from each subplot in year 2000 were evaluated to estimate species cover. After 2000, cover was too high to allow accuracy using digital images; cover was instead determined in the field. Taxonomic classification to species was determined using dichotomous keys (Weishaupt, 1971).

Plant Abundance. The number of individual plants (N) per species was counted. For bunch grasses (e.g. *Lolium perenne*, ryegrass) the number of individuals was estimated based on an average of three counts per given unit of area. A few of the species on site reproduce asexually through underground stems. In these cases, (N) reflects the number

of ramets rather than number of individuals. Number is measured as the ramet emerging from its own rooting mass. Hence, stoloniferous plants (goldenrods, willows) were counted as individuals, yet their ramets could emerge from the same genet; causing an overestimation of the number of individuals. Plants whose crown partially covered an area in the subplot were only counted if root was found within the subplot. Vines (e.g., honeysuckle), even at high coverage appeared to be from an interconnected individual. To count vines, attempts were made to isolate individuals from the mass. These numbers may underestimate where cover is high. All seedlings were counted. Mosses and plant litter were not counted. Accurate species identification requires a flower, but in many cases a flower was not present. When possible, a sample of similar specimen growing outside of plot was collected, labeled, dried and stored. Back in the lab, dried samples were compared to known samples for leaf margins and hair patterning to key out to species. There were three species without final identification, but these species represented low cover and abundance. Plant density and frequency were also calculated on a per-species basis where:

$$\text{Density} = \text{total \# plants} / m^2$$

$$\text{Frequency} = \text{\# subplots present} / \text{total number of subplots}$$

Biomass. Biomass is plant dry weight per unit area (Barbour *et al*., 1999) and an indication of system production. At the end of the growing season, vegetation was harvested along the outside perimeter of each plot. Three subplots ($0.33 m^2$) per plot were located and stratified with respect to the berm (nearest edge vegetation). Cover of vegetation within that area was noted. Plants were cut at the soil-level, separated by

species, placed in a bag, and transferred to U.C. The bags were emptied into stainless steel containers and dried at 45º C to a constant weight.

Cover to Biomass. In 2003, the most abundant (in terms of cover and number) of each of the trees (n = 8), grasses (n = 5) and forbs (n = 10) were sampled to estimate the relationship between cover and biomass. Grasses and forbs were collected just prior to peak flowering for that species. At this time in the plant lifecycle, root growth is thought to have reached the maximum as allocation of photosynthates begins to shift towards flower production. Woody plant species (trees, vines and shrubs) were collected in September. Within each quadrat (n = 4), the tallest and smallest specimens per species were selected based on both height and cover (using the same process as with the summer sampling). The plants were removed by loosening dirt around the root, shaking off excess soil, and placing the root ball in a bag. At the lab, crown height and root length was measured. Extra dirt was loosened from the root and washed out under running water until no visible dirt remained. Then the plant was soaked in 3 washes of DDI water on a shaker for 3 hours followed by a final DDI rinse (Azcue and Mudroch 1994). The plants were left to dry to the touch, and then separated by root, midsection, and flower/fruit. The root section was separated from clinging dirt by using a 2mm sieve taking care to not lose any root material. Plant material was dried 70°C oven overnight (12 hrs), and the dry weight was recorded.

Diversity. There are two applications for diversity in this study: (1) to compare diversity across the site within a growing season, and (2) to compare changes in diversity in

subsequent growing seasons. Richness, defined as the number of species present in a defined area (S), is the simplest estimate of diversity (Magurran, 1988). Other indices of diversity provide an indication of the balance between number and relative abundance of species. Table 2 lists the indices for richness and diversity used in this study.

Generally, the term "species density" refers to the number of species per meter square, or in this study, the subplot. Alpha Diversity will be used in this study on a per-plot basis representing the cumulative number of species from each of three subplots. Beta diversity (β) may be used to determine the number of distinct communities in multivariate space or for a large sample area (McCune & Grace, 2002). Hence, β will be calculated on a plot, per month and per year basis. The Shannon-Weiner (H') index is useful in discriminating subtle differences in diversity between sites (Barbour et al., 1999; Magurran, 1988). The log base used to determine H' varies within the literature depending on the sample size, the distribution of species and the total number of species. The $\log_2$ (as opposed to base 10 or e calculation) provides the most variation across years and within subplots of this study site, as would be expected from a study with the comparatively small sample size ($1m^2$). Ecostat was used to calculate diversity measures on the plot, month and year scale. The Dice Similarity test was conducted in NTSYS to determine similarity plant species within plots. This index was also used to determine co-occurrences among plant species based on the plots in which they were found.

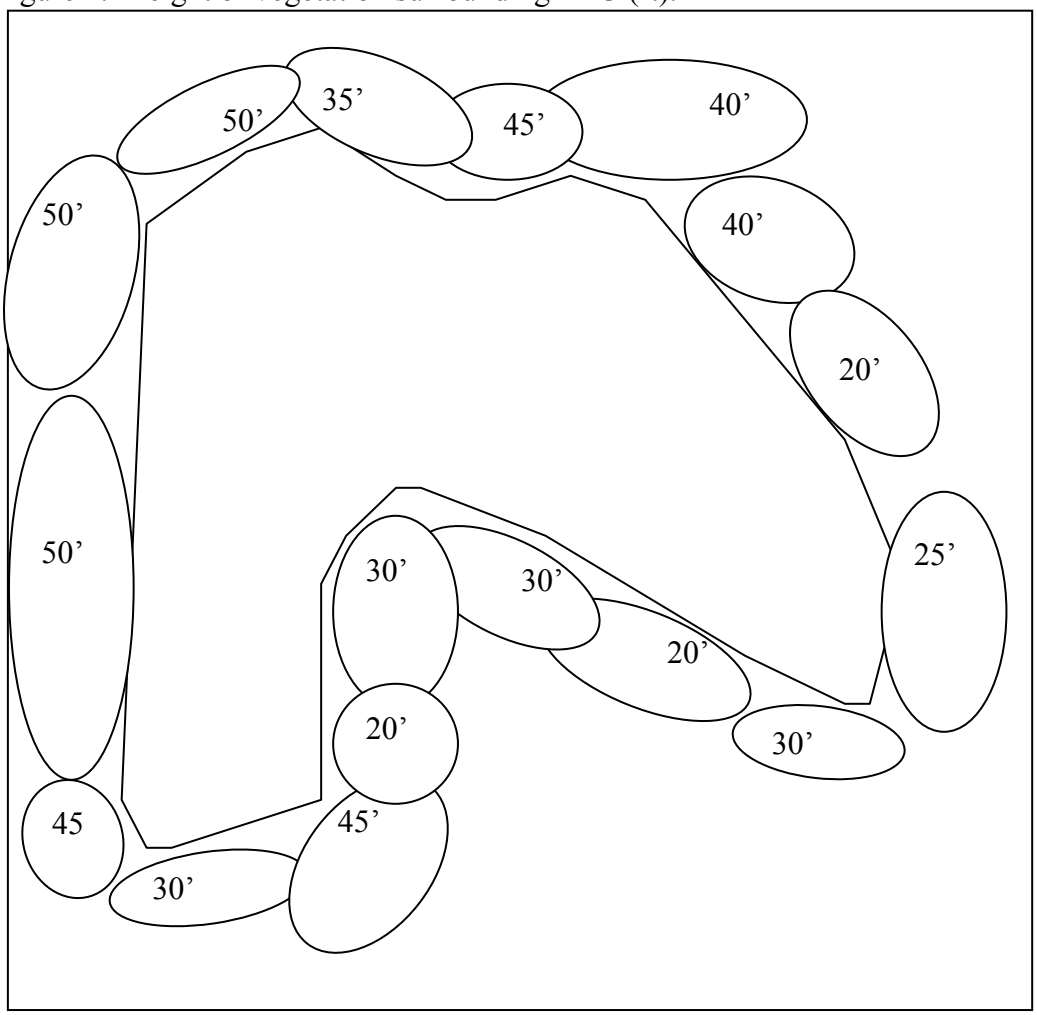
Comparison to Surrounding Community. In September of 2003, a survey of the surrounding community was conducted. By observation of satellite photos, the forest community immediately adjacent to the LTU has been left undisturbed ranging from 20

to 50+ years. Unlike the offsite community (essentially at the same successional stage), the surrounding community species composition is stable. In practical terms, it is not efficient to use the same plot and subplot technique employed above on the surrounding community because forest communities require a much larger plot size to assess diversity. Therefore, for each plot, the nearest edge composition of species, listed in order of importance (that is, the most common), was recorded. From this, a comparison can be made between the presence and absence of the dominant species in the plots to the nearest edge (Table 3 and Figure 1).

Table 3. Distance to Edge and Description of Edge Vegetation. Height of Overstory Trees estimated the distance to tree by sighting tree crown with 45° protractor. Direction and distance to edge calculated from GPS readings.

Plot	Description of Nearest Edge Species listed in order of most common followed height (m)
1,1	Sycamore (15.25m), Black locust 15.25m), Willow (4.575m), Snakeroot, Honeysuckle vine
1,2	Sycamore (15.25m), Black Locust (7.625m), Honeysuckle Bush (1.83m), Black Cherry (13.725m)
1,3	Ash (7.625m), Sycamore (9.15m), Black Locust (9.15m), Chronus spp. (4.575m), Honey vine, Honey Bush, Eupatorium, Snakeroot
1,4	Cottonwood (6.1m), Black Locust (3.05m), Honeybush (2.745m), Ash (6.1m), Goldenrod
2,1	Willow (9.15m), Black Locust (10.675m), Sycamore (10.675m), Cherry (10.675m)
2,4	Sycamore (9.15m), Black Locust (9.15m), Boxelder (9.15m)
3,1	Elm (9.15m), Black Locust (9.15m), Honey bush (4.575m)
3,2	Black Locust (4.575m), Willow (4.575m), Honey bush (3.05m)
3,3	White Pine (9.15m), Sycamore (6.1m), Black Locust (10.675m) Cherry (13.725m), Hackberry (13.725m)
3,4	Black Locust (9.15m), Ailanthus (9.15m), Honey bush (2.135m)
4,1	Black Locust (6.1m), Honey bush (2.135m)
4,2	Ash (12.2m), Cherry (12.2m), Black Locust (12.2m), Willow (3.05m)
4,3	Black Locust (10.675m), Cherry (12.2m), Willow (4.575m), Cottonwood (6.1m)
4,4	Cottonwood (7.625m), Black Locust (7.625m), Sycamore (9.15m), Cottonwood (13.725m), Cherry (7.625m)

Figure 1. Height of vegetation surrounding LTU (ft).



Comparison to Early Succession Off-site Community. Similarity between communities on and offsite was evaluated the year following the three year community study (in September 2003). This offsite community will be used to compare diversity, number, cover and proportion of invasive species. Since the end of the 2002 growing season, the on-site control plots had been left to grow fallow. An offsite location was chosen of the same total area (5.5 acres) that had areas representing the same year of succession as onsite (1-year, and roughly 4-year succession) with plots at similar distances from the nearest edge. The site is located in Union, Kentucky in a home development project.

Fourteen plots (20' x 20') were established in an area left to grow fallow for 4 years. As determined by satellite photos and interview with site developers, the years of succession of these 11 plots with 5 plots growing in an old-field left to grow fallow (since 2000). Triplicate random subplots were used to count vegetation and assess cover. Soil samples (0-10 cm composite) were taken for soil nutrient analysis.

Content in Plant Tissue: Onsite Relative to Offsite. Of the species collected for the cover to biomass assessment, the nine species with highest cover were also collected from an offsite location. Using the same criteria as above (see "cover to biomass"), a pair of individuals was removed on an offsite 1 acre development site* also in its fourth year of succession. Plants were processed as above, including the digging, cleaning and drying. Plants from onsite and offsite were then processed for metal content in plant tissue using an acid digestion procedure. Soil samples were taken for soil nutrient analysis and for metal content using the same procedures as for onsite soil.

Acid digestion of plant material was conducted according to Keane *et al*. (2001) (Keane et al., 2001). Plant tissue was soaked in distilled water* for approximately 3 hours. Leaves were then rinsed 10 times with distilled water, oven dried at 60°C for 48 h and ground in a mill. Powdered leaf tissue (2g) was ashed in a muffle furnace at 550°C for 5 h to maximize for the metals in question (Azcue and Mudroch 1994). The ash was dissolved in 2 ml concentrated HCl and heated to boiling on a hot plate to extract total leaf metals. Samples were filtered (Whatman 41), brought to 20 ml with 4 M HNO_3 and stored in plastic bottles at 4°C until analysis. Leaf digests were analyzed for Cr, Cu, Zn, Pb and Ni by inductively coupled plasma atomic emission spectrometry (ICP-AES,

Elan). Each instrument was standardized on a curve using stock metal concentrations in a 4 M HNO_3 solution (Azcue & Mudroch, 1994).

Dried plant material collected for the Biomass Study (above) was also analyzed for metal using the same acid digestion and ICP-MS analysis mentioned above. Because this plant material was not washed prior to metal determination, it does not represent uptake of metals, but rather the level of metals from soil adhering their surfaces. The measure, however, does provide a reasonable estimate of metal exposure of herbivores on LTU plants (Azcue & Mudroch, 1994).

Ecological Function. Classifications of species were made to assess changes throughout succession. The rationale for classifying species was a combination of 1) testing ecological hypotheses, 2) micro-habitat indicators, 3) quality assessment and 4) for use in phytoremediation (Table 4).

Table 4. Characterization of Plant Species.

Rationale	Category	Relevance to Study	Range of Values
Ecological Theory Testing	Lifespan[1]	Ho: Annuals become replaced by perennials.	Annual, Biennial, Perennial
	Successional Sere[1]	Ho: Early succession species replaced by later succession species (Bengtsson, 1998).	Early Pioneer, Later Pioneer, Mid-Succession, Late Succession
	Longevity[1]	Ho: Short lived species replaced by species with longer lifespans.	Short, Moderate, Long, Unknown
	Seasonal Growth[1]	Species that are most active in particular season may dominate during certain monthly counts.	Spring, Summer, Fall
Phyto-remediation	Allelopath[1]	Secondary plant compound link to enhanced degradation of organic contaminants.	Yes, No
	Nitrogen Fixation[1]	Links of nitrogen fixation and enhanced phytoremediation. Range is relative to other species.	None, low, moderate, high
	Vegetative Spread Rate[1]	Practical qualities for establishing at other phytoremediation sites.	
Quality	Invasive[3]	Plants considered invasive by Ohio Department of Natural Resources.	Yes, No
	U.S. Nativity[1]	Native species are desired for ecological restoration.	Native, Assimilated, Non-Native Invasive
	FQAI Rating[2] (Ohio 2004)	Quality rating on a per-species basis that measures fidelity of species to its habitat. High number indicates stable plant community.	0 – 10
	Noxious State Plant[1]	Not a desired quality for ecological restoration.	Yes, No
Source: 1) (USDA, 2002), 3) (ODNR, 2004), 4) (ODNR, 2000)			

Plant Species Specific Characteristics. Similarity between species establishment across the site can also be quantified based on co-occurrences within plots. Similarity between species is determined in the same way as similarity between plots (described in Chapter

3, Table 3.2). This assessment of the vegetative community will further determine if plot similarities may be compounded by plant species co-occurrences. Other species-specific traits include plant density, frequency and distribution. These can be calculated based on the count data collected per plot.

Characterization of the LTU Environment and Soil Nutrients

Total Organic Carbon (Colorimetric). The same sample of soil used for PAH extraction and analysis was extracted for total organic carbon (TOC). TOC was determined according to the method of Heanes (1984) which measures the carbon-induced reduction of Cr^{+6} to Cr^{+3} (Heanes, 1984). Dry soil (50-100 mg) or extract (1 ml) was digested with concentrated H_2SO_4 (1 ml) in the presence of 0.33 N $K_2Cr_2O_7$ for 30 min at 140-150^o C. When cool, samples were diluted with 3.5 ml dH_20, then TOC was measured spectrophotometrically at 600 nm. TOC was quantified using a standard curve prepared from D-glucose. TOC was adjusted for chloride (if chloride is greater than 0.5%) by subtracting 1/12 of the chloride value.

Chloride Determination. Chloride was determined according to the method described by Adriano, et al (Adriano & Doner, 1982). Dry soil (50-100 mg) or extract (1 ml) was reacted with 1 ml each of $Hg(SCN)_2$ and $Fe(NO_3)_3$ for 10 min. Absorbance was measured at 460 nm and chloride quantified based on a standard curve prepared from NaCl.

Carbon, Hydrogen, Nitrogen and C:N. Elemental Analysis was conducted to determine C,H and N (percent by weight) using a Perkin Elmer Series II Elemental Analyzer 2400 with combustion oven at 640ºC and reduction at 925ºC, according to specifications. Oven-dried soil (the same subsample used for PAH, TOC and chloride) was ground to a fine powder, measured to 2mg, and run, calibrated against an acetanilide K-factor. Average of duplicates was reported for all cores, for both upper and lower layers. The ratio of carbon to nitrogen (C:N) was determined on a per sample, then averaged amongst replicates.

Moisture. Soil was collected across the site for analysis of moisture. Triplicate 12cm-deep surface samples were collected (approximately 15g) and bagged in separate sealed containers, weighed, dried in 40°C oven for 3 days and reweighed. The % moisture was calculated from the difference in wet and dry over the initial wet weight. This process was repeated twice per growing season – once in wet conditions and once in dry conditions.

Agricultural Quality Characteristics. Soil samples collected for field soil moisture from summer 2001 and 2002 were combined by plot and sent to an agricultural station for nutritional status of the soil for plant growth. The qualities measured were as follows: pH, % organic matter, phosphorus, potassium, magnesium, calcium, CEC, %K, %Mg, %Ca. A qualitative measure was also reported, indicating whether the level of nutrients was low, medium, good or very high relative to general nutritional needs of plants.

Environmental Parameters. The following edaphic and climatic parameters were measured to assess variation across plots: ambient temperature, soil temperature, light intensity, light exposure. These measurements were taken periodically throughout the growing seasons representing dry conditions and wet conditions to account for the compounding effect of moisture on these parameters.

Temperature was recorded at waist height (1 meter), at ground and 16 cm underground (Atkins Temperature Probe 396K series).

Light intensity was measured in Lux using a light meter (Extech Instruments) and averaged from triplicate readings taken in the plot center at midday. The instrument was held at both waist level and at the ground level to account for variation caused by vegetation interference.

Light exposure (in hours) was calculated using azimuth pathway maps for 40°N based on the projected angle of light interference (e.g. the angle at which the surrounding forest blocks sunlight from the horizon). Angle of light interference was calculated using the tangent of the distance over height of vegetative interference and was calculated for compass directions East, South and West.

$$\text{Angle of Light Interference} = \text{Arctan}(\text{height}/\text{distance}) * (360° / 2\Pi \text{ rad})$$

Whole-site Environmental Assessment. In addition to plot data, in 2002 the entire LTU was divided into a grid and measurements of light intensity, elevation (GPS12, Garmin) and relative humidity (Cold/Heat Hygrometer, Taylor) taken at regularly-spaced locations. These data were used to generate GIS profiles and combined with plot data to map out ecological regions.

Seasonal Data. Temperature and precipitation were obtained from local databases to compare differences between months and years. These values are reported as the average and departure from normal of temperature and precipitation on a monthly basis. For a yearly average of the growing season, only summer months sampled (June through September) were averaged.

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APPENDIX B

RAW DATA

Environmental Variables. Light and moisture values for yearly sampling across LTU. Factor loadings correspond to the factor analyses conducted in Chapter 3. Methodology for all variables outlined in Detailed Methods Appendix.

PLOT	Distance to Edge (m)	Total Sunlight Hours (h)	LUX @ 1m '00	% Moisture '00	LUX @ 1m '01	% Moisture '01	LUX @ 1m '02	% Moisture '02	Factor Loading LUX Axis1	Factor Loading LUX Axis2	Factor Loading % Moist Axis1	Factor Loading % Moist Axis2	Factor Loading Initial Env. Axis1	Factor Loading Initial Env. Axis2	Factor Loading Aug '01 Axis1	Factor Loading Aug '01 Axis2	Factor Loading Aug '02 Axis1	Factor Loading Aug '02 Axis2
1,1	5.0	13.8	1300	19.3	975	26.8	963	13.5	-1.65	-0.45	0.67	-0.61	-1.29	0.79	-0.66	-1.31	-1.17	1.08
1,2	17.0	19.8	1200	21.0	984	24.8	995	14.4	-1.53	1.26	0.61	-0.83	-1.08	1.81	-0.37	-0.51	-0.29	1.43
1,3	5.0	13.8	1300	17.2	997	23.6	991	14.3	-1.15	0.22	-0.56	-1.32	-1.47	-1.51	-1.46	0.29	1.21	1.45
1,4	4.0	16.3	1350	17.8	995	26.4	1012	9.2	-0.78	-0.05	-0.14	0.91	-1.26	0.07	-0.99	-0.77	1.11	-0.34
2,1	10.0	19.5	1500	21.7	1029	23.9	1016	11.2	0.03	-1.28	0.28	0.49	0.27	1.17	-1.30	0.42	-0.50	0.30
2,4	39.0	22.8	1450	20.0	1033	27.1	1008	14.9	-0.18	-0.80	1.04	-1.08	1.05	0.51	-1.03	-0.46	-0.58	0.89
3,1	21.0	21.3	1500	20.1	1017	28.4	1028	15.1	0.06	-1.24	1.40	-1.11	0.95	0.02	0.45	-0.77	-0.19	0.97
3,2	39.0	23.0	1550	21.2	1055	26.3	1028	7.4	0.55	-1.37	0.47	2.06	1.48	0.22	1.12	-0.01	-1.48	-1.59
3,3	26.0	23.5	1400	21.0	1039	26.7	1056	10.9	0.25	0.56	0.81	0.66	0.77	0.36	1.89	-1.22	0.80	-0.53
3,4	13.0	19.8	1350	21.0	1077	24.8	1053	10.6	0.38	1.51	0.29	0.70	0.11	0.24	0.93	0.15	-0.52	-0.90
4,1	10.5	18.0	1400	18.9	1053	25.0	1031	8.9	0.09	0.34	-0.28	1.11	0.03	0.19	0.28	-0.32	-0.31	-0.91
4,2	26.0	23.0	1600	17.1	1088	23.1	1085	11.8	1.62	-0.69	-0.92	-0.37	0.90	-1.37	0.51	1.05	0.97	-0.33
4,3	24.0	21.3	1450	13.8	1113	23.0	1091	10.6	1.43	1.37	-1.80	-0.28	0.33	-1.24	0.60	1.14	1.83	-0.94
4,4	3.0	14.0	1450	16.3	1118	20.2	1037	11.2	0.87	0.62	-1.89	-0.32	-0.79	-1.24	0.02	2.32	-0.88	-0.58

Soil Variables. Methodology outlined in Detailed Methods Appendix.

PLOT	pH '01	pH '02	% OM '01	% OM '02	CEC '01	CEC '02	% K '01	% K '02	% mg '01	% mg '02	% Ca '01	% Ca '02	P '01	P '02	K '01	K '02	Mg '01	Mg '02	Ca '01	Ca '02
1,1	7.4	7.7	5.7	5.5	22.9	17.4	1.3	1.5	11.3	12.2	65.5	86.3	31	23	236	203	621	508	10850	11350
1,2	7.1	7.7	6.3	4.8	20.4	18.2	1.3	1.2	16.1	16.3	73.5	82.5	24	29	214	165	789	711	12416	11613
1,3	7.6	7.8	5.9	5.7	18.5	17.8	2.0	1.9	17.1	14.0	80.9	84.1	30	33	295	259	761	601	15090	12710
1,4	7.6	7.6	6.8	5.0	17.8	17.5	1.6	1.9	14.3	12.2	84.1	86.0	30	25	226	257	612	509	14048	12653
2,1	7.6	7.5	5.1	4.6	17.6	17.4	1.4	1.5	13.5	12.4	85.1	86.1	40	43	196	208	572	518	12337	11326
2,4	7.5	7.4	7.0	4.8	18.7	17.9	2.0	1.6	17.6	14.8	80.4	83.6	24	27	289	223	790	638	14326	11927
3,1	7.6	7.5	5.0	4.8	20.1	18.8	2.0	1.4	23.3	18.9	74.7	79.7	25	24	313	199	1120	855	16775	13593
3,2	7.7	7.4	6.2	4.6	18.2	18.0	2.6	2.0	15.1	14.9	82.4	83.1	25	17	366	282	658	645	15467	13893
3,3	7.0	7.4	5.5	4.9	20.0	17.6	1.1	1.9	10.9	13.0	74.9	85.1	22	19	172	266	525	549	11312	13258
3,4	7.5	7.5	5.3	4.3	17.8	17.9	1.5	1.4	14.2	15.0	84.3	83.6	21	25	209	194	604	645	12755	13078
4,1	7.8	7.5	5.7	4.8	17.4	17.6	1.2	2.0	12.5	12.7	86.4	85.3	19	25	156	270	519	536	12848	13516
4,2	7.8	7.6	5.7	5.1	18.4	18.0	1.3	1.4	17.0	15.0	81.7	83.6	23	26	185	203	748	646	13866	12364
4,3	7.9	7.6	5.1	4.1	18.4	18.1	1.6	1.4	16.9	15.9	81.5	82.7	28	29	230	201	744	693	13205	11923
4,4	7.7	7.9	6.3	5.1	17.8	17.8	1.2	1.2	14.4	14.4	84.4	84.4	31	27	171	164	614	613	12445	13231

Biomass Data. Details are outlined in the Detailed Methods Appendix.

	Biomass (g/m^2)			Percent Cover (per m^2)		
PLOT	2000	2001	2002	2000	2001	2002
1,1	38.9	46.1	193.0	38.4	86.9	100.0
1,2	25.0	36.2	55.2	31.3	38.4	60.6
1,3	24.3	68.0	160.0	33.3	81.8	93.9
1,4	50.8	38.6	172.1	37.4	89.9	80.8
2,1	58.4	53.7	122.9	66.7	69.7	98.0
2,4	23.2	17.3	45.1	36.4	48.5	64.6
3,1	28.9	48.3	77.3	43.4	62.6	73.7
3,2	83.0	75.7	67.4	70.7	92.9	85.9
3,3	49.9	114.0	154.0	62.6	97.0	83.8
3,4	30.8	47.3	120.0	41.4	65.7	65.7
4,1	40.9	66.1	148.4	41.4	69.7	50.5
4,2	18.2	27.3	64.6	31.3	46.5	49.5
4,3	31.8	31.9	214.6	40.4	83.8	84.8
4,4	30.8	26.2	211.2	27.3	79.8	80.8

Average Cover per Species, Plot, Study Year (n = 5 months). Species acronym from USDA PlantsDatabase (www.plants.USDA.gov).

YEAR	PLOT	Total	ACENEG	ACERUB	AGRPER	AGRSTO	AILALT	AMBART	APOCAN	ASTPIL	BIDBIP	BIDFRO	CARNUT	CONCAN	CXSART	CYPSTR	DACGLO	DIPFUL	ECHCRU	EPICOL
2001	1,1	79.33	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.00	0.07	0.67	0.00	0.00	0.00
2001	1,2	39.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.20	0.00	0.00	1.20	0.00
2001	1,4	72.47	0.40	0.27	0.00	0.00	0.07	30.07	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.20	0.00	0.00	0.07	0.00
2001	2,1	65.27	0.60	0.00	0.00	0.00	0.33	0.00	0.00	0.40	0.00	0.00	0.00	6.60	0.00	0.87	0.00	0.67	2.40	0.00
2001	2,4	52.67	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2001	3,2	65.87	0.00	0.00	1.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.40	0.00	0.33	0.00	0.00	0.60	0.00
2001	3,3	87.33	0.40	0.00	0.00	0.00	0.00	0.07	0.00	1.13	0.00	1.33	1.87	17.73	0.00	0.00	0.00	0.00	0.53	0.00
2001	3,4	52.40	0.00	0.00	0.00	0.00	0.47	0.00	0.00	0.00	0.00	0.00	0.00	1.13	0.00	0.33	0.00	0.00	0.13	0.20
2001	4,1	62.93	0.00	0.00	0.00	0.00	0.27	0.00	0.00	0.00	0.00	0.00	0.00	1.93	0.00	0.00	0.00	0.00	0.00	0.00
2001	4,2	48.67	0.00	0.00	0.00	0.00	0.27	0.00	0.00	0.00	0.00	0.00	0.00	0.27	0.00	0.07	0.00	0.67	0.20	0.00
2001	4,3	67.47	0.20	0.00	0.00	0.00	0.53	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.80	0.00	0.00	10.53	0.00
Mean		63.07	0.16	0.02	0.12	0.00	0.19	2.74	0.00	0.14	0.00	0.12	0.17	2.67	0.00	0.44	0.06	0.12	1.42	0.02
Std. Dev.		14.02	0.22	0.08	0.40	0.00	0.20	9.06	0.00	0.35	0.00	0.40	0.56	5.35	0.00	0.66	0.20	0.27	3.11	0.06
2002	1,1	97.93	1.27	0.07	0.00	0.00	0.00	0.00	0.00	5.40	0.00	0.40	0.00	0.20	0.07	0.00	0.93	0.00	0.00	0.00
2002	1,2	60.00	0.00	0.00	0.00	0.00	0.07	0.33	0.00	3.27	0.07	1.07	0.00	0.27	0.00	3.20	0.00	0.07	3.27	0.00
2002	1,4	90.67	0.40	0.13	0.00	0.00	0.00	15.53	0.00	4.73	0.00	0.00	0.00	4.33	0.00	0.07	0.27	0.00	0.00	0.00
2002	2,1	90.40	1.13	0.20	0.00	0.00	0.00	0.00	0.00	14.33	0.00	0.33	0.00	5.27	0.00	0.13	0.00	0.00	0.07	0.07
2002	2,4	44.27	0.00	0.07	0.00	0.00	0.13	0.00	0.00	0.20	0.00	0.40	0.00	0.00	0.00	0.27	0.00	0.00	0.20	0.00
2002	3,2	82.00	0.33	0.20	0.80	0.00	0.07	0.00	0.00	0.00	0.00	0.00	0.07	0.93	0.00	0.00	0.00	0.00	3.60	0.00
2002	3,3	93.87	0.27	0.00	0.00	0.00	0.13	0.00	0.40	3.60	0.00	0.47	0.07	0.07	0.07	0.00	0.00	0.07	0.13	0.00
2002	3,4	82.20	0.07	0.00	0.07	0.00	0.07	0.00	0.00	0.53	0.00	0.00	0.07	0.87	0.00	0.00	0.00	0.13	0.20	0.00
2002	4,1	95.87	1.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.47	0.00	0.00	0.00	0.00	0.00	0.00
2002	4,2	56.40	0.00	0.07	0.00	0.00	0.27	0.00	0.00	1.13	0.00	0.13	0.00	0.40	0.00	0.00	0.00	0.07	0.27	0.00
2002	4,3	75.27	0.80	0.00	1.40	1.13	0.07	0.00	0.00	0.20	0.00	0.00	0.07	0.20	0.00	0.00	0.00	0.00	1.00	0.00
Mean		78.99	0.51	0.07	0.21	0.10	0.07	1.44	0.04	3.04	0.01	0.25	0.02	1.36	0.01	0.33	0.11	0.03	0.79	0.01
Std. Dev.		18.00	0.53	0.08	0.46	0.34	0.08	4.67	0.12	4.25	0.02	0.33	0.03	1.85	0.03	0.95	0.28	0.05	1.34	0.02

Average Cover Per Species Per Plot Within Study Year (n = 5 months)…continued.

YEAR	PLOT	ERIANN	EUPHMA	EUPRUG	EUPSER	EUTGRA	FESELA	FRAAME	FRAVES	JUNVIR	LACBIE	LACSER	LOLPER	LONJAP	LONMAA	MELOFF	MENSPP	MORALB
2001	1,1	0.00	0.00	0.07	0.00	3.87	33.93	0.00	0.00	0.00	0.00	0.00	15.60	20.27	0.00	0.00	0.00	0.00
2001	1,2	0.27	0.00	0.00	0.00	0.07	7.13	0.00	0.00	0.00	0.00	0.07	27.33	0.00	0.00	0.00	0.00	0.00
2001	1,4	0.20	0.00	0.00	0.00	1.33	6.74	0.20	0.00	0.00	0.00	0.53	23.00	0.00	0.07	2.13	0.00	0.00
2001	2,1	0.87	0.07	0.47	0.00	4.73	0.67	0.27	0.00	0.00	0.00	0.33	34.80	0.93	0.47	0.00	0.00	0.00
2001	2,4	0.00	0.00	0.00	0.00	0.00	1.80	0.07	0.00	0.00	0.00	0.00	49.94	0.00	0.20	0.00	0.00	0.00
2001	3,2	0.00	0.13	0.13	0.00	0.00	3.93	0.00	0.00	0.00	0.00	0.00	56.00	0.00	0.60	0.00	0.00	0.00
2001	3,3	0.00	0.20	0.13	0.13	2.40	6.67	0.00	0.00	0.00	0.00	0.33	34.53	0.33	0.00	0.00	0.00	0.00
2001	3,4	0.00	0.00	0.07	0.00	0.07	4.20	0.00	0.00	0.00	0.00	0.13	31.07	0.00	0.53	0.13	0.00	0.00
2001	4,1	0.13	0.00	0.53	1.33	0.27	1.73	0.27	0.07	0.00	0.00	0.00	26.20	0.00	0.20	1.07	0.00	0.00
2001	4,2	0.00	0.00	0.07	0.00	0.00	0.40	0.00	0.00	0.00	0.00	0.00	38.40	0.00	0.60	0.00	0.00	0.00
2001	4,3	0.20	0.00	0.00	0.00	0.33	0.80	0.33	0.00	0.00	0.00	0.00	29.87	0.00	0.07	10.53	0.00	0.00
Mean		0.15	0.04	0.13	0.13	1.19	6.18	0.10	0.01	0.00	0.00	0.13	33.34	1.96	0.25	1.26	0.00	0.00
Std. Dev.		0.26	0.07	0.19	0.40	1.72	9.55	0.13	0.02	0.00	0.00	0.19	11.59	6.08	0.25	3.15	0.00	0.00
2002	1,1	0.00	0.00	0.13	0.07	1.80	24.47	0.60	0.00	0.00	0.00	0.00	4.60	45.20	0.07	0.00	0.00	0.00
2002	1,2	0.00	0.00	0.13	0.07	0.00	12.20	6.20	0.00	0.00	0.00	0.20	24.53	0.00	0.33	0.00	0.00	0.00
2002	1,4	9.40	0.00	0.13	0.00	0.33	8.73	2.14	0.00	0.00	0.00	0.00	16.87	1.67	1.80	2.67	0.00	0.00
2002	2,1	5.93	0.00	0.93	0.33	0.60	0.07	1.47	0.00	0.00	0.00	0.07	21.13	5.80	0.93	0.00	0.27	0.00
2002	2,4	0.07	0.00	0.00	0.00	0.00	3.60	1.40	0.00	0.07	0.00	0.13	31.93	0.00	0.60	0.00	0.00	0.00
2002	3,2	0.07	0.13	0.13	0.07	0.00	8.34	0.20	0.00	0.13	0.00	0.00	27.67	0.00	2.73	0.00	0.00	0.00
2002	3,3	0.13	0.00	0.07	1.00	0.20	4.20	0.93	0.00	0.00	0.00	0.33	12.60	0.00	1.47	0.00	0.00	0.00
2002	3,4	0.33	0.00	0.00	0.00	0.53	11.67	0.47	0.00	0.00	0.13	0.00	26.33	0.00	1.33	3.53	0.20	0.00
2002	4,1	0.07	0.00	0.80	0.53	0.93	2.47	0.67	0.00	0.00	0.00	0.00	13.93	0.47	1.33	0.33	0.00	0.00
2002	4,2	0.00	0.00	0.00	0.00	0.00	4.27	1.33	0.00	0.00	0.00	0.67	27.47	0.00	1.33	0.00	0.00	0.00
2002	4,3	0.00	0.00	0.20	0.00	3.53	5.20	0.80	0.00	0.00	0.00	0.07	22.53	0.00	1.07	17.07	0.00	0.07
Mean		1.45	0.01	0.23	0.19	0.72	7.75	1.47	0.00	0.02	0.01	0.13	20.87	4.83	1.18	2.15	0.04	0.01
Std. Dev.		3.17	0.04	0.32	0.32	1.08	6.72	1.66	0.00	0.04	0.04	0.21	8.10	13.50	0.73	5.10	0.10	0.02

Average Cover Per Species Per Plot Within Study Year (n = 5 months)…continued.

YEAR	PLOT	OENBIE	PARQUI	PLAOCC	PLGCON	PLGPER	POPDEL	PRUSER	ROBPSE	RUDHIR	SCHSCO	SETGLA	SLXEXI	SOLCAN	SPOVAG	TAROFF	TOXRAD	ULMAME
2001	1,1	0.00	0.00	1.13	0.00	0.00	0.07	0.00	0.00	0.00	0.00	0.00	0.00	2.67	0.00	0.00	0.00	0.67
2001	1,2	0.00	0.00	0.13	0.00	0.00	0.27	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.00	0.00	0.33
2001	1,4	0.00	0.07	0.13	0.00	0.00	0.07	0.00	0.00	0.00	0.00	0.00	0.00	6.33	0.00	0.20	0.00	0.20
2001	2,1	0.00	0.33	1.73	0.00	0.00	0.27	0.00	0.00	0.00	0.00	0.20	1.07	5.40	0.00	0.00	0.00	0.20
2001	2,4	0.00	0.00	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.33
2001	3,2	0.53	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.00	0.00	0.53	0.00	0.00	0.00	0.13
2001	3,3	3.93	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.00	14.40	0.00	0.13	0.00	0.53
2001	3,4	0.00	0.00	0.87	0.00	0.00	0.13	0.00	0.07	0.00	0.00	0.00	0.00	12.07	0.00	0.33	0.00	0.40
2001	4,1	0.53	0.00	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	28.07	0.00	0.20	0.00	0.07
2001	4,2	0.07	0.00	0.27	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.93	0.00	6.00	0.00	0.00	0.00	0.20
2001	4,3	0.00	0.27	0.20	0.00	0.00	0.20	0.00	0.00	0.00	0.00	0.07	0.00	12.27	0.00	0.13	0.00	0.13
Mean		0.46	0.06	0.42	0.00	0.00	0.12	0.01	0.01	0.00	0.00	0.11	0.10	8.02	0.00	0.09	0.00	0.29
Std. Dev.		1.17	0.12	0.57	0.00	0.00	0.10	0.02	0.02	0.00	0.00	0.28	0.32	8.35	0.00	0.12	0.00	0.18
2002	1,1	0.00	0.07	1.80	0.00	0.00	0.47	0.00	0.00	0.00	0.00	0.00	0.40	6.93	0.00	0.07	0.00	1.27
2002	1,2	0.00	0.07	1.07	0.00	0.20	1.47	0.00	0.00	0.00	0.27	0.00	0.00	1.33	0.00	0.07	0.00	0.26
2002	1,4	0.00	0.00	0.33	0.00	0.07	0.00	0.00	0.07	0.00	0.00	0.00	0.00	20.20	0.00	0.27	0.00	0.47
2002	2,1	0.20	0.33	1.93	0.00	0.00	0.20	0.13	0.00	0.00	0.33	0.00	7.53	17.60	0.00	0.20	0.00	0.74
2002	2,4	0.00	0.87	0.73	0.00	0.00	0.07	0.00	0.00	0.00	0.27	0.00	0.00	2.87	0.00	0.00	0.00	0.27
2002	3,2	4.53	0.13	0.40	0.00	0.00	0.00	0.00	0.00	0.00	5.40	0.00	0.00	16.27	9.20	0.00	0.00	0.53
2002	3,3	1.60	0.27	0.00	0.00	0.00	0.13	0.00	0.00	0.13	0.40	0.00	0.00	63.93	0.00	0.20	0.07	0.67
2002	3,4	0.40	0.07	0.33	0.00	0.07	0.20	0.07	0.00	0.00	0.07	0.00	0.13	32.93	0.00	0.33	0.00	0.87
2002	4,1	1.40	0.00	0.07	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	67.20	0.00	0.07	0.00	0.60
2002	4,2	0.47	0.20	0.33	0.00	0.00	0.33	0.00	0.00	0.00	0.07	0.20	0.00	16.80	0.00	0.46	0.00	0.13
2002	4,3	0.53	0.13	0.33	0.07	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	18.07	0.00	0.00	0.00	0.40
Mean		0.83	0.19	0.67	0.01	0.05	0.26	0.02	0.01	0.01	0.62	0.02	0.73	24.01	0.84	0.15	0.01	0.56
Std. Dev.		1.35	0.25	0.66	0.03	0.08	0.43	0.04	0.02	0.04	1.59	0.06	2.26	22.35	2.77	0.15	0.02	0.32

Average Cover Per Species Per Plot Within Study Year (n = 5 months)…continued.

YEAR	PLOT	VERGIG	VINMIN	VITVUL	VULBRO	UNKBUR	UNKGR1	UNKGR2
2001	1,1	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2001	1,2	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2001	1,4	0.00	0.00	0.07	0.00	0.00	0.00	0.00
2001	2,1	0.27	0.00	0.33	0.00	0.00	0.00	0.00
2001	2,4	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2001	3,2	0.00	0.00	0.13	0.00	0.00	0.00	0.00
2001	3,3	0.00	0.13	0.20	0.00	0.00	0.00	0.00
2001	3,4	0.07	0.00	0.00	0.00	0.00	0.00	0.00
2001	4,1	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2001	4,2	0.00	0.00	0.13	0.00	0.00	0.00	0.00
2001	4,3	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean		0.03	0.01	0.08	0.00	0.00	0.00	0.00
Std. Dev.		0.08	0.04	0.11	0.00	0.00	0.00	0.00
2002	1,1	0.80	0.00	0.47	0.00	0.00	0.40	0.00
2002	1,2	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2002	1,4	0.07	0.00	0.00	0.00	0.00	0.00	0.00
2002	2,1	1.27	0.00	0.33	0.13	0.00	0.00	0.40
2002	2,4	0.00	0.00	0.13	0.00	0.00	0.00	0.00
2002	3,2	0.00	0.00	0.07	0.00	0.00	0.00	0.00
2002	3,3	0.00	0.07	0.20	0.00	0.00	0.00	0.00
2002	3,4	0.20	0.00	0.00	0.00	0.00	0.00	0.00
2002	4,1	0.40	0.00	0.13	0.33	0.27	0.00	0.00
2002	4,2	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2002	4,3	0.13	0.00	0.00	0.00	0.00	0.00	0.00
Mean		0.26	0.01	0.12	0.04	0.02	0.04	0.04
Std. Dev.		0.41	0.02	0.16	0.10	0.08	0.12	0.12

Sum of Number of Individuals by Plot within Study Year.

YEAR	PLOT	ACENEG	ACERUB	AGRPER	AGRSTO	AILALT	AMBART	APOCAN	ASTPIL	BIDBIP	BIDFRO	CARNUT	CONCAN	CXSART	CYPSTR	DACGLO	DIPFUL
2000	1,1	3	2	0	0	4	0	0	0	0	0	0	4	0	0	4	0
2000	1,2	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0
2000	1,4	2	3	0	0	12	152	0	0	0	0	0	10	0	0	0	0
2000	2,1	2	4	0	0	0	0	0	0	0	0	0	3	0	0	0	0
2000	2,4	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
2000	3,2	2	0	131	0	2	0	0	0	0	0	0	1	0	0	0	0
2000	3,3	1	0	0	0	2	0	0	0	0	0	0	33	0	0	0	0
2000	3,4	0	1	0	0	5	0	0	1	0	0	0	1	0	0	0	0
2000	4,1	0	0	0	0	5	0	0	0	0	0	0	8	0	0	0	0
2000	4,2	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0
2000	4,3	1	0	0	0	14	0	0	0	0	0	0	1	0	0	0	0
Total 2000		11	10	131	0	53	152	0	1	0	0	0	61	0	0	4	0
2001	1,1	1	3	0	0	0	0	0	0	0	0	0	6	0	2	119	0
2001	1,2	0	0	0	0	1	0	0	0	0	0	0	1	0	31	0	0
2001	1,4	1	3	0	0	4	174	0	0	0	0	0	15	0	10	0	0
2001	2,1	5	0	0	0	5	0	0	1	0	0	0	124	0	55	0	4
2001	2,4	0	1	0	0	4	0	0	0	0	0	0	0	0	0	0	0
2001	3,2	0	1	1400	0	0	0	0	0	0	0	0	73	0	13	0	0
2001	3,3	2	0	0	0	0	3	0	3	0	2	6	471	0	0	0	0
2001	3,4	0	0	0	0	13	0	0	0	0	0	0	31	0	8	10	0
2001	4,1	0	0	0	0	6	0	0	0	0	0	0	46	0	0	0	0
2001	4,2	0	0	0	0	8	0	0	0	0	0	0	22	0	1	0	1
2001	4,3	1	1	0	0	10	0	0	0	0	0	0	0	0	10	0	0
Total 2001		10	9	1400	0	51	177	0	4	0	2	6	789	0	130	129	5
2002	1,1	2	1	0	0	0	0	0	28	0	6	0	4	3	0	182	0
2002	1,2	0	0	0	0	1	4	0	10	1	21	0	7	0	252	0	1
2002	1,4	1	2	0	0	0	839	0	15	0	0	0	205	0	1	52	0
2002	2,1	5	2	0	0	8	0	0	410	0	4	0	501	0	6	0	0
2002	2,4	0	1	0	0	2	0	0	2	0	3	0	0	0	18	0	0
2002	3,2	1	2	840	0	1	0	0	0	0	0	1	40	0	0	0	0
2002	3,3	1	0	0	0	1	0	2	39	0	24	1	2	12	0	0	3
2002	3,4	1	0	70	0	2	0	0	4	0	0	1	38	0	0	0	1
2002	4,1	2	0	0	0	0	0	0	0	0	0	0	123	0	0	0	0
2002	4,2	0	1	0	0	3	0	0	4	0	1	0	11	0	0	0	2
2002	4,3	2	0	1470	1190	1	0	0	1	0	0	1	16	0	0	0	0
Total 2002		15	9	2380	1190	19	843	2	513	1	59	4	947	15	277	234	7

Sum of Number of Individuals and Richness Per Plot within Study Year…continued.

YEAR	PLOT	ECHCRU	EPICOL	ERIANN	EUPHMA	EUPRUG	EUPSER	EUTGRA	FESELA	FRAAME	FRAVES	JUNVIR	LACBIE	LACSER
2000	1,1	6	0	26	0	0	0	0	877	4	0	0	0	0
2000	1,2	5	0	0	0	0	0	0	164	3	0	0	0	0
2000	1,4	0	0	13	0	0	0	0	249	5	0	0	0	1
2000	2,1	18	0	23	0	11	0	0	122	4	0	0	0	0
2000	2,4	1	0	0	0	0	0	0	0	0	0	0	0	0
2000	3,2	56	0	6	17	0	0	0	64	0	0	0	0	3
2000	3,3	20	0	13	2	1	0	0	193	1	0	0	0	1
2000	3,4	0	0	7	0	0	0	0	98	0	0	0	0	6
2000	4,1	1	0	21	0	1	0	0	395	2	0	0	0	0
2000	4,2	7	0	1	0	0	0	0	4	0	0	0	0	0
2000	4,3	224	0	2	0	10	0	0	109	0	0	0	0	0
Total 2000		338	0	112	19	23	0	0	2275	19	0	0	0	11
2001	1,1	0	0	0	0	2	0	26	6617	0	0	0	0	0
2001	1,2	47	0	4	0	0	0	1	1393	0	0	0	0	0
2001	1,4	13	0	1	0	1	0	5	1316	3	0	0	0	0
2001	2,1	346	0	8	1	13	0	17	130	2	1	0	0	7
2001	2,4	0	0	0	0	0	0	0	351	1	0	0	0	0
2001	3,2	74	0	0	5	2	0	0	768	0	0	0	0	0
2001	3,3	23	0	0	1	6	1	17	1308	0	0	0	0	5
2001	3,4	1	1	0	0	1	0	1	836	0	0	0	0	5
2001	4,1	0	0	2	0	7	4	2	348	1	1	0	0	10
2001	4,2	2	0	0	0	5	0	0	82	0	0	0	0	4
2001	4,3	1247	0	3	0	0	0	1	166	5	0	0	0	1
Total 2001		1753	1	18	7	37	5	70	13315	12	2	0	0	32
2002	1,1	0	0	0	0	2	1	8	4771	13	0	0	0	0
2002	1,2	524	0	0	0	2	1	0	2379	487	0	0	0	3
2002	1,4	0	0	271	0	4	0	2	1703	53	0	0	0	0
2002	2,1	1	0	179	0	22	2	4	13	31	0	0	0	3
2002	2,4	19	0	1	0	0	0	0	670	31	0	1	0	1
2002	3,2	543	0	1	2	2	1	0	1625	3	0	1	0	0
2002	3,3	15	0	2	0	1	5	10	819	5	0	0	0	5
2002	3,4	36	0	4	0	0	0	4	2275	5	0	0	1	0
2002	4,1	0	0	1	0	14	3	6	481	3	0	0	0	0
2002	4,2	21	0	0	0	0	0	0	832	35	0	0	0	14
2002	4,3	80	0	0	0	1	0	28	1014	6	0	0	0	1
Total 2002		1239	0	459	2	48	13	62	16582	672	0	2	1	27

Sum of Number of Individuals and Richness Per Plot within Study Year…continued.

YEAR	PLOT	LOLPER	LONJAP	LONMAA	MELOFF	MENSPP	MORALB	OENBIE	PARQUI	PLAOCC	PLGCON	PLGPER	POPDEL	PRUSER
2000	1,1	27237	23	0	2	0	0	0	2	69	0	9	2	0
2000	1,2	17246	3	0	1	0	0	0	1	11	0	0	0	0
2000	1,4	23182	22	0	0	0	0	0	0	7	0	1	0	0
2000	2,1	60754	5	0	0	0	0	0	0	29	0	0	1	0
2000	2,4	30397	1	0	0	0	0	0	0	0	0	0	0	0
2000	3,2	51327	3	0	0	0	0	0	0	2	0	0	0	0
2000	3,3	54694	1	0	3	0	0	1	0	3	0	0	1	0
2000	3,4	28556	7	0	1	0	0	0	0	6	0	1	0	0
2000	4,1	28141	7	0	12	0	0	0	0	2	0	12	0	0
2000	4,2	23876	2	0	0	0	0	0	0	1	0	24	0	0
2000	4,3	33127	4	0	0	0	0	0	0	1	0	0	0	0
Total 2000		378537	78	0	19	0	0	1	3	131	0	47	4	0
2001	1,1	18252	21	0	0	0	0	0	0	34	0	11	6	0
2001	1,2	31980	0	1	0	0	0	0	0	8	0	11	16	0
2001	1,4	26910	0	5	15	0	0	0	1	4	0	0	2	0
2001	2,1	40716	7	4	0	0	0	0	2	42	0	5	8	0
2001	2,4	58422	0	4	0	0	0	0	0	2	0	4	0	0
2001	3,2	65520	0	8	0	0	0	5	0	3	0	0	0	1
2001	3,3	40404	2	2	0	0	0	17	0	1	0	0	2	0
2001	3,4	36348	0	5	2	0	0	1	1	20	0	1	3	0
2001	4,1	30654	0	6	15	0	0	7	0	1	0	0	0	0
2001	4,2	44928	0	12	0	0	0	1	0	3	1	0	2	0
2001	4,3	34944	0	6	86	0	0	0	6	2	0	0	4	0
Total 2001		429078	30	53	118	0	0	31	10	120	1	32	43	1
2002	1,1	5382	17	1	0	0	0	0	1	17	0	0	3	0
2002	1,2	28704	0	10	0	0	0	0	1	55	0	8	40	0
2002	1,4	19734	4	17	62	0	0	0	0	5	0	1	0	0
2002	2,1	24726	12	14	0	6	0	2	5	37	0	0	3	3
2002	2,4	37362	0	18	0	0	0	0	24	29	0	0	1	0
2002	3,2	32370	0	5	0	0	0	37	2	12	0	0	0	0
2002	3,3	14742	0	6	0	0	0	15	3	0	0	0	1	0
2002	3,4	30810	0	11	3	21	0	2	1	9	0	1	2	1
2002	4,1	16302	2	5	12	0	0	25	0	3	1	0	0	0
2002	4,2	32136	0	7	0	0	0	6	2	21	0	0	4	0
2002	4,3	26364	0	10	72	0	1	58	2	18	1	2	0	0
Total 2002		268632	35	104	149	27	1	145	41	206	2	12	54	4

Sum of Number of Individuals and Richness Per Plot within Study Year…continued.

YEAR	PLOT	ROBPSE	RUDHIR	SCHSCO	SETGLA	SLXEXI	SOLCAN	SPOVAG	TAROFF	TOXRAD	ULMAME	VERGIG	VINMIN	VITVUL
2000	1,1	0	0	0	0	0	51	0	0	0	24	0	0	1
2000	1,2	0	0	0	0	0	0	0	0	0	2	0	0	0
2000	1,4	0	0	0	0	0	107	0	0	0	4	0	0	0
2000	2,1	0	0	0	0	0	16	0	0	0	7	0	0	1
2000	2,4	0	0	0	0	0	0	0	0	0	1	0	0	0
2000	3,2	0	0	0	0	0	14	0	0	0	2	0	0	2
2000	3,3	0	0	0	0	0	79	0	1	0	3	0	0	3
2000	3,4	0	0	0	0	0	70	0	0	0	4	0	0	0
2000	4,1	0	0	0	0	0	374	0	1	0	2	0	0	1
2000	4,2	0	0	0	0	0	0	0	0	0	5	0	0	2
2000	4,3	0	0	0	0	0	13	0	0	0	3	0	0	2
Total 2000		0	0	0	0	0	724	0	2	0	57	0	0	12
2001	1,1	0	0	0	0	0	72	0	0	0	25	0	0	1
2001	1,2	0	0	0	0	0	6	0	0	0	20	0	0	0
2001	1,4	0	0	0	0	0	148	0	6	0	7	0	0	4
2001	2,1	0	0	0	12	5	124	0	1	0	18	1	0	6
2001	2,4	0	0	0	0	0	4	0	0	0	11	0	0	0
2001	3,2	0	0	0	0	0	20	0	0	0	5	0	0	4
2001	3,3	0	1	0	0	0	341	0	3	0	6	0	3	2
2001	3,4	1	2	0	0	0	207	0	41	0	13	2	0	1
2001	4,1	0	0	0	0	0	605	0	4	0	2	0	0	0
2001	4,2	0	0	0	56	0	147	0	0	0	4	0	0	3
2001	4,3	0	0	0	4	0	193	0	52	0	6	0	0	0
Total 2001		1	3	0	72	5	1867	0	107	0	117	3	3	21
2002	1,1	0	0	0	0	4	839	0	1	0	11	4	0	5
2002	1,2	0	0	24	0	0	137	0	1	0	7	0	0	0
2002	1,4	1	0	0	0	0	1686	0	3	0	6	1	0	0
2002	2,1	0	0	32	0	29	1951	0	3	0	12	5	0	2
2002	2,4	0	0	21	0	0	431	0	0	0	8	0	0	2
2002	3,2	0	0	482	0	0	419	10764	0	0	5	0	0	1
2002	3,3	0	2	63	0	0	1492	0	3	1	6	0	1	3
2002	3,4	0	0	3	0	2	1859	0	3	0	12	1	0	0
2002	4,1	0	0	0	0	0	1802	0	1	0	5	3	0	2
2002	4,2	0	0	1	12	0	792	0	6	0	3	0	0	0
2002	4,3	0	0	0	0	0	381	0	0	0	6	1	0	0
Total 2002		1	2	626	12	35	11789	10764	21	1	81	15	1	15

Sum of Number of Individuals and Richness Per Plot within Study Year…continued.

YEAR	PLOT	VULBRO	UNKBUR	UNKGR1	UNKGR2	Richness	β Month
2000	1,1	31	0	0	0	20	2.0
2000	1,2	0	0	0	0	10	2.0
2000	1,4	5	0	0	0	16	1.6
2000	2,1	666	0	0	0	16	1.8
2000	2,4	566	0	0	0	6	2.5
2000	3,2	0	0	0	0	15	2.0
2000	3,3	0	0	0	0	20	2.3
2000	3,4	0	0	0	0	14	1.9
2000	4,1	0	0	0	0	16	2.1
2000	4,2	15	0	0	0	11	2.5
2000	4,3	41	0	0	0	14	1.9
Total 2000		1324	0	0	0	Total Species = 28	
2001	1,1	0	0	0	0	16	1.6
2001	1,2	0	0	0	0	14	1.7
2001	1,4	0	0	0	0	22	1.7
2001	2,1	0	0	0	0	29	1.8
2001	2,4	0	0	0	0	10	1.8
2001	3,2	0	0	0	0	16	1.7
2001	3,3	0	0	0	0	25	2.0
2001	3,4	0	0	0	0	25	2.0
2001	4,1	0	0	0	0	18	1.8
2001	4,2	0	0	0	0	18	2.0
2001	4,3	0	0	0	0	20	1.7
Total 2001		0	0	0	0	Total Species = 44	
2002	1,1	0	0	384	0	25	1.7
2002	1,2	0	0	0	0	24	1.6
2002	1,4	0	0	0	0	23	1.6
2002	2,1	140	0	0	492	33	1.6
2002	2,4	0	0	0	0	20	1.8
2002	3,2	0	0	0	0	24	2.0
2002	3,3	0	0	0	0	30	2.2
2002	3,4	0	0	0	0	29	2.4
2002	4,1	350	4	0	0	22	2.2
2002	4,2	0	0	0	0	21	1.8
2002	4,3	0	0	0	0	25	2.0
Total 2002		490	4	384	492	Total Species = 57	

Core Sampling Times and Geographical Characteristics. Details in Methods Appendix.

PLOT	upper layer ID	lowerlayerID	Year	months	QUAD	elev(m)	Plot Northing	Plot Easting	Core y-coord (m, - Northing)	Core x-coord (m, + Easting)	Core northing (y)	core easting (x)
1,1	1	85	0	0	1	206.3	693092	4340036	0.6	0.6	693091.39	4340036.6
1,2	2	86	0	0	1	205.9	693104	4340063	0.6	0.6	693103.39	4340063.6
1,3	3	87	0	0	1	206.0	693134	4340087	0.6	0.6	693133.39	4340087.6
1,4	4	88	0	0	1	206.2	693136	4340067	0.6	0.6	693135.39	4340067.6
2,1	5	89	0	0	2	205.9	693096	4340128	0.6	0.6	693095.39	4340128.6
2,4	6	90	0	0	2	205.7	693125	4340132	0.6	0.6	693124.39	4340132.6
3,1	7	91	0	0	3	205.6	693158	4340136	0.6	0.6	693157.39	4340136.6
3,2	8	92	0	0	3	205.9	693139	4340162	0.6	0.6	693138.39	4340162.6
3,3	9	93	0	0	3	205.9	693156	4340175	0.6	0.6	693155.39	4340175.6
3,4	10	94	0	0	3	205.9	693174	4340128	0.6	0.6	693173.39	4340128.6
4,1	11	95	0	0	4	205.7	693205	4340117	0.6	0.6	693204.39	4340117.6
4,2	12	96	0	0	4	205.3	693228	4340122	0.6	0.6	693227.39	4340122.6
4,3	13	97	0	0	4	205.3	693263	4340111	0.6	0.6	693262.39	4340111.6
4,4	14	98	0	0	4	205.9	693282	4340081	0.6	0.6	693281.39	4340081.6
1,1	15	99	0	6	1	206.3	693092	4340036	1.2	1.2	693090.78	4340037.2
1,2	16	100	0	6	1	205.9	693104	4340063	1.2	1.2	693102.78	4340064.2
1,3	17	101	0	6	1	206.0	693134	4340087	1.2	1.2	693132.78	4340088.2
1,4	18	102	0	6	1	206.2	693136	4340067	1.2	1.2	693134.78	4340068.2
2,1	19	103	0	6	2	205.9	693096	4340128	1.2	1.2	693094.78	4340129.2
2,4	20	104	0	6	2	205.7	693125	4340132	1.2	1.2	693123.78	4340133.2
3,1	21	105	0	6	3	205.6	693158	4340136	1.2	1.2	693156.78	4340137.2
3,2	22	106	0	6	3	205.9	693139	4340162	1.2	1.2	693137.78	4340163.2
3,3	23	107	0	6	3	205.9	693156	4340175	1.2	1.2	693154.78	4340176.2
3,4	24	108	0	6	3	205.9	693174	4340128	1.2	1.2	693172.78	4340129.2
4,1	25	109	0	6	4	205.7	693205	4340117	1.2	1.2	693203.78	4340118.2
4,2	26	110	0	6	4	205.3	693228	4340122	1.2	1.2	693226.78	4340123.2
4,3	27	111	0	6	4	205.3	693263	4340111	1.2	1.2	693261.78	4340112.2
4,4	28	112	0	6	4	205.9	693282	4340081	1.2	1.2	693280.78	4340082.2
1,1	29	113	1	18	1	206.3	693092	4340036	4.6	3.1	693087.43	4340039.1
1,2	30	114	1	18	1	205.9	693104	4340063	4.6	3.1	693099.43	4340066.1
1,3	31	115	1	18	1	206.0	693134	4340087	1.5	3.1	693132.48	4340090.1
1,4	32	116	1	18	1	206.2	693136	4340067	1.5	3.1	693134.48	4340070.1
2,1	33	117	1	18	2	205.9	693096	4340128	4.6	3.1	693091.43	4340131.1
2,4	34	118	1	18	2	205.7	693125	4340132	1.5	3.1	693123.48	4340135.1
3,1	35	119	1	18	3	205.6	693158	4340136	3.1	1.5	693154.95	4340137.5
3,2	36	120	1	18	3	205.9	693139	4340162	3.1	4.6	693135.95	4340166.6
3,3	37	121	1	18	3	205.9	693156	4340175	3.1	4.6	693152.95	4340179.6
3,4	38	122	1	18	3	205.9	693174	4340128	3.1	1.5	693170.95	4340129.5
4,1	39	123	1	18	4	205.7	693205	4340117	3.1	1.5	693201.95	4340118.5
4,2	40	124	1	18	4	205.3	693228	4340122	3.1	4.6	693224.95	4340126.6
4,3	41	125	1	18	4	205.3	693263	4340111	3.1	4.6	693259.95	4340115.6
4,4	42	126	1	18	4	205.9	693282	4340081	3.1	1.5	693278.95	4340082.5

Core Sampling Times and Geographical Characteristics…continued.

PLOT	upper layer ID	lowerlayerID	Year	months	QUAD	elev(m)	Plot Northing	Plot Easting	Core y-coord (m, - Northing)	Core x-coord (m, + Easting)	Core northing (y)	core easting (x)
1,1	43	127	1	18	1	206.3	693092	4340036	1.5	3.1	693090.48	4340039.1
1,2	44	128	1	18	1	205.9	693104	4340063	1.5	3.1	693102.48	4340066.1
1,3	45	129	1	18	1	206.0	693134	4340087	4.6	3.1	693129.43	4340090.1
1,4	46	130	1	18	1	206.2	693136	4340067	4.6	3.1	693131.43	4340070.1
2,1	47	131	1	18	2	205.9	693096	4340128	1.5	3.1	693094.48	4340131.1
2,4	48	132	1	18	2	205.7	693125	4340132	4.6	3.1	693120.43	4340135.1
3,1	49	133	1	18	3	205.6	693158	4340136	3.1	4.6	693154.95	4340140.6
3,2	50	134	1	18	3	205.9	693139	4340162	3.1	1.5	693135.95	4340163.5
3,3	51	135	1	18	3	205.9	693156	4340175	3.1	1.5	693152.95	4340176.5
3,4	52	136	1	18	3	205.9	693174	4340128	3.1	4.6	693170.95	4340132.6
4,1	53	137	1	18	4	205.7	693205	4340117	3.1	4.6	693201.95	4340121.6
4,2	54	138	1	18	4	205.3	693228	4340122	3.1	1.5	693224.95	4340123.5
4,3	55	139	1	18	4	205.3	693263	4340111	3.1	1.5	693259.95	4340112.5
4,4	56	140	1	18	4	205.9	693282	4340081	3.1	4.6	693278.95	4340085.6
1,1	57	141	2	30	1	206.3	693092	4340036	5.2	3.1	693086.82	4340039.1
1,2	58	142	2	30	1	205.9	693104	4340063	5.2	3.1	693098.82	4340066.1
1,3	59	143	2	30	1	206.0	693134	4340087	0.9	3.1	693133.09	4340090.1
1,4	60	144	2	30	1	206.2	693136	4340067	0.9	3.1	693135.09	4340070.1
2,1	61	145	2	30	2	205.9	693096	4340128	5.2	3.1	693090.82	4340131.1
2,4	62	146	2	30	2	205.7	693125	4340132	0.9	3.1	693124.09	4340135.1
3,1	63	147	2	30	3	205.6	693158	4340136	3.1	0.9	693154.95	4340136.9
3,2	64	148	2	30	3	205.9	693139	4340162	3.1	5.2	693135.95	4340167.2
3,3	65	149	2	30	3	205.9	693156	4340175	3.1	5.2	693152.95	4340180.2
3,4	66	150	2	30	3	205.9	693174	4340128	3.1	0.9	693170.95	4340128.9
4,1	67	151	2	30	4	205.7	693205	4340117	3.1	0.9	693201.95	4340117.9
4,2	68	152	2	30	4	205.3	693228	4340122	3.1	0.9	693224.95	4340122.9
4,3	69	153	2	30	4	205.3	693263	4340111	3.1	0.9	693259.95	4340111.9
4,4	70	154	2	30	4	205.9	693282	4340081	3.1	0.9	693278.95	4340081.9
1,1	71	155	2	30	1	206.3	693092	4340036	0.9	3.1	693091.09	4340039.1
1,2	72	156	2	30	1	205.9	693104	4340063	0.9	3.1	693103.09	4340066.1
1,3	73	157	2	30	1	206.0	693134	4340087	5.2	3.1	693128.82	4340090.1
1,4	74	158	2	30	1	206.2	693136	4340067	5.2	3.1	693130.82	4340070.1
2,1	75	159	2	30	2	205.9	693096	4340128	0.9	3.1	693095.09	4340131.1
2,4	76	160	2	30	2	205.7	693125	4340132	5.2	3.1	693119.82	4340135.1
3,1	77	161	2	30	3	205.6	693158	4340136	3.1	5.2	693154.95	4340141.2
3,2	78	162	2	30	3	205.9	693139	4340162	3.1	0.9	693135.95	4340162.9
3,3	79	163	2	30	3	205.9	693156	4340175	3.1	0.9	693152.95	4340175.9
3,4	80	164	2	30	3	205.9	693174	4340128	3.1	5.2	693170.95	4340133.2
4,1	81	165	2	30	4	205.7	693205	4340117	3.1	5.2	693201.95	4340122.2
4,2	82	166	2	30	4	205.3	693228	4340122	3.1	5.2	693224.95	4340127.2
4,3	83	167	2	30	4	205.3	693263	4340111	3.1	5.2	693259.95	4340116.2
4,4	84	168	2	30	4	205.9	693282	4340081	3.1	5.2	693278.95	4340086.2

Core Physical Characteristics. Details in Methods Appendix.

PLOT	Upper Layer ID	Lower Layer ID	% Moisture Upper	% Moisture Lower	Total Mass Upper (gDW)	Total Mass Lower (gDW)	% Core Compaction	Top depth	Black Depth	Total Lower	Total Depth to Clay	Densely Rooted Depth	Sparsely Rooted Depth	Either Rooting Depth
1,1	1	85	0.19	0.13	138.3	18.9	0.15	25.79	3.22	3.22	29.01	0.00	21.49	21.49
1,2	2	86	0.21	0.17	122.5	46.3	0.05	28.90	8.43	8.43	37.33	0.00	36.13	36.13
1,3	3	87	0.17	0.14	148.4	69.3	0.10	30.81	4.56	5.71	36.52	0.00	18.26	18.26
1,4	4	88	0.18	0.14	145.6	46.3	0.05	32.73	8.49	8.49	41.22	16.97	38.79	38.79
2,1	5	89	0.22	0.20	105.5	11.6	0.12	21.16	2.23	4.45	25.61	0.00	21.16	21.16
2,4	6	90	0.20	0.14	139.2	72.5	0.09	31.35	13.93	13.93	45.28	4.64	25.55	25.55
3,1	7	91	0.20	0.19	133.0	97.6	0.00	30.48	20.32	20.32	50.80	0.00	26.67	26.67
3,2	8	92	0.21	0.15	129.5	64.0	0.06	29.99	11.99	11.99	41.98	15.59	22.79	22.79
3,3	9	93	0.21	0.18	131.8	63.1	0.10	31.00	12.63	12.63	43.63	4.59	27.56	27.56
3,4	10	94	0.21	0.17	121.9	53.5	0.15	22.60	7.53	7.53	30.13	0.00	21.52	21.52
4,1	11	95	0.19	0.15	130.3	28.1	0.07	29.51	5.90	5.90	35.42	29.51	0.00	29.51
4,2	12	96	0.17	0.17	113.2	5.4	0.08	25.61	1.16	1.16	26.78	3.49	25.61	25.61
4,3	13	97	0.14	0.19	112.3	26.6	0.04	24.28	7.28	7.28	31.56	9.71	23.07	23.07
4,4	14	98	0.16	0.17	132.9	12.2	0.06	29.91	2.39	2.39	32.30	11.96	25.12	25.12
1,1	15	99	0.21	0.19	131.4	41.3	0.06	30.98	7.75	7.75	38.73	16.68	30.98	30.98
1,2	16	100	0.18	0.17	159.1	8.6	0.02	37.49	3.12	3.12	40.61	37.49	0.00	37.49
1,3	17	101	0.18	0.17	86.7	36.8	0.07	23.73	7.71	7.71	31.45	23.73	30.85	30.85
1,4	18	102	0.17	0.16	131.0	60.4	0.12	33.50	5.58	5.58	39.09	33.50	39.09	39.09
2,1	19	103	0.20	0.19	97.2	47.5	0.06	21.43	7.14	7.14	28.58	0.00	21.43	21.43
2,4	20	104	0.23	0.15	137.2	100.0	0.09	28.75	17.82	17.82	46.57	23.00	0.00	23.00
3,1	21	105	0.21	0.19	56.4	187.9	0.18	11.52	33.50	33.50	45.02	11.52	45.02	45.02
3,2	22	106	0.23	0.19	122.0	88.4	-0.01	29.41	20.46	20.46	49.86	5.11	29.41	29.41
3,3	23	107	0.22	0.19	150.9	65.7	0.03	36.81	15.95	15.95	52.77	17.18	0.00	17.18
3,4	24	108	0.20	0.17	123.0	51.5	0.03	28.42	11.12	11.12	39.54	28.42	0.00	28.42
4,1	25	109	0.19	0.20	114.4	30.8	0.08	26.84	5.84	5.84	32.68	19.84	32.68	32.68
4,2	26	110	0.19	0.16	112.5	7.3	0.13	20.96	1.10	1.10	22.06	4.41	20.96	20.96
4,3	27	111	0.14	0.16	21.6	26.7	0.26	6.58	2.82	2.82	9.39	0.00	5.64	5.64
4,4	28	112	0.18	0.17	119.1	27.5	0.08	23.31	5.83	5.83	29.14	3.50	15.15	15.15
1,1	29	113	0.24	0.17	142.3	54.9	0.03	33.34	8.64	8.64	41.98	19.76	33.34	33.34
1,2	30	114	0.22	0.17	140.4	27.5	0.05	30.21	4.23	4.23	34.44	14.50	30.21	30.21
1,3	31	115	0.22	0.19	79.6	55.9	0.15	17.27	9.72	9.72	26.99	10.80	17.27	17.27
1,4	32	116	0.23	0.16	130.5	35.5	0.13	29.82	5.52	5.52	35.34	29.82	0.00	29.82
2,1	33	117	0.15	0.17	152.2	21.2	0.05	29.46	4.81	4.81	34.28	16.84	29.46	29.46
2,4	34	118	0.24	0.18	131.4	95.2	0.09	31.35	17.42	17.42	48.77	31.35	0.00	31.35
3,1	35	119	0.23	0.17	154.7	107.4	0.04	30.58	20.80	20.80	51.38	30.58	0.00	30.58
3,2	36	120	0.24	0.20	149.4	62.3	0.04	36.66	14.66	14.66	51.32	0.00	17.11	17.11
3,3	37	121	0.23	0.25	135.7	3.3	0.16	25.48	0.53	0.53	26.01	25.48	0.00	25.48
3,4	38	122	0.24	0.19	133.3	57.6	0.19	24.82	7.24	7.24	32.06	0.00	24.82	24.82
4,1	39	123	0.21	0.15	179.9	7.4	0.05	38.44	1.80	1.80	40.25	0.00	24.03	24.03
4,2	40	124	0.22	0.20	111.0	39.7	0.06	24.95	7.72	7.72	32.67	7.13	24.95	24.95
4,3	41	125	0.21	0.22	36.9	67.4	0.10	9.16	1.14	12.59	21.75	0.00	10.30	10.30
4,4	42	126	0.21	0.18	138.3	16.9	0.00	33.02	3.81	3.81	36.83	0.00	0.00	0.00

Core Physical Characteristics...continued.

PLOT	upper layer ID	lowerlayerID	% Moisture Upper	% Moisture Lower	Total Mass Upper (gDW)	Total Mass Lower (gDW)	% Core Compaction	Top depth	Black Depth	Total Lower	Total Depth to Clay	Densely Rooted Depth	Sparsely Rooted Depth	Either Rooting Depth
1,1	43	127	0.22	0.18	127.3	50.6	0.04	30.37	4.86	4.86	35.23	0.00	30.37	30.37
1,2	44	128	0.22	0.21	146.0	46.4	0.03	29.51	9.22	9.22	38.74	17.22	29.51	29.51
1,3	45	129	0.22	0.17	134.3	46.2	0.05	37.37	8.44	8.44	45.81	0.00	37.37	37.37
1,4	46	130	0.22	0.21	113.9	22.3	0.09	28.88	2.89	2.89	31.77	28.88	0.00	28.88
2,1	47	131	0.22	0.18	183.6	49.1	0.01	41.30	7.51	7.51	48.81	0.00	41.30	41.30
2,4	48	132	0.22	0.16	151.3	82.3	0.09	32.27	16.13	16.13	48.40	18.44	32.27	32.27
3,1	49	133	0.22	0.15	133.5	42.9	0.05	26.54	7.24	7.24	33.78	0.00	9.65	9.65
3,2	50	134	0.22	0.21	137.6	70.1	0.00	33.02	15.24	15.24	48.26	0.00	33.02	33.02
3,3	51	135	0.22	0.18	158.3	49.5	0.14	28.30	8.71	8.71	37.01	0.00	28.30	28.30
3,4	52	136	0.22	0.17	161.2	73.1	-0.02	38.94	15.58	15.58	54.52	0.00	20.77	20.77
4,1	53	137	0.22	0.16	120.4	94.8	0.06	26.27	19.11	19.11	45.38	0.00	26.27	26.27
4,2	54	138	0.22	0.22	144.7	9.0	0.00	27.94	1.27	1.27	29.21	0.00	27.94	27.94
4,3	55	139	0.22	0.19	150.6	7.7	0.09	26.67	1.16	1.16	27.83	2.32	0.00	2.32
4,4	56	140	0.22	0.18	127.8	25.2	0.00	27.31	4.45	4.45	31.75	0.00	25.40	25.40
1,1	57	141	0.24	0.24	83.3	68.4	0.03	20.98	14.81	14.81	35.79	35.79	49.37	49.37
1,2	58	142	0.24	0.18	81.6	76.6	0.20	15.24	13.21	13.21	28.45	28.45	0.00	28.45
1,3	59	143	0.26	0.18	111.0	38.0	0.15	21.53	6.46	6.46	27.98	16.14	21.53	21.53
1,4	60	144	0.29	NA	148.7	NA	0.06	42.82	0.00	0.00	42.82	42.82	45.20	45.20
2,1	61	145	0.19	0.19	147.7	57.8	0.03	31.88	8.58	8.58	40.46	20.85	0.00	20.85
2,4	62	146	0.26	0.16	164.2	52.3	0.07	35.26	10.58	10.58	45.83	25.85	35.26	35.26
3,1	63	147	0.26	0.15	119.6	94.9	0.00	34.93	18.42	18.42	53.34	0.00	10.16	10.16
3,2	64	148	0.27	0.17	135.1	64.9	0.09	31.12	11.53	11.53	42.65	25.36	32.28	32.28
3,3	65	149	0.25	0.22	137.3	8.9	0.09	31.31	1.16	1.16	32.47	31.31	40.58	40.58
3,4	66	150	0.25	0.21	131.1	20.8	0.06	33.44	2.39	2.39	35.83	26.27	33.44	33.44
4,1	67	151	0.22	0.17	124.2	22.6	0.10	27.43	3.43	3.43	30.86	30.86	0.00	30.86
4,2	68	152	0.21	0.19	104.5	39.3	0.26	20.67	5.64	5.64	26.30	18.79	22.55	22.55
4,3	69	153	0.20	0.18	118.9	25.0	0.07	25.94	4.72	4.72	30.66	16.51	30.66	30.66
4,4	70	154	0.18	0.18	122.2	18.2	0.23	26.45	1.96	1.96	28.41	0.00	19.59	19.59
1,1	71	155	0.19	0.27	145.4	35.6	0.03	28.38	8.64	8.64	37.02	28.38	37.02	37.02
1,2	72	156	0.23	0.16	132.7	29.5	0.01	30.24	5.04	5.04	35.28	20.16	35.28	35.28
1,3	73	157	0.25	0.18	89.1	52.6	0.10	21.67	11.40	11.40	33.07	0.00	21.67	21.67
1,4	74	158	0.24	0.17	135.6	18.1	0.16	29.71	2.12	2.12	31.84	14.86	29.71	29.71
2,1	75	159	0.24	0.19	132.1	34.9	0.07	33.12	3.55	3.55	36.67	36.67	0.00	36.67
2,4	76	160	0.25	0.19	131.0	56.6	0.05	33.81	12.08	12.08	45.89	26.57	0.00	26.57
3,1	77	161	0.24	0.18	91.8	55.2	0.13	21.98	9.89	9.89	31.87	0.00	17.58	17.58
3,2	78	162	0.25	0.20	185.4	96.4	-0.10	54.57	8.39	8.39	62.96	39.18	54.57	54.57
3,3	79	163	0.25	0.22	136.5	33.5	0.17	33.67	3.16	3.16	36.83	33.67	0.00	33.67
3,4	80	164	0.21	0.15	157.0	14.8	-0.03	37.88	13.06	13.06	50.95	24.82	50.95	50.95
4,1	81	165	0.21	0.17	108.3	88.7	0.11	32.74	11.29	11.29	44.03	31.61	0.00	31.61
4,2	82	166	0.24	0.22	155.2	5.6	-0.03	39.24	1.31	1.31	40.55	5.23	39.24	39.24
4,3	83	167	0.21	NA	118.9	NA	0.09	25.55	0.00	0.00	25.55	25.55	32.51	32.51
4,4	84	168	0.20	0.17	120.5	18.9	-0.11	39.57	2.83	2.83	42.40	14.13	56.54	56.54

Core Contamination Characteristics: PAHs Part 1. Values listed in ug/g of dry weight soil.

UC PLOT	YEAR	MONTH	Core Number	LAYER	TREATMENT	WT PAHSAM	TPH	TOTAL PAH	ACENAPHTHY	FLUORINE	PHENANTHRE	ANTHRACENE	PYRENE	BENZAANTHR
1,1	0	0	1	U	V	3.29	22811.1	87.69	0.00	0.38	0.00	0.00	0.00	0.00
1,2	0	0	2	U	V	3.36	7847.1	39.41	1.64	0.00	0.79	0.46	2.70	3.79
1,3	0	0	3	U	C	3.48	9681.0	108.39	0.00	0.00	0.00	0.00	3.51	0.00
1,4	0	0	4	U	V	3.41	9682.1	114.61	1.52	5.13	10.95	0.00	8.43	8.50
2,1	0	0	5	U	V	3.21	8985.9	124.71	0.00	8.74	2.16	0.00	5.61	0.00
2,4	0	0	6	U	V	3.17	44977.0	201.02	0.00	14.69	0.00	0.00	48.57	97.43
3,1	0	0	7	U	C	3.29	6810.5	64.60	0.12	0.00	2.30	1.11	2.78	13.12
3,2	0	0	8	U	V	3.20	12319.5	121.65	4.62	4.35	6.19	7.06	0.00	11.24
3,3	0	0	9	U	V	3.21	5368.3	23.42	1.45	0.00	0.00	0.98	0.00	5.59
3,4	0	0	10	U	V	3.23	7312.9	90.50	1.77	25.28	1.91	0.00	0.00	0.00
4,1	0	0	11	U	V	3.35	12800.0	165.50	0.00	22.31	7.18	0.00	4.12	8.67
4,2	0	0	12	U	V	3.32	9898.2	77.54	0.00	0.00	0.60	0.00	0.00	15.50
4,3	0	0	13	U	V	3.50	7697.7	50.76	0.00	0.00	7.10	1.44	8.35	19.47
4,4	0	0	14	U	C	3.39	13451.2	166.22	7.76	23.52	3.70	0.00	10.60	32.28
1,1	0	6	15	U	V	3.15	10957.8	132.80	0.00	0.00	2.22	3.69	20.32	29.29
1,2	0	6	16	U	V	3.31	9260.3	121.94	1.64	0.00	3.27	2.83	6.12	16.08
1,3	0	6	17	U	C	3.30	4021.0	71.80	0.68	1.34	0.67	4.66	6.92	5.74
1,4	0	6	18	U	V	3.32	5031.0	102.32	4.60	0.19	0.00	0.86	6.48	23.93
2,1	0	6	19	U	V	3.35	2898.2	73.41	4.17	1.42	2.42	1.38	0.81	0.11
2,4	0	6	20	U	V	3.07	9048.5	159.24	0.00	0.00	4.01	8.45	3.77	43.05
3,1	0	6	21	U	C	3.25	8067.2	82.74	0.57	0.00	1.66	1.91	21.11	33.99
3,2	0	6	22	U	V	3.11	4514.7	67.03	0.62	0.88	0.88	7.61	3.45	0.00
3,3	0	6	23	U	V	3.13	3849.4	125.10	0.00	1.13	0.00	14.37	0.00	0.00
3,4	0	6	24	U	V	3.19	3944.4	56.36	1.47	0.68	0.65	9.42	0.00	4.11
4,1	0	6	25	U	V	3.27	5765.1	67.27	0.00	0.00	12.72	0.95	5.55	6.12
4,2	0	6	26	U	V	3.27	6501.0	140.38	0.48	0.00	11.18	0.98	2.42	45.67
4,3	0	6	27	U	V	3.53	4956.9	105.31	1.39	0.43	1.24	2.49	2.60	10.10
4,4	0	6	28	U	C	3.29	11700.9	238.47	3.26	1.22	1.12	2.28	4.93	0.00
1,1	2	30	57	U	V	3.10	1602.8	10.66	1.42	0.00	1.07	2.48	1.41	1.80
1,2	2	30	58	U	V	3.08	1550.7	10.82	1.46	0.61	0.00	1.71	1.91	0.41
1,3	2	30	59	U	C	3.00	3041.4	60.00	0.00	0.00	0.00	7.50	9.63	8.02
1,4	2	30	60	U	V	3.13	1104.4	46.46	1.58	0.63	0.05	0.34	0.00	8.72
2,1	2	30	61	U	V	3.29	4651.4	63.33	1.05	0.00	0.00	0.00	7.78	0.00
2,4	2	30	62	U	V	2.69	1344.5	33.91	3.89	0.89	2.52	1.29	0.89	1.34
3,1	2	30	63	U	C	3.10	1154.5	21.28	2.20	0.04	2.14	0.19	0.56	0.00
3,2	2	30	64	U	V	2.95	1882.4	99.79	13.61	10.05	20.29	0.14	17.64	19.44
3,3	2	30	65	U	V	3.00	2947.2	39.54	1.85	3.49	1.74	0.67	4.89	0.90
3,4	2	30	66	U	V	3.09	3310.1	62.33	1.82	4.30	1.44	1.72	1.06	6.22
4,1	2	30	67	U	V	3.13	1180.1	18.89	0.54	0.46	0.80	0.92	0.51	0.09
4,2	2	30	68	U	V	3.22	3508.2	73.45	8.35	3.66	8.91	25.76	0.00	4.76
4,3	2	30	69	U	V	3.20	4241.7	40.66	3.75	0.00	5.48	6.31	0.42	0.76
4,4	2	30	70	U	C	3.27	786.1	18.76	0.00	0.00	0.00	0.00	2.32	0.00
1,1	2	30	71	U	V	3.08	6275.8	48.02	0.00	3.07	0.00	11.10	11.53	5.28

UC PLOT	YEAR	MONTH	Core Number	LAYER	TREATMENT	WT PAHSAM	TPH	TOTAL PAH	ACENAPHTHY	FLUORINE	PHENANTHRE	ANTHRACENE	PYRENE	BENZAANTHR
1,2	2	30	72	U	V	1.74	2673.5	93.38	2.02	1.84	0.63	0.74	1.25	3.11
1,3	2	30	73	U	C	3.04	1828.6	11.55	0.16	0.00	0.00	1.41	2.63	1.62
1,4	2	30	74	U	V	3.07	1213.4	74.46	21.27	10.28	1.97	4.36	0.69	1.20
2,1	2	30	75	U	V	3.10	3580.9	181.22	0.00	5.89	8.68	7.90	11.86	0.00
2,4	2	30	76	U	V	3.00	1450.9	38.39	0.00	0.00	1.88	0.00	12.71	0.00
3,1	2	30	77	U	C	3.00	1360.8	16.52	2.33	0.55	0.00	2.45	1.49	1.51
3,2	2	30	78	U	V	3.34	37562.0	115.17	12.89	0.20	0.00	4.33	2.87	51.60
3,3	2	30	79	U	V	3.02	1357.5	51.18	1.88	1.20	3.99	0.68	0.53	0.60
3,4	2	30	80	U	V	3.18	3212.8	50.61	0.13	0.00	4.19	0.00	6.36	2.23
4,1	2	30	81	U	V	3.16	1352.9	16.19	2.83	0.58	0.00	0.00	1.39	2.23
4,2	2	30	82	U	V	1.82	17298.2	108.68	0.64	1.32	3.76	9.11	15.66	3.26
4,3	2	30	83	U	V	3.18	3722.6	127.83	0.39	32.89	45.44	11.04	1.19	4.98
4,4	2	30	84	U	C	3.27	901.8	20.94	0.10	0.60	0.28	0.13	1.82	0.00
1,1	0	0	85	L	V	0.92	144028.1	2111.84	0.00	370.73	0.00	0.00	183.50	0.00
1,2	0	0	86	L	V	3.52	49149.7	359.61	2.30	26.49	7.76	0.00	13.37	0.00
1,3	0	0	87	L	C	3.51	9467.5	74.08	2.36	13.88	1.40	0.00	4.05	8.92
1,4	0	0	88	L	V	3.56	44182.4	465.76	4.11	9.00	5.03	0.00	63.86	25.87
2,1	0	0	89	L	V	0.89	161317.4	1293.71	9.57	31.62	19.21	0.00	46.75	102.56
2,4	0	0	90	L	V	3.55	15052.8	266.86	0.00	21.36	0.00	54.06	26.02	0.00
3,1	0	0	91	L	C	3.46	23674.3	85.71	0.00	10.74	0.00	0.00	4.97	0.00
3,2	0	0	92	L	V	3.57	85856.6	430.93	6.68	19.60	0.00	0.00	171.53	0.00
3,3	0	0	93	L	V	3.34	20351.2	140.51	0.00	19.24	5.24	0.00	9.43	1.08
3,4	0	0	94	L	V	3.43	5016.5	61.31	0.00	18.10	0.00	0.00	3.33	0.00
4,1	0	0	95	L	V	1.79	61362.8	3577.60	8.84	60.55	201.99	9.04	369.49	943.13
4,2	0	0	96	L	V									
4,3	0	0	97	L	V	1.78	99086.7	1053.97	3.74	218.04	52.79	0.00	0.00	485.41
4,4	0	0	98	L	C	0.95	621304.7	1884.47	13.49	153.26	175.89	41.19	145.62	196.20
1,1	0	6	99	L	V	3.31	13659.2	287.65	1.04	0.14	2.20	1.17	1.05	125.10
1,2	0	6	100	L	V	2.73	12662.1	205.03	5.10	2.40	0.34	2.83	0.04	89.52
1,3	0	6	101	L	C	3.42	6500.7	99.27	1.33	0.92	0.74	0.63	2.15	14.58
1,4	0	6	102	L	V	3.43	10044.8	96.94	3.55	0.52	9.32	1.97	5.99	28.48
2,1	0	6	103	L	V	3.20	7407.1	227.14	0.82	0.09	0.00	1.25	2.36	51.88
2,4	0	6	104	L	V	3.37	31681.0	509.63	0.92	1.05	0.00	23.42	35.06	149.08
3,1	0	6	105	L	C	3.25	18954.9	333.89	1.32	1.95	1.28	8.52	61.78	28.92
3,2	0	6	106	L	V	3.27	60652.3	1302.18	0.73	8.57	0.74	3.80	94.56	661.17
3,3	0	6	107	L	V	3.21	30385.3	691.82	1.64	4.18	1.06	3.59	30.86	295.71
3,4	0	6	108	L	V	3.42	13843.1	148.94	7.11	2.48	2.81	0.50	10.49	7.34
4,1	0	6	109	L	V	3.44	13285.3	196.43	5.01	2.82	1.94	1.04	9.23	65.03
4,2	0	6	110	L	V	1.45	5639.7	184.82	15.22	0.12	0.98	1.85	4.90	13.04
4,3	0	6	111	L	V	3.69	6736.7	458.06	6.89	3.48	15.24	10.06	10.88	374.41
4,4	0	6	112	L	C	3.46	6844.0	178.57	0.93	0.25	0.20	0.96	1.32	10.51
1,1	2	30	141	L	V	3.13	12366.1	244.94	2.21	0.50	1.19	30.54	16.73	83.88
1,2	2	30	142	L	V	3.3	43959.0	699.69	1.30	8.07	17.97	24.59	236.99	187.76
1,3	2	30	143	L	C	3.43	56497.2	374.89	0.28	7.76	8.92	3.89	25.55	29.12

UC PLOT	YEAR	MONTH	Core Number	LAYER	TREATMENT	WT PAHSAM	TPH	TOTAL PAH	ACENAPHTHY	FLUORINE	PHENANTHRE	ANTHRACENE	PYRENE	BENZAANTHR
2,1	2	30	145	L	V	3.41	5861.8	81.08	0.49	0.37	0.43	0.81	7.06	2.59
2,4	2	30	146	L	V	3.51	79968.2	918.56	1.41	27.71	71.39	73.84	251.93	26.06
3,1	2	30	147	L	C	3.38	21097.2	273.29	1.11	7.56	24.80	27.08	73.09	45.04
3,2	2	30	148	L	V	3.36	29343.8	579.23	0.47	16.94	27.71	8.01	142.53	38.03
3,3	2	30	149	L	V	1.75	8928.2	44.17	2.32	1.01	0.54	1.07	3.09	5.26
3,4	2	30	150	L	V	3.36	14064.3	164.30	1.11	1.25	5.26	4.78	50.38	17.12
4,1	2	30	151	L	V	3.43	37322.4	280.96	0.57	9.44	22.91	4.85	29.92	31.20
4,2	2	30	152	L	V	3.37	22022.3	221.26	1.12	2.80	3.83	3.78	1.23	30.43
4,3	2	30	153	L	V	3.38	11158.1	96.34	0.37	1.72	4.39	0.52	17.93	0.73
4,4	2	30	154	L	C	3.37	25986.6	242.23	0.14	1.91	0.69	4.29	38.82	16.16
1,1	2	30	155	L	V	3.34	38863.3	411.27	0.00	6.97	7.68	2.85	21.70	0.78
1,2	2	30	156	L	V	3.4	17648.9	220.39	0.97	1.32	0.00	5.17	29.41	20.96
1,3	2	30	157	L	C	3.39	21113.8	222.12	2.18	0.19	5.80	3.10	74.08	6.31
1,4	2	30	158	L	V	3.4	12875.9	89.59	1.26	2.92	6.20	0.08	25.02	1.62
2,1	2	30	159	L	V	3.31	65193.3	412.18	0.00	58.64	23.31	0.00	41.82	16.69
2,4	2	30	160	L	V	3.28	17777.3	271.09	0.37	4.46	3.70	5.61	46.39	18.07
3,1	2	30	161	L	C	3.35	119254.9	1697.05	0.00	93.31	75.34	0.00	279.55	89.84
3,2	2	30	162	L	V	2.94	15517.0	263.78	4.58	0.97	0.00	0.00	10.25	35.34
3,3	2	30	163	L	V	3.27	21985.6	231.53	4.01	9.60	10.70	3.70	105.33	5.92
3,4	2	30	164	L	V	3.54	18537.9	300.92	0.19	2.90	6.40	14.35	53.52	33.40
4,1	2	30	165	L	V	3.45	98475.7	1779.98	11.57	37.67	153.39	102.51	1083.19	29.48
4,2	2	30	166	L	V	3.12	3239.7	344.19	6.37	0.55	15.51	8.74	10.55	25.03
4,4	2	30	168	L	C	3.51	15123.0	105.46	1.81	1.07	2.78	5.37	37.87	10.34

Core Contamination Characteristics: PAHs part 2. Values listed in ug/g dry weight soil.

PLOT	YEAR	MONTH	Core Number	LAYER	TREATMENT	CHRYSENE	BENZOBFLUO	BENZOKFLUO	BENZOAPYRE	DIBENZAHAN	INDENO123C	BENZOGHIPE	ORGC	C	H	N
1,1	0	0	1	U	V	4.87	36.50	1.34	1.34	30.03	3.57	9.65	0.20	10.30	1.29	0.39
1,2	0	0	2	U	V	0.00	7.95	0.92	8.27	6.98	2.70	3.21	0.23	11.15	1.25	0.34
1,3	0	0	3	U	C	1.45	18.11	4.55	80.77	0.00	0.00	0.00	0.16	11.51	1.23	0.34
1,4	0	0	4	U	V	16.48	11.13	14.19	10.74	16.37	9.55	1.63	0.24	11.37	1.18	0.32
2,1	0	0	5	U	V	79.20	4.17	0.00	11.71	3.03	6.57	3.53	0.23	10.50	1.15	0.26
2,4	0	0	6	U	V	0.00	5.82	4.56	29.97	0.00	0.00	0.00	0.23	12.24	1.17	0.49
3,1	0	0	7	U	C	0.00	6.33	1.24	22.98	1.11	6.88	6.63	0.16	10.93	1.43	2.00
3,2	0	0	8	U	V	5.86	16.07	5.39	12.67	21.15	12.46	14.59	0.19	11.98	1.12	0.57
3,3	0	0	9	U	V	1.47	5.85	1.29	4.35	0.00	1.74	0.72	0.21	10.77	0.87	0.60
3,4	0	0	10	U	V	31.56	2.62	0.00	3.75	0.00	3.95	19.67	0.25	8.62	0.52	0.60
4,1	0	0	11	U	V	57.57	11.22	3.70	8.94	24.12	3.09	14.58	0.19	11.00	1.08	0.36
4,2	0	0	12	U	V	0.00	43.79	4.44	9.45	0.80	0.21	2.75	0.19	9.44	1.05	0.29
4,3	0	0	13	U	V	0.31	0.00	4.83	1.26	2.97	0.27	4.75	0.14	7.70	0.99	0.32
4,4	0	0	14	U	C	0.00	11.46	0.00	23.25	52.03	1.61	0.00	0.17	9.58	1.04	0.40
1,1	0	6	15	U	V	19.40	15.22	6.44	13.23	19.79	1.70	1.51	0.19	9.75	1.07	0.34
1,2	0	6	16	U	V	30.50	14.84	4.33	9.23	23.22	8.02	1.86	0.19	10.58	1.18	0.36
1,3	0	6	17	U	C	4.52	4.60	0.73	8.30	20.90	2.96	9.78	0.20	12.02	1.42	0.44
1,4	0	6	18	U	V	2.35	2.15	1.99	0.00	35.86	8.23	15.69	0.21	10.94	1.27	0.44
2,1	0	6	19	U	V	13.83	0.41	0.34	18.28	16.07	0.45	13.73	0.17	10.29	1.02	0.45
2,4	0	6	20	U	V	33.78	14.41	4.08	9.96	27.19	8.30	2.24	0.18	12.68	1.49	0.41
3,1	0	6	21	U	C	1.78	5.98	1.08	2.22	5.28	6.89	0.28	0.23	10.84	1.20	0.38
3,2	0	6	22	U	V	2.49	6.17	1.60	7.47	8.72	1.21	25.94	0.23	11.32	1.32	0.46
3,3	0	6	23	U	V	20.31	0.00	1.55	11.44	17.97	18.51	39.83	0.21	10.74	1.29	0.42
3,4	0	6	24	U	V	0.33	0.00	1.13	8.31	7.51	1.83	20.91	0.22	8.75	1.08	0.38
4,1	0	6	25	U	V	15.62	13.46	3.82	4.96	0.00	0.00	4.08	0.17	10.52	1.17	0.36
4,2	0	6	26	U	V	1.73	25.91	8.58	36.05	6.02	0.00	1.38	0.15	11.36	1.27	0.38
4,3	0	6	27	U	V	0.88	20.83	0.52	47.24	0.04	16.06	1.48	0.18	10.38	1.08	0.45
4,4	0	6	28	U	C	6.19	198.98	6.17	3.97	6.76	0.00	3.59	0.14	10.13	1.23	0.35
1,1	2	30	57	U	V	1.09	0.00	0.11	1.30	0.00	0.00	0.00	0.22	10.25	1.30	0.30
1,2	2	30	58	U	V	2.23	0.00	0.00	2.12	0.00	0.00	0.37	0.21	10.53	1.31	0.25
1,3	2	30	59	U	C	0.00	0.00	0.00	0.68	20.89	3.22	10.06	0.17	11.73	1.06	0.45
1,4	2	30	60	U	V	1.92	0.62	0.90	21.56	2.37	0.29	7.46	0.20	10.76	0.89	0.36
2,1	2	30	61	U	V	6.61	1.35	2.76	3.94	11.08	8.56	20.22	0.18	7.39	0.76	0.37
2,4	2	30	62	U	V	1.22	1.84	4.29	6.47	3.43	3.56	2.28	0.14	12.82	1.15	0.44
3,1	2	30	63	U	C	4.27	4.09	1.09	3.90	1.30	1.51	0.00	0.16	9.67	0.98	0.36
3,2	2	30	64	U	V	3.04	5.57	3.06	0.93	5.51	0.44	0.09	0.23	10.44	0.90	0.44
3,3	2	30	65	U	V	2.54	9.13	2.15	4.77	3.46	1.09	2.86	0.20	10.01	0.94	0.40
3,4	2	30	66	U	V	0.08	6.71	4.52	12.66	10.73	3.65	7.43	0.15	8.71	0.84	0.35
4,1	2	30	67	U	V	3.86	2.96	0.09	1.97	3.16	1.90	1.62	0.23	10.29	0.84	0.40
4,2	2	30	68	U	V	9.65	0.31	0.00	0.72	4.41	6.34	0.58	0.24	10.55	1.02	0.43
4,3	2	30	69	U	V	0.00	6.56	1.18	11.06	2.81	0.94	1.39	0.20	9.18	0.97	0.42
4,4	2	30	70	U	C	4.81	0.00	0.00	2.00	1.42	0.25	7.96	0.15	10.48	1.00	0.38
1,1	2	30	71	U	V	5.74	0.88	1.98	6.77	1.66	0.00	0.00	0.21	7.97	0.92	0.31
1,2	2	30	72	U	V	37.61	3.44	0.87	8.88	2.96	12.00	18.04	0.15	9.52	0.90	0.28

PLOT	YEAR	MONTH	Core Number	LAYER	TREATMENT	CHRYSENE	BENZOBFLUO	BENZOKFLUO	BENZOAPYRE	DIBENZAHAN	INDENO123C	BENZOGHIPE	ORGC	C	H	N
1,3	2	30	73	U	C	0.23	0.00	0.00	4.30	0.00	1.20	0.00	0.24	12.15	1.37	0.35
1,4	2	30	74	U	V	4.40	4.33	1.37	5.08	10.79	2.57	6.16	0.18	11.72	0.97	0.44
2,1	2	30	75	U	V	31.18	19.19	5.47	11.53	55.39	21.12	3.01	0.25	10.64	1.26	0.31
2,4	2	30	76	U	V	3.85	1.88	1.59	12.19	3.74	0.53	0.00	0.20	11.87	0.97	0.48
3,1	2	30	77	U	C	1.33	3.04	0.44	3.38	0.00	0.00	0.00	0.23	10.17	0.93	0.36
3,2	2	30	78	U	V	26.13	0.18	0.24	4.99	3.09	2.90	5.75	0.16	12.06	1.48	0.39
3,3	2	30	79	U	V	6.74	7.53	3.99	4.62	2.23	0.61	16.59	0.19	10.32	1.08	0.46
3,4	2	30	80	U	V	10.79	2.07	1.23	10.99	0.00	0.00	12.63	0.24	10.22	0.91	0.40
4,1	2	30	81	U	V	2.62	3.45	0.00	0.00	0.99	0.99	1.12	0.26	10.95	1.22	0.33
4,2	2	30	82	U	V	29.08	14.89	1.32	5.38	6.01	4.82	13.42	0.20	12.69	1.66	0.33
4,3	2	30	83	U	V	4.84	2.37	0.00	4.62	11.03	6.10	2.95	0.20	7.70	0.77	0.33
4,4	2	30	84	U	C	4.09	0.41	0.17	7.05	0.81	1.21	4.26	0.20	10.25	0.97	0.34
1,1	0	0	85	L	V	63.44	99.02	1266.19	58.06	10.28	0.00	60.63	0.09	3.51	0.38	0.35
1,2	0	0	86	L	V	177.33	61.29	22.26	41.75	0.00	1.41	5.65	0.19	3.24	0.64	0.18
1,3	0	0	87	L	C	27.18	7.05	1.86	7.39	0.00	0.00	0.00	0.14	9.73	0.61	0.20
1,4	0	0	88	L	V	175.03	41.45	18.50	44.61	72.76	5.54	0.00	0.19	6.77	1.03	0.22
2,1	0	0	89	L	V	392.05	258.57	49.92	198.51	0.00	0.00	184.97	0.20	13.63	1.38	0.29
2,4	0	0	90	L	V	97.98	15.82	4.46	14.78	24.00	0.00	8.38	0.22	8.03	0.91	0.34
3,1	0	0	91	L	C	0.00	0.00	4.14	18.52	25.76	21.59	0.00	0.18	7.21	0.89	0.36
3,2	0	0	92	L	V	129.17	91.24	0.56	1.54	0.00	7.37	3.24	0.19	13.29	1.48	0.34
3,3	0	0	93	L	V	54.84	0.63	7.17	21.22	12.12	4.75	4.80	0.25	8.44	0.91	0.46
3,4	0	0	94	L	V	19.96	2.24	0.00	7.36	7.53	0.00	2.79	0.25	5.42	0.43	0.37
4,1	0	0	95	L	V	949.80	0.00	203.99	630.28	0.00	30.03	170.44	0.16	14.53	1.33	0.40
4,2	0	0	96	L	V								0.20	9.29	1.25	0.46
4,3	0	0	97	L	V	123.95	44.38	17.78	55.21	0.00	0.00	52.68	0.16	8.79	0.96	0.30
4,4	0	0	98	L	C	343.66	103.16	39.62	217.49	0.00	22.20	432.69	0.11	6.45	0.86	0.34
1,1	0	6	99	L	V	10.23	136.34	0.08	0.09	2.12	7.33	0.76	0.18	12.38	1.28	0.43
1,2	0	6	100	L	V	0.00	11.35	9.52	16.49	48.58	18.56	0.31	0.19	12.24	1.19	0.47
1,3	0	6	101	L	C	1.43	38.57	0.63	20.06	1.58	16.08	0.59	0.14	6.83	0.79	0.39
1,4	0	6	102	L	V	5.68	0.04	15.50	1.21	4.22	1.06	19.40	0.19	10.53	1.07	0.40
2,1	0	6	103	L	V	6.72	53.55	9.34	5.49	41.87	10.21	43.56	0.23	10.60	1.32	0.38
2,4	0	6	104	L	V	16.72	248.03	10.33	10.42	9.24	3.38	1.96	0.19	10.90	1.24	0.30
3,1	0	6	105	L	C	73.95	69.89	4.58	40.40	10.37	7.13	23.82	0.20	10.91	1.37	0.39
3,2	0	6	106	L	V	37.69	410.82	18.51	28.40	26.77	5.87	4.55	0.19	11.86	1.35	0.32
3,3	0	6	107	L	V	18.20	294.36	8.72	11.65	16.23	3.02	2.59	0.16	9.98	1.20	0.36
3,4	0	6	108	L	V	6.81	25.62	1.12	67.55	13.85	2.40	0.86	0.17	11.07	1.32	0.37
4,1	0	6	109	L	V	0.10	0.00	33.06	57.61	2.08	7.22	11.29	0.17	11.49	1.17	0.45
4,2	0	6	110	L	V	83.75	0.76	2.83	21.51	5.83	5.44	28.59	0.24	8.51	0.34	1.27
4,3	0	6	111	L	V	0.54	5.30	3.71	12.06	9.55	4.36	1.59	0.18	8.31	1.07	0.36
4,4	0	6	112	L	C	85.18	17.80	0.38	38.25	17.07	2.96	2.77	0.17	11.97	1.28	0.42
1,1	2	30	141	L	V	2.08	12.86	7.22	22.57	24.94	14.00	26.22	0.22	11.24	1.11	0.43
1,2	2	30	142	L	V	46.62	27.74	49.07	30.81	27.06	13.78	27.94	0.21	13.39	1.51	0.33
1,3	2	30	143	L	C	109.85	0.26	0.33	64.57	120.08	4.30	0.00	0.18	15.57	1.89	0.44
2,1	2	30	145	L	V	40.21	6.80	1.48	9.26	0.79	0.26	10.54	0.19	8.40	1.18	0.36
2,4	2	30	146	L	V	218.39	15.37	32.80	98.96	8.01	56.68	36.01	0.16	15.75	1.79	0.30

PLOT	YEAR	MONTH	Core Number	LAYER	TREATMENT	CHRYSENE	BENZOBFLUO	BENZOKFLUO	BENZOAPYRE	DIBENZAHAN	INDENO123C	BENZOGHIPE	ORGC	C	H	N
3,1	2	30	147	L	C	51.18	0.00	5.24	18.21	9.31	8.05	2.63	0.19	10.24	1.04	0.30
3,2	2	30	148	L	V	80.79	82.53	21.48	40.85	58.53	18.50	42.88	0.18	14.28	1.85	0.31
3,3	2	30	149	L	V	10.85	4.64	1.26	7.74	1.30	2.00	3.10	0.15	10.97	1.14	0.41
3,4	2	30	150	L	V	48.77	19.49	1.41	12.35	1.56	0.51	0.31	0.16	9.53	1.17	0.34
4,1	2	30	151	L	V	56.10	4.44	24.17	44.00	50.18	3.16	0.00	0.20	13.21	1.58	0.39
4,2	2	30	152	L	V	77.60	33.97	8.49	34.40	18.86	0.56	4.20	0.21	12.88	1.58	0.38
4,3	2	30	153	L	V	41.24	15.51	0.10	9.15	2.01	1.39	1.29	0.15	8.14	0.87	0.32
4,4	2	30	154	L	C	99.22	31.51	2.66	39.05	6.71	0.26	0.80	0.15	13.11	1.41	0.35
1,1	2	30	155	L	V	5.03	11.31	38.14	33.77	19.28	20.29	243.46	0.20	11.60	1.42	0.36
1,2	2	30	156	L	V	64.87	30.32	13.82	24.89	10.05	3.32	15.30	0.26	12.68	1.59	0.37
1,3	2	30	157	L	C	39.39	0.00	21.46	1.03	33.98	0.41	34.20	0.13	8.06	1.00	0.36
1,4	2	30	158	L	V	37.32	5.16	3.19	1.15	4.97	0.00	0.70	0.17	9.00	0.99	0.36
2,1	2	30	159	L	V	28.86	16.63	90.55	44.83	23.76	0.00	67.10	0.15	11.04	1.27	0.40
2,4	2	30	160	L	V	134.92	16.77	6.78	26.49	2.17	1.39	3.96	0.19	14.37	1.70	0.37
3,1	2	30	161	L	C	205.32	27.69	756.06	77.21	27.97	14.71	50.04	0.14	12.15	1.37	0.36
3,2	2	30	162	L	V	22.13	47.21	5.03	18.70	32.00	4.39	83.17	0.22	11.35	1.05	0.45
3,3	2	30	163	L	V	50.61	0.00	0.00	0.26	0.92	0.00	40.49	0.15	12.19	1.47	0.42
3,4	2	30	164	L	V	61.46	49.54	13.89	24.08	25.33	1.12	14.74	0.26	8.50	1.19	0.34
4,1	2	30	165	L	V	187.92	0.00	19.21	73.30	25.37	23.13	33.23	0.19	10.97	1.28	0.37
4,2	2	30	166	L	V	29.31	14.48	4.29	200.20	0.69	12.17	16.32	0.21	11.61	1.15	0.43
4,4	2	30	168	L	C	10.26	13.00	1.95	11.97	4.34	0.22	4.50	0.22	6.89	0.91	0.24

Core Contamination Characteristics: Metals part 1. Extraction procedure reported in Methods Appendix. Values listed in ug/g soil dry weight.

UC PLOT	YEAR	MONTH	Core Number	LAYER	TREATMENT	CR BIOAVAI	NI BIOAVAI	CU BIOAVAI	ZN BIOAVAI	PB BIOAVAI	CR ORGANIC	NI ORGANIC	CU ORGANIC	ZN ORGANIC	PB ORGANIC
1,1	0	0	1	U	V	0.37	0.61	3.08	2.76	0.77	12.39	2.06	13.66	13.57	77.55
1,2	0	0	2	U	V	0.45	1.30	3.55	2.81	0.81	6.22	2.34	16.47	15.63	146.41
1,3	0	0	3	U	C	0.86	0.92	4.68	2.73	0.65	8.05	2.67	19.82	16.94	188.94
1,4	0	0	4	U	V	1.53	0.65	4.22	3.36	0.93	7.38	2.34	18.95	16.49	114.92
2,1	0	0	5	U	V	0.34	0.92	1.31	2.75	0.83	5.93	2.67	16.07	13.09	121.69
2,4	0	0	6	U	V	0.89	0.99	2.09	2.89	1.18	6.65	3.31	14.44	15.03	120.08
3,1	0	0	7	U	C	0.60	0.73	2.10	2.82	0.27	2.80	0.60	14.16	9.43	106.66
3,2	0	0	8	U	V	0.59	1.09	4.65	9.68	0.89	4.23	0.90	13.12	16.30	189.02
3,3	0	0	9	U	V	0.72	0.79	2.53	3.40	0.12	3.06	0.69	16.41	11.75	129.95
3,4	0	0	10	U	V	0.46	1.15	1.99	6.93	0.00	0.75	0.52	11.07	7.17	75.43
4,1	0	0	11	U	V	1.36	1.45	4.59	3.23	0.96	15.95	4.93	31.65	12.76	216.56
4,2	0	0	12	U	V	0.80	0.96	4.92	5.44	0.63	2.21	0.64	15.11	8.06	103.30
4,3	0	0	13	U	V	0.80	0.71	4.90	2.96	0.54	5.61	1.36	15.92	6.76	209.64
4,4	0	0	14	U	C	0.66	0.48	3.14	2.59	0.47	1.00	0.52	12.38	6.94	52.58
1,1	0	6	15	U	V	0.27	1.35	2.82	9.17	0.44	3.49	1.16	16.90	10.60	42.26
1,2	0	6	16	U	V	0.21	0.47	2.31	3.42	0.50	3.17	1.28	16.67	10.12	49.18
1,3	0	6	17	U	C	0.57	0.12	2.54	2.21	0.08	6.81	1.49	21.29	15.40	81.44
1,4	0	6	18	U	V	0.38	0.27	1.72	2.36	0.07	6.26	1.23	22.88	16.54	83.19
2,1	0	6	19	U	V	0.43	0.00	1.51	2.20	0.00	3.59	1.09	17.79	8.78	39.94
2,4	0	6	20	U	V	1.25	0.34	2.87	7.94	0.74	5.45	1.26	20.59	12.75	63.29
3,1	0	6	21	U	C	0.60	0.29	3.46	4.49	0.46	5.89	0.91	19.47	10.60	129.12
3,2	0	6	22	U	V	0.74	0.37	2.32	5.22	0.31	11.06	1.86	26.63	22.83	158.11
3,3	0	6	23	U	V	0.52	0.36	3.30	4.63	0.69	14.87	1.49	20.80	21.55	138.33
3,4	0	6	24	U	V	0.55	0.45	1.52	1.99	0.27	5.04	1.04	17.01	9.70	79.57
4,1	0	6	25	U	V	0.53	0.38	2.21	1.27	0.00	4.24	1.01	20.76	10.98	87.74
4,2	0	6	26	U	V	0.54	0.56	3.03	2.72	0.30	3.63	1.08	17.05	8.58	72.45
4,3	0	6	27	U	V	0.38	0.31	1.85	3.95	0.00	4.49	0.79	9.44	5.32	40.70
4,4	0	6	28	U	C	0.64	0.72	2.08	1.88	0.13	3.89	1.01	18.13	9.41	67.40
1,1	1	18	29	U	V	0.00	0.15	1.32	2.19	0.07	5.78	2.01	15.02	12.79	61.47
1,2	1	18	30	U	V	0.00	0.00	1.23	1.55	0.18	6.27	2.22	18.58	14.62	75.21
1,3	1	18	31	U	C	1.26	0.38	2.39	3.56	0.37	8.24	2.43	23.24	15.35	81.84
1,4	1	18	32	U	V	0.46	0.40	2.97	3.12	0.73	7.60	2.63	19.93	13.34	66.90
2,1	1	18	33	U	V	1.35	0.39	2.91	1.42	0.00	3.92	2.56	14.89	8.57	26.87
2,4	1	18	34	U	V	0.62	0.77	3.27	2.33	0.09	10.71	2.78	26.74	18.15	64.24
3,1	1	18	35	U	C	0.75	0.85	2.07	2.81	1.07	5.58	2.50	19.91	11.38	41.34
3,2	1	18	36	U	V	0.84	0.77	2.43	5.99	2.54	11.80	2.98	29.10	16.39	107.22
3,3	1	18	37	U	V	0.38	0.16	0.40	1.77	0.26	10.01	3.51	20.67	17.91	88.41
3,4	1	18	38	U	V	0.96	0.54	1.23	2.21	0.05	5.74	2.61	23.72	14.15	65.49
4,1	1	18	39	U	V	0.47	0.50	0.69	1.35	1.42	4.33	0.67	18.16	9.87	32.62
4,2	1	18	40	U	V	0.47	0.64	2.07	3.11	1.42	4.48	0.84	18.33	7.78	67.01
4,3	1	18	41	U	V	0.84	0.73	2.41	3.14	0.92	2.54	0.68	15.44	5.21	69.90

UC PLOT	YEAR	MONTH	Core Number	LAYER	TREATMENT	CR BIOAVAI	NI BIOAVAI	CU BIOAVAI	ZN BIOAVAI	PB BIOAVAI	CR ORGANIC	NI ORGANIC	CU ORGANIC	ZN ORGANIC	PB ORGANIC
4,4	1	18	42	U	C	0.63	0.54	1.61	4.14	1.59	2.88	0.68	14.55	6.76	26.69
1,1	1	18	43	U	V	0.51	0.46	2.39	3.17	0.20	6.71	1.11	21.44	13.86	51.43
1,2	1	18	44	U	V	0.64	0.15	1.47	1.80	0.06	3.63	0.59	15.96	10.01	53.89
1,3	1	18	45	U	C	0.75	0.11	1.12	2.71	0.27	6.72	0.83	19.20	14.12	75.92
1,4	1	18	46	U	V	1.04	0.51	3.49	5.30	1.65	5.85	0.76	18.37	11.75	100.58
2,1	1	18	47	U	V	0.56	0.67	2.38	1.55	0.00	3.90	0.94	17.21	11.43	24.86
2,4	1	18	48	U	V	0.42	0.65	1.71	1.80	0.00	5.25	0.81	19.20	14.84	94.65
3,1	1	18	49	U	C	0.54	0.22	1.27	4.38	0.70	3.75	0.72	18.96	9.11	94.72
3,2	1	18	50	U	V	1.23	0.13	1.10	2.92	0.03	6.34	0.96	24.81	15.95	96.41
3,3	1	18	51	U	V	0.82	0.41	2.75	2.49	1.02	6.33	0.94	20.95	12.90	47.68
3,4	1	18	52	U	V	0.91	0.50	3.35	2.64	0.32	4.83	0.73	19.48	14.29	52.51
4,1	1	18	53	U	V	0.92	1.03	4.60	7.36	2.57	6.45	0.89	24.12	11.91	94.93
4,2	1	18	54	U	V	0.21	1.08	2.90	3.36	1.02	3.27	0.66	17.72	7.09	85.39
4,3	1	18	55	U	V	0.99	0.46	4.02	3.72	1.72	2.11	0.55	12.57	7.28	12.52
4,4	1	18	56	U	C	0.89	0.50	3.04	4.04	1.38	2.90	0.52	16.22	7.35	49.23
1,1	2	30	57	U	V	0.60	0.46	2.15	2.10	0.23	5.42	1.80	18.68	11.07	100.87
1,2	2	30	58	U	V	0.60	0.46	2.70	3.16	0.29	4.55	1.73	17.35	9.60	83.36
1,3	2	30	59	U	C	0.86	0.60	1.79	8.56	0.46	9.21	1.61	21.02	14.48	101.39
1,4	2	30	60	U	V	1.12	0.70	2.93	2.32	0.19	5.60	1.71	17.25	13.20	113.50
2,1	2	30	61	U	V	0.55	0.71	2.83	5.79	0.41	3.76	1.33	10.55	10.40	55.71
2,4	2	30	62	U	V	0.76	0.43	1.44	1.37	0.43	11.41	1.66	19.46	22.40	149.37
3,1	2	30	63	U	C	0.91	0.67	2.20	2.92	0.44	11.41	1.41	16.75	9.93	47.41
3,2	2	30	64	U	V	1.12	0.92	2.06	1.44	0.52	7.04	1.80	23.13	21.47	136.36
3,3	2	30	65	U	V	0.50	0.63	1.69	2.11	0.37	4.31	1.48	18.82	15.63	83.75
3,4	2	30	66	U	V	0.87	0.59	2.52	2.69	0.27	3.95	1.50	18.50	6.73	42.10
4,1	2	30	67	U	V	0.94	0.48	2.58	2.69	0.21	3.17	1.39	19.36	6.70	43.59
4,2	2	30	68	U	V	0.60	0.44	1.42	1.77	0.39	6.81	1.34	17.19	7.02	65.24
4,3	2	30	69	U	V	0.24	0.76	2.47	2.45	0.14	4.68	1.21	9.75	4.59	19.84
4,4	2	30	70	U	C	1.07	1.23	2.32	3.08	0.32	4.63	1.02	12.79	8.09	39.93
1,1	2	30	71	U	V	1.04	0.98	5.99	6.94	1.20	3.60	2.18	16.64	8.31	21.31
1,2	2	30	72	U	V	0.49	0.42	2.25	1.88	0.16	5.65	1.48	14.67	13.49	65.95
1,3	2	30	73	U	C	1.03	0.69	3.52	2.50	0.33	20.69	1.72	19.38	12.49	56.08
1,4	2	30	74	U	V	0.69	0.66	2.29	1.95	0.26	15.96	2.17	18.93	11.65	46.69
2,1	2	30	75	U	V	0.61	0.54	1.57	1.91	0.30	4.96	1.61	14.33	10.89	65.94
2,4	2	30	76	U	V	1.15	0.80	4.79	5.26	1.22	9.48	1.95	18.08	13.01	82.75
3,1	2	30	77	U	C	0.43	1.36	1.03	2.23	0.13	3.04	0.75	7.00	7.39	97.90
3,2	2	30	78	U	V	0.90	1.09	3.17	4.25	0.60	12.78	2.35	23.38	10.56	65.78
3,3	2	30	79	U	V	1.10	0.58	3.19	3.42	0.43	7.53	2.18	25.71	11.34	77.38
3,4	2	30	80	U	V	0.83	0.43	2.41	2.18	0.24	5.25	1.61	18.05	11.66	83.57
4,1	2	30	81	U	V	1.85	1.38	4.46	8.65	0.80	9.33	2.21	22.21	10.32	43.40
4,2	2	30	82	U	V	0.49	0.66	2.17	2.69	0.22	5.34	2.22	14.27	7.59	119.32
4,3	2	30	83	U	V	1.00	0.57	2.48	2.50	0.51	4.29	1.02	12.27	4.27	24.25
4,4	2	30	84	U	C	1.09	0.71	3.52	3.56	0.30	6.44	1.39	14.92	4.60	16.90

UC PLOT	YEAR	MONTH	Core Number	LAYER	TREATMENT	CR BIOAVAI	NI BIOAVAI	CU BIOAVAI	ZN BIOAVAI	PB BIOAVAI	CR ORGANIC	NI ORGANIC	CU ORGANIC	ZN ORGANIC	PB ORGANIC
1,1	0	0	85	L	V	0.15	1.74	2.50	7.93	0.62	3.20	2.27	12.46	8.90	48.87
1,2	0	0	86	L	V	1.05	1.62	5.06	4.25	0.68	5.64	2.34	13.62	14.83	137.28
1,3	0	0	87	L	C	0.33	0.94	1.97	7.18	0.12	2.74	2.18	4.51	11.16	17.58
1,4	0	0	88	L	V	0.50	1.06	2.66	4.66	0.00	3.47	2.26	4.23	14.71	174.77
2,1	0	0	89	L	V	0.18	0.59	1.24	2.53	0.00	4.36	3.33	14.63	10.95	192.45
2,4	0	0	90	L	V	0.49	0.85	0.58	2.96	0.19	3.72	3.49	4.28	11.70	23.46
3,1	0	0	91	L	C	0.00	0.82	0.85	4.12	0.00	0.00	0.16	2.15	6.13	11.03
3,2	0	0	92	L	V	0.00	0.90	1.09	3.17	0.00	0.00	0.42	3.47	10.25	7.85
3,3	0	0	93	L	V	0.00	0.95	1.84	2.29	0.00	0.04	0.43	2.40	14.11	11.87
3,4	0	0	94	L	V	0.00	0.90	1.13	3.81	0.00	0.00	0.16	1.94	5.17	19.39
4,1	0	0	95	L	V	0.18	0.74	1.97	2.24	0.47	7.18	2.79	11.55	19.10	76.49
4,2	0	0	96	L	V	0.57	1.10	5.11	9.55	0.62	0.00	0.48	5.64	9.55	25.97
4,3	0	0	97	L	V	0.47	0.60	2.27	3.37	0.41	6.33	1.43	16.92	8.21	89.33
4,4	0	0	98	L	C	0.30	0.67	1.80	2.76	0.28	0.05	0.25	4.16	7.35	17.80
1,1	0	6	99	L	V	0.00	0.31	2.17	3.46	0.00	2.83	1.75	8.32	14.17	57.77
1,2	0	6	100	L	V	0.14	0.24	2.16	4.92	0.13	5.54	1.48	17.00	20.02	53.85
1,3	0	6	101	L	C	0.09	0.44	1.80	3.65	0.16	3.27	0.74	6.27	11.10	17.68
1,4	0	6	102	L	V	0.08	0.52	1.18	3.42	0.47	5.01	1.47	10.73	17.35	60.33
2,1	0	6	103	L	V	0.05	0.00	1.26	2.27	0.06	3.25	1.24	12.09	11.19	53.62
2,4	0	6	104	L	V	0.27	0.26	2.17	4.85	0.08	1.51	1.01	3.25	13.32	3.83
3,1	0	6	105	L	C	0.10	0.33	2.05	5.11	0.36	2.49	0.92	5.50	14.97	38.02
3,2	0	6	106	L	V	0.13	0.82	2.55	7.36	0.47	3.99	1.79	5.92	23.99	23.80
3,3	0	6	107	L	V	0.05	0.50	1.30	6.12	0.31	3.21	0.90	5.73	18.54	47.35
3,4	0	6	108	L	V	0.00	0.16	0.75	2.87	0.16	1.76	1.11	5.19	9.21	12.18
4,1	0	6	109	L	V	0.24	0.40	2.02	3.12	0.15	2.28	1.12	11.29	8.46	21.40
4,2	0	6	110	L	V	0.45	0.78	8.56	9.13	0.21	1.57	0.99	3.56	11.71	6.35
4,3	0	6	111	L	V	0.23	0.80	2.84	8.70	0.64	3.79	0.96	10.63	11.13	59.40
4,4	0	6	112	L	C	0.00	0.56	1.22	4.17	0.00	4.63	0.87	9.50	17.32	84.71
1,1	1	18	113	L	V	0.00	0.21	1.79	3.77	0.55	1.00	1.90	8.19	4.34	6.29
1,2	1	18	114	L	V	0.14	0.53	1.23	4.94	1.13	4.18	2.59	5.58	17.02	43.80
1,3	1	18	115	L	C	0.19	0.42	1.65	7.42	0.16	6.42	2.81	7.61	18.24	71.77
1,4	1	18	116	L	V	0.00	0.46	1.87	1.85	0.00	3.32	2.80	4.02	12.28	6.65
2,1	1	18	117	L	V	0.95	0.94	3.99	10.20	0.16	4.90	3.33	8.80	15.32	143.71
2,4	1	18	118	L	V	1.08	0.85	2.72	3.55	0.07	5.23	2.64	7.73	21.55	68.67
3,1	1	18	119	L	C	0.37	0.85	0.81	3.43	0.19	2.41	2.18	3.60	10.66	9.90
3,2	1	18	120	L	V	0.51	1.13	2.09	4.77	0.27	4.51	3.39	4.71	18.01	41.52
3,3	1	18	121	L	V	0.94	0.73	2.55	3.19	0.75	7.65	2.91	24.74	12.44	57.89
3,4	1	18	122	L	V	0.06	0.44	1.12	2.45	0.00	3.45	2.60	6.85	13.39	0.50
4,1	1	18	123	L	V	0.39	0.62	0.66	2.15	0.36	3.01	1.19	3.32	11.84	21.12
4,2	1	18	124	L	V	0.18	0.57	0.64	2.48	0.26	2.90	1.06	6.55	10.37	104.26
4,3	1	18	125	L	V	0.58	0.52	2.36	3.61	3.14	2.46	0.59	15.05	5.94	57.50
4,4	1	18	126	L	C	0.21	0.58	0.96	3.12	1.16	1.54	0.54	2.25	8.61	5.30
1,1	1	18	127	L	V	0.13	0.27	0.75	4.51	0.10	1.50	0.93	6.26	10.47	20.02

UC PLOT	YEAR	MONTH	Core Number	LAYER	TREATMENT	CR BIOAVAI	NI BIOAVAI	CU BIOAVAI	ZN BIOAVAI	PB BIOAVAI	CR ORGANIC	NI ORGANIC	CU ORGANIC	ZN ORGANIC	PB ORGANIC
1,2	1	18	128	L	V	0.54	0.63	2.75	5.21	1.09	4.04	0.97	8.38	15.18	61.16
1,3	1	18	129	L	C	0.27	0.00	0.65	1.79	0.05	1.75	0.60	2.72	13.49	5.34
1,4	1	18	130	L	V	0.14	0.14	0.69	2.44	0.00	3.59	1.51	4.68	16.92	63.65
2,1	1	18	131	L	V	0.16	0.97	2.37	2.87	0.19	0.26	0.57	4.93	6.97	7.03
2,4	1	18	132	L	V	0.14	0.76	1.31	2.65	0.00	2.06	0.85	4.30	15.90	36.44
3,1	1	18	133	L	C	0.10	0.37	0.55	3.91	0.45	1.37	0.88	4.10	9.61	12.49
3,2	1	18	134	L	V	0.25	0.49	0.93	3.63	0.16	3.19	1.13	3.03	19.55	34.72
3,3	1	18	135	L	V	0.00	0.85	1.69	3.61	0.17	2.56	0.92	5.06	12.27	9.56
3,4	1	18	136	L	V	0.00	0.58	0.77	2.85	0.00	1.44	0.90	2.10	13.19	9.45
4,1	1	18	137	L	V	0.11	0.66	1.53	2.00	0.41	2.13	0.86	2.89	14.98	41.85
4,2	1	18	138	L	V	0.26	0.69	3.14	4.16	0.00	2.43	0.99	4.47	11.24	24.12
4,3	1	18	139	L	V	0.21	0.96	2.47	8.89	0.94	1.36	0.62	3.71	6.85	38.78
4,4	1	18	140	L	C	0.00	0.50	1.89	3.62	0.76	1.21	0.51	2.58	8.76	9.73
1,1	2	30	141	L	V	0.62	0.40	1.89	1.89	0.23	5.02	1.50	15.25	10.45	69.28
1,2	2	30	142	L	V	0.24	0.56	2.37	2.47	0.18	3.75	1.40	5.38	10.83	46.75
1,3	2	30	143	L	C	0.41	1.19	3.03	3.07	0.15	4.20	2.09	6.32	15.56	60.23
2,1	2	30	145	L	V	0.00	0.57	1.13	3.10	0.00	4.52	1.93	7.22	22.62	111.25
2,4	2	30	146	L	V	0.09	0.59	1.96	2.56	0.00	3.22	2.05	5.21	27.68	98.32
3,1	2	30	147	L	C	0.12	0.71	2.10	3.95	0.33	2.08	0.89	2.63	14.85	2.95
3,2	2	30	148	L	V	0.23	0.79	1.67	2.22	0.18	6.35	2.31	5.85	48.06	56.57
3,3	2	30	149	L	V	0.75	0.56	2.20	4.34	0.35	5.31	1.46	13.19	7.11	27.41
3,4	2	30	150	L	V	0.24	0.74	1.95	2.49	0.00	2.41	1.00	4.63	6.38	1.78
4,1	2	30	151	L	V	0.41	0.87	3.54	2.96	0.14	2.01	1.62	5.66	8.73	5.64
4,2	2	30	152	L	V	0.31	0.60	1.61	2.38	0.33	3.59	1.36	5.46	8.29	36.07
4,3	2	30	153	L	V	0.31	1.17	2.96	3.90	0.35	1.87	0.60	4.17	5.52	27.26
4,4	2	30	154	L	C	0.47	0.75	2.07	1.82	0.00	4.28	0.70	6.38	11.32	36.96
1,1	2	30	155	L	V	0.36	0.77	2.54	3.95	0.43	7.09	2.01	10.38	17.90	52.23
1,2	2	30	156	L	V	0.10	0.82	2.15	2.20	0.31	8.14	1.87	5.21	22.21	74.43
1,3	2	30	157	L	C	0.45	0.81	2.57	2.26	0.19	10.11	1.26	5.50	21.01	13.64
1,4	2	30	158	L	V	0.44	0.73	2.34	3.23	0.29	5.53	1.73	5.50	14.88	25.54
2,1	2	30	159	L	V	0.60	0.62	2.54	3.58	0.41	6.68	1.57	11.54	10.08	28.40
2,4	2	30	160	L	V	0.13	0.65	1.08	4.55	1.00	1.57	1.33	2.67	9.65	7.71
3,1	2	30	161	L	C	0.00	0.54	2.08	1.43	0.00	4.43	1.33	5.71	11.55	19.86
3,2	2	30	162	L	V	0.45	0.86	2.34	3.45	0.69	3.41	1.75	4.04	16.60	14.28
3,3	2	30	163	L	V	0.45	0.72	2.70	3.63	0.48	5.13	1.63	10.13	10.96	27.99
3,4	2	30	164	L	V	0.00	0.43	2.07	1.69	0.17	4.57	2.19	8.50	23.76	73.18
4,1	2	30	165	L	V	0.00	0.57	1.18	1.51	0.00	1.12	0.94	1.76	15.32	2.22
4,2	2	30	166	L	V	0.57	0.87	2.76	7.57	0.50	1.87	0.97	2.00	3.95	1.56
4,4	2	30	168	L	C	0.00	0.70	2.88	2.17	0.24	2.82	0.26	2.25	3.88	1.19

Core Contamination Characteristics: Metals part 2. Details in Metals part 1.

UC PLOT	YEAR	MONTH	Core Number	LAYER	TREATMENT	CR OXIDE	NI OXIDE	CU OXIDE	ZN OXIDE	PB OXIDE	CR RESID	NI RESID	CU RESID	ZN RESID	PB RESID
1,1	0	0	1	U	V	14.41	1.46	7.94	30.55	61.76	385.17	59.46	53.47	289.04	273.34
1,2	0	0	2	U	V	22.58	2.31	12.18	50.41	110.20	408.31	63.65	57.02	299.49	253.94
1,3	0	0	3	U	C	21.93	2.20	8.92	53.38	109.25	507.75	64.01	59.46	328.25	371.24
1,4	0	0	4	U	V	21.52	2.26	15.13	63.99	142.00	523.08	60.58	56.62	325.35	311.52
2,1	0	0	5	U	V	13.74	1.53	8.92	31.14	68.23	392.58	68.37	53.64	286.21	278.71
2,4	0	0	6	U	V	10.54	1.76	7.16	27.98	83.57	529.07	68.28	75.60	378.13	378.64
3,1	0	0	7	U	C	17.47	2.44	14.63	47.04	199.97	466.20	57.02	54.95	289.57	468.20
3,2	0	0	8	U	V	19.25	1.98	6.02	39.77	119.86	595.57	52.87	71.43	352.37	750.76
3,3	0	0	9	U	V	18.97	2.02	13.02	46.67	131.06	538.67	56.50	55.35	320.46	513.96
3,4	0	0	10	U	V	12.41	1.05	11.21	31.01	107.16	402.97	46.44	50.19	256.77	415.92
4,1	0	0	11	U	V	16.05	7.87	16.77	81.06	300.04	516.90	72.44	46.10	291.70	441.37
4,2	0	0	12	U	V	6.22	0.89	4.97	15.18	47.74	425.75	73.29	58.36	291.21	527.20
4,3	0	0	13	U	V	19.96	4.25	13.05	59.01	241.33	268.25	48.84	27.42	175.04	144.86
4,4	0	0	14	U	C	17.02	2.43	12.98	38.22	112.31	360.40	68.18	53.59	263.63	237.59
1,1	0	6	15	U	V	16.48	6.84	10.26	36.20	92.73	420.64	73.75	52.02	287.09	264.53
1,2	0	6	16	U	V	9.13	7.59	10.15	38.05	116.23	431.41	61.36	55.25	293.84	261.53
1,3	0	6	17	U	C	13.57	8.07	12.07	43.91	145.00	523.83	52.20	61.68	332.00	347.08
1,4	0	6	18	U	V	10.31	8.61	15.02	46.46	151.79	561.83	52.14	63.95	338.46	346.25
2,1	0	6	19	U	V	13.47	8.02	14.13	36.70	125.04	435.13	88.23	58.15	301.29	266.67
2,4	0	6	20	U	V	23.02	12.15	18.19	54.73	159.27	588.06	77.64	68.87	379.07	476.26
3,1	0	6	21	U	C	14.34	13.57	22.25	53.00	209.55	520.81	59.33	58.17	308.83	467.59
3,2	0	6	22	U	V	13.66	14.36	20.25	75.60	268.13	655.25	50.40	55.76	345.54	603.33
3,3	0	6	23	U	V	12.29	15.92	24.04	75.86	214.15	611.46	65.45	53.98	332.06	462.63
3,4	0	6	24	U	V	17.13	13.83	20.00	53.82	187.39	470.33	52.12	47.45	275.46	417.27
4,1	0	6	25	U	V	14.48	14.09	24.56	60.28	235.25	530.29	61.42	52.16	302.50	494.18
4,2	0	6	26	U	V	20.14	13.24	19.21	47.48	182.81	455.42	75.01	58.16	286.90	430.10
4,3	0	6	27	U	V	7.85	10.66	11.38	30.52	109.88	291.44	69.26	33.24	202.19	226.88
4,4	0	6	28	U	C	17.70	13.10	19.17	42.96	102.37	381.08	56.30	48.42	260.45	264.56
1,1	1	18	29	U	V	24.17	3.41	14.12	56.87	129.32	430.56	67.56	56.13	292.19	291.53
1,2	1	18	30	U	V	21.96	3.81	16.48	60.65	136.29	406.38	62.55	55.68	285.88	282.92
1,3	1	18	31	U	C	22.36	3.94	20.75	70.28	184.74	527.27	89.19	81.67	342.50	426.84
1,4	1	18	32	U	V	20.40	2.97	14.22	60.93	134.08	524.58	68.68	61.12	332.17	359.63
2,1	1	18	33	U	V	18.66	2.98	15.66	40.49	111.83	305.37	65.15	44.02	220.60	234.21
2,4	1	18	34	U	V	12.04	3.27	24.55	97.49	258.48	562.06	81.04	62.91	352.93	471.67
3,1	1	18	35	U	C	29.99	3.74	23.74	69.32	214.60	503.08	63.24	63.50	312.06	561.13
3,2	1	18	36	U	V	21.27	3.03	22.84	89.25	255.36	641.20	57.94	61.94	343.94	627.53
3,3	1	18	37	U	V	17.11	1.43	17.29	71.67	201.84	457.34	59.05	46.81	240.76	271.52
3,4	1	18	38	U	V	27.20	5.58	29.44	72.59	211.19	456.56	55.03	56.29	280.30	448.15
4,1	1	18	39	U	V	31.83	6.05	21.97	75.44	210.63	521.38	61.20	66.76	312.42	495.83
4,2	1	18	40	U	V	23.48	5.03	24.51	67.63	187.05	458.70	92.43	57.43	292.18	431.84
4,3	1	18	41	U	V	24.87	4.53	23.88	57.19	144.39	361.48	65.13	46.79	240.51	292.77
4,4	1	18	42	U	C	28.99	3.85	23.43	64.17	98.69	406.95	83.27	55.21	289.65	264.77

UC PLOT	YEAR	MONTH	Core Number	LAYER	TREATMENT	CR OXIDE	NI OXIDE	CU OXIDE	ZN OXIDE	PB OXIDE	CR RESID	NI RESID	CU RESID	ZN RESID	PB RESID
1,1	1	18	43	U	V	11.49	3.10	19.59	71.44	141.84	415.11	65.80	46.37	270.62	257.46
1,2	1	18	44	U	V	27.06	3.78	18.68	57.31	117.21	399.39	62.01	51.27	287.97	280.50
1,3	1	18	45	U	C	26.32	3.72	20.14	73.40	192.18	528.30	76.75	58.15	328.01	477.50
1,4	1	18	46	U	V	23.92	2.72	17.07	64.69	127.37	513.93	73.67	53.69	332.37	362.88
2,1	1	18	47	U	V	24.66	4.20	20.03	64.03	136.21	387.70	70.73	61.86	278.56	281.62
2,4	1	18	48	U	V	13.55	2.95	19.24	64.74	230.65	548.67	69.40	62.99	360.20	546.03
3,1	1	18	49	U	C	22.78	2.90	16.48	49.15	191.81	489.27	63.92	60.27	302.90	624.51
3,2	1	18	50	U	V	16.61	3.28	21.71	72.34	255.58	595.04	61.91	55.37	345.41	647.86
3,3	1	18	51	U	V	17.12	1.43	17.29	71.38	201.83	590.13	52.49	54.00	319.67	516.79
3,4	1	18	52	U	V	10.19	1.20	18.85	58.57	264.54	495.78	51.99	48.59	278.08	537.41
4,1	1	18	53	U	V	19.52	1.40	19.19	55.89	303.56	500.05	50.93	51.99	290.89	735.06
4,2	1	18	54	U	V	19.48	1.26	15.24	37.60	133.76	409.14	74.84	52.32	268.99	426.35
4,3	1	18	55	U	V	15.06	1.35	12.71	30.77	80.93	304.55	61.07	42.95	214.06	259.19
4,4	1	18	56	U	C	17.26	1.18	14.08	37.34	83.78	387.15	71.97	50.69	276.47	290.39
1,1	2	30	57	U	V	18.04	7.49	9.94	39.80	72.64	475.39	74.25	55.28	386.25	323.63
1,2	2	30	58	U	V	16.11	7.49	9.13	35.93	75.68	431.93	73.70	47.30	370.50	301.98
1,3	2	30	59	U	C	7.78	7.97	12.23	50.99	120.75	591.06	87.18	56.65	424.50	391.55
1,4	2	30	60	U	V	17.91	8.26	9.70	46.34	98.59	552.28	68.89	58.71	412.50	418.75
2,1	2	30	61	U	V	18.18	9.10	10.78	37.75	103.49	299.54	63.39	38.91	274.50	227.67
2,4	2	30	62	U	V	8.45	11.08	12.41	56.00	222.32	554.95	72.60	62.29	412.50	471.67
3,1	2	30	63	U	C	7.93	8.31	10.27	35.20	169.18	422.57	53.21	47.03	300.00	428.33
3,2	2	30	64	U	V	9.96	12.11	15.09	61.31	254.26	635.19	68.61	68.06	409.50	600.00
3,3	2	30	65	U	V	8.00	11.22	12.78	47.47	172.19	566.99	61.46	49.36	370.50	460.15
3,4	2	30	66	U	V	9.65	8.05	10.51	34.57	136.56	494.78	52.39	45.51	346.50	423.33
4,1	2	30	67	U	V	8.49	11.71	16.50	55.19	202.32	529.55	62.43	51.70	345.00	468.08
4,2	2	30	68	U	V	3.99	6.84	9.86	27.81	146.97	429.25	88.96	58.58	324.00	389.26
4,3	2	30	69	U	V	9.75	6.61	8.17	22.43	77.22	290.18	62.01	33.83	240.00	215.63
4,4	2	30	70	U	C	5.18	6.93	10.54	25.81	83.12	382.45	72.05	42.76	282.00	270.00
1,1	2	30	71	U	V	9.33	5.89	5.74	30.51	70.21	449.31	73.15	52.39	297.00	269.14
1,2	2	30	72	U	V	10.25	4.68	5.33	22.62	56.60	410.53	67.79	59.13	340.50	305.83
1,3	2	30	73	U	C	5.57	6.52	9.08	43.21	133.39	583.04	59.40	61.19	432.00	393.75
1,4	2	30	74	U	V	9.59	8.81	10.20	47.51	111.94	378.44	54.04	39.88	294.00	307.25
2,1	2	30	75	U	V	10.59	12.71	13.48	47.91	125.71	429.25	77.96	62.15	367.50	317.27
2,4	2	30	76	U	V	10.94	13.22	13.62	57.69	200.67	538.91	80.16	61.19	379.50	463.33
3,1	2	30	77	U	C	17.33	7.23	6.93	29.07	102.67	230.01	31.90	25.30	175.50	189.17
3,2	2	30	78	U	V	8.30	9.57	13.02	57.51	224.26	625.83	55.96	58.30	415.50	538.75
3,3	2	30	79	U	V	7.06	8.30	12.16	51.79	160.74	629.84	61.33	54.86	400.50	522.50
3,4	2	30	80	U	V	8.49	12.46	17.57	60.75	248.63	554.95	60.64	65.73	385.50	514.13
4,1	2	30	81	U	V	9.15	6.90	11.43	39.92	187.92	478.73	56.24	40.01	318.00	524.17
4,2	2	30	82	U	V	28.72	16.91	13.59	57.97	197.92	456.00	87.59	61.05	318.00	382.61
4,3	2	30	83	U	V	8.37	8.16	10.93	29.79	123.62	343.67	46.06	35.89	246.00	269.59
4,4	2	30	84	U	C	4.12	6.42	9.25	32.39	86.10	386.46	72.88	42.01	311.25	263.33
1,1	0	0	85	L	V	11.21	3.03	12.47	39.86	65.39	129.40	23.60	22.48	93.69	55.51

UC PLOT	YEAR	MONTH	Core Number	LAYER	TREATMENT	CR OXIDE	NI OXIDE	CU OXIDE	ZN OXIDE	PB OXIDE	CR RESID	NI RESID	CU RESID	ZN RESID	PB RESID
1,2	0	0	86	L	V	13.32	2.53	14.36	66.82	151.40	391.37	61.97	56.82	265.23	200.94
1,3	0	0	87	L	C	13.36	1.94	4.23	34.78	27.27	144.76	19.36	24.89	113.46	46.80
1,4	0	0	88	L	V	21.91	2.45	6.07	49.11	36.35	220.46	24.37	34.44	152.05	73.97
2,1	0	0	89	L	V	17.86	3.04	15.10	48.03	105.99	276.70	56.32	41.18	201.57	158.49
2,4	0	0	90	L	V	3.58	0.80	1.90	12.99	7.06	233.16	26.69	47.89	194.34	91.03
3,1	0	0	91	L	C	11.34	2.15	6.07	40.65	36.99	225.76	34.02	57.14	170.69	100.34
3,2	0	0	92	L	V	18.94	2.66	5.60	60.06	38.85	335.30	44.57	88.09	241.46	140.91
3,3	0	0	93	L	V	6.26	0.88	1.37	19.41	19.69	314.24	28.98	52.76	230.28	186.31
3,4	0	0	94	L	V	3.09	0.53	2.54	12.69	18.13	174.69	25.75	27.06	122.69	114.22
4,1	0	0	95	L	V	34.02	10.78	11.21	100.52	71.39	421.65	74.76	65.87	256.89	192.14
4,2	0	0	96	L	V	9.55	3.21	10.87	36.46	41.43	222.85	69.29	42.81	178.02	122.00
4,3	0	0	97	L	V	30.86	6.05	15.64	70.09	213.88	290.62	52.47	28.21	185.23	166.28
4,4	0	0	98	L	C	8.38	2.42	12.99	39.30	55.09	169.92	35.74	32.68	141.72	83.74
1,1	0	6	99	L	V	15.21	6.77	4.05	43.24	75.09	413.80	53.62	74.37	276.20	282.86
1,2	0	6	100	L	V	18.06	9.24	11.67	52.69	136.67	438.83	75.27	47.61	273.50	212.50
1,3	0	6	101	L	C	11.33	5.35	8.37	34.18	49.46	230.70	41.09	45.58	170.90	105.49
1,4	0	6	102	L	V	8.23	5.61	3.21	27.93	30.44	445.83	47.92	65.64	275.62	315.72
2,1	0	6	103	L	V	14.56	7.91	11.32	41.19	129.02	424.95	92.90	60.12	288.62	255.40
2,4	0	6	104	L	V	16.34	11.56	3.86	55.02	32.74	315.02	40.84	72.18	246.30	155.58
3,1	0	6	105	L	C	21.89	13.68	9.37	60.63	124.58	384.21	33.57	65.17	230.21	290.42
3,2	0	6	106	L	V	26.23	13.32	4.41	73.64	59.58	523.63	48.40	72.19	306.71	245.00
3,3	0	6	107	L	V	23.26	12.63	9.56	69.76	104.47	439.62	37.27	71.94	246.02	340.54
3,4	0	6	108	L	V	15.41	10.84	7.61	50.27	51.50	325.35	71.59	75.49	235.64	185.24
4,1	0	6	109	L	V	16.09	11.56	16.37	62.21	113.21	460.88	56.12	52.05	264.71	267.92
4,2	0	6	110	L	V	13.73	11.74	6.69	48.91	32.10	282.15	108.58	50.17	212.97	190.92
4,3	0	6	111	L	V	21.42	12.95	15.97	56.26	154.25	316.46	61.32	39.58	207.21	226.67
4,4	0	6	112	L	C	11.92	11.49	14.48	48.17	107.60	306.58	53.90	43.15	203.13	211.23
1,1	1	18	113	L	V	2.81	2.04	4.58	15.13	13.89	27.14	10.34	25.97	50.29	21.97
1,2	1	18	114	L	V	14.36	3.30	2.90	40.24	57.91	417.60	62.95	107.79	293.10	436.34
1,3	1	18	115	L	C	28.49	4.69	6.88	67.88	83.02	448.34	66.44	81.57	303.08	354.65
1,4	1	18	116	L	V	23.51	4.10	2.93	55.98	13.50	270.76	31.02	46.19	190.67	82.86
2,1	1	18	117	L	V	5.87	1.91	4.57	28.15	14.98	282.94	84.36	65.64	221.04	185.40
2,4	1	18	118	L	V	24.98	4.46	8.52	98.25	114.44	405.38	48.01	82.63	261.35	348.19
3,1	1	18	119	L	C	16.01	2.96	3.83	39.81	21.01	261.82	31.98	80.80	195.39	134.43
3,2	1	18	120	L	V	28.94	4.19	4.11	66.34	30.72	434.44	45.03	65.06	260.87	175.72
3,3	1	18	121	L	V	23.74	5.68	25.81	83.35	188.33	540.46	51.98	57.10	304.58	399.75
3,4	1	18	122	L	V	26.19	6.66	7.04	64.67	78.40	395.31	47.57	118.58	264.06	257.04
4,1	1	18	123	L	V	25.08	7.60	6.48	52.75	33.09	430.17	61.97	65.06	265.13	215.97
4,2	1	18	124	L	V	32.62	5.96	9.50	86.79	154.02	437.73	81.19	85.64	282.46	458.53
4,3	1	18	125	L	V	28.98	3.85	23.50	58.12	111.25	362.08	80.10	49.94	254.50	307.67
4,4	1	18	126	L	C	19.48	4.51	6.91	65.78	18.46	338.63	37.89	51.12	214.79	91.13
1,1	1	18	127	L	V	14.55	4.45	8.33	55.64	40.38	113.99	19.77	29.55	88.55	56.62
1,2	1	18	128	L	V	22.00	3.76	7.59	50.34	73.35	434.52	60.29	81.29	308.33	355.66

UC PLOT	YEAR	MONTH	Core Number	LAYER	TREATMENT	CR OXIDE	NI OXIDE	CU OXIDE	ZN OXIDE	PB OXIDE	CR RESID	NI RESID	CU RESID	ZN RESID	PB RESID
1,3	1	18	129	L	C	28.39	3.85	5.22	68.54	30.82	295.30	30.76	53.02	215.55	121.70
1,4	1	18	130	L	V	21.83	4.31	4.27	73.80	22.37	390.29	53.63	57.46	278.30	140.69
2,1	1	18	131	L	V	4.63	4.13	5.89	19.05	25.05	37.61	12.33	11.99	42.85	19.97
2,4	1	18	132	L	V	26.36	4.95	3.52	81.24	52.78	427.30	57.61	101.93	316.65	260.40
3,1	1	18	133	L	C	19.52	4.23	3.61	48.24	41.15	396.18	64.98	93.37	270.88	306.13
3,2	1	18	134	L	V	29.84	2.13	1.66	71.52	70.32	576.13	53.95	79.51	314.31	541.71
3,3	1	18	135	L	V	20.21	1.89	2.35	46.85	22.33	342.11	48.97	80.15	248.12	202.85
3,4	1	18	136	L	V	24.30	3.44	2.84	70.79	50.69	404.62	70.34	70.49	240.40	246.71
4,1	1	18	137	L	V	23.64	3.62	4.20	72.97	71.88	412.26	71.25	94.20	257.88	244.46
4,2	1	18	138	L	V	11.97	1.93	5.23	53.44	54.30	393.66	58.09	53.43	233.11	160.56
4,3	1	18	139	L	V	14.12	2.16	7.33	38.39	101.56	219.50	40.09	29.68	147.19	108.19
4,4	1	18	140	L	C	20.78	1.84	2.25	48.38	17.40	399.00	37.99	51.43	241.88	108.51
1,1	2	30	141	L	V	12.52	7.19	9.67	42.97	86.19	486.75	69.85	53.90	393.00	290.83
1,2	2	30	142	L	V	11.07	7.34	4.03	42.02	62.47	397.16	54.59	70.68	342.00	298.75
1,3	2	30	143	L	C	10.78	6.41	3.74	47.99	30.61	339.66	71.09	60.78	330.00	164.66
2,1	2	30	145	L	V	15.85	8.76	7.70	48.13	76.97	339.66	81.26	57.48	291.00	277.64
2,4	2	30	146	L	V	15.32	9.36	2.43	47.17	36.34	414.54	103.13	89.51	429.00	275.83
3,1	2	30	147	L	C	6.89	4.66	3.58	24.91	19.52	337.65	53.21	79.68	276.00	175.42
3,2	2	30	148	L	V	22.25	11.99	1.65	66.40	32.75	552.28	61.05	69.58	375.00	273.22
3,3	2	30	149	L	V	0.00	0.00	0.00	0.00	0.00	469.37	57.34	47.03	337.50	346.25
3,4	2	30	150	L	V	9.60	8.00	5.98	43.90	40.82	406.52	40.70	48.40	268.50	142.08
4,1	2	30	151	L	V	11.10	12.14	7.45	82.63	55.93	550.94	55.41	69.03	357.00	222.95
4,2	2	30	152	L	V	12.86	12.40	6.27	52.17	81.49	334.31	63.11	59.26	298.50	157.92
4,3	2	30	153	L	V	9.87	6.56	7.01	26.64	77.13	243.38	48.40	31.63	207.00	245.00
4,4	2	30	154	L	C	8.35	6.63	6.97	33.45	95.28	307.57	58.85	46.61	252.00	155.63
1,1	2	30	155	L	V	9.95	4.48	3.64	32.21	45.65	386.46	69.85	50.46	312.00	242.50
1,2	2	30	156	L	V	7.02	5.71	1.73	36.71	45.29	390.47	58.03	59.54	328.50	281.67
1,3	2	30	157	L	C	8.15	9.25	5.92	60.75	44.48	296.87	31.49	33.14	252.00	116.25
1,4	2	30	158	L	V	10.11	9.18	5.30	67.44	41.62	331.64	38.78	54.86	294.00	135.00
2,1	2	30	159	L	V	8.48	9.97	10.97	45.11	103.05	305.34	74.57	47.21	281.00	197.08
2,4	2	30	160	L	V	18.83	8.31	6.06	43.23	85.00	442.63	85.53	69.16	375.00	443.75
3,1	2	30	161	L	C	13.77	8.56	4.47	37.79	76.52	362.39	44.28	51.15	262.50	285.42
3,2	2	30	162	L	V	16.27	7.34	2.08	51.34	92.81	575.01	42.63	85.53	376.50	715.83
3,3	2	30	163	L	V	6.17	9.34	8.85	70.24	121.82	454.66	57.61	47.99	321.00	305.42
3,4	2	30	164	L	V	14.96	8.72	3.30	38.02	34.10	344.71	63.48	55.53	175.80	202.08
4,1	2	30	165	L	V	9.24	7.10	1.80	39.39	19.37	369.08	57.61	67.38	306.00	184.78
4,2	2	30	166	L	V	4.58	3.81	2.48	18.86	13.77	239.37	33.14	44.83	187.50	106.25
4,4	2	30	168	L	C	8.85	4.24	3.62	20.82	16.00	180.53	27.09	28.46	162.00	66.25