ABSTRACT

EVALUATING RHIZODEGRADATION OF PETROLEUM HYDROCARBONS AND POLYCYCLIC AROMATIC HYDROCARBONS IN WETLANDS SEDIMENT USING *SPARTINA PATENS*

by
Robert J. Lippencott

Biodegradation of petroleum hydrocarbons (PHC) and 16 polycyclic aromatic hydrocarbon (PAH) compounds was evaluated in sediments from a stormwater basin that was a former salt marsh at an active oil refinery. *Spartina patens* were grown in basin sediments during a field study and a greenhouse study over three consecutive growing seasons. Ninety percent (90%) survival was observed in field study plots containing sediments with <12% PHC. In the greenhouse study, planted and bulk sediments were monitored for PHC, PAH, microbial density, nutrients, pH, redox and root biomass at 2.5 and 7.5 cm in sediments with <12% PHC. The sediments contained on average 6.5% to 9.5% PHC and 56 to 124 parts per million (ppm) total PAH. The plants exhibited a 95% survival rate and added from 1% to 2% root biomass to the sediment. Microbial densities in planted sediments were significantly higher than in bulk sediments ($p<0.05$). Oxidizing conditions were prevalent in all treatments at both depths; however, reducing conditions developed in the planted sediments at 7.5 cm. PHC was 35% to 37% lower in the planted sediments vs. bulk sediment at the end of the study. Planted sediment PAH concentrations were generally lower than in bulk sediments. Low MW PAHs (2 and 3-Rings) were either absent or at relatively low concentrations in all treatments. Phenanthrene concentrations in bulk sediments did not change, but were reduced by 95% in planted sediments ($p<0.01$), with half-life estimates

of 141 to 165 days at 2.5 cm. Temporal changes in high MW PAHs (≥4-Rings) were not observed over the greenhouse study period. Differences in PAH concentrations between planted and bulk sediments exhibited a decreasing trend with PAH molecular weight. Evaluation of differences in planted and bulk sediment PAH concentrations suggests that lower MW PAHs degrade better under oxidizing conditions, and higher MW PAHs under reducing conditions in the planted sediments. Comparison of planted and bulk sediment PAH concentrations links depth and redox conditions to decreases in PAH as a function of PAH MW. Therefore, it was concluded that spatio-temporal variations and cycling of redox conditions should be considered to properly evaluate biodegradation of PAH.

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EVALUATING RHIZODEGRADATION OF PETROLEUM HYDROCARBONS AND POLYCYCLIC AROMATIC HYDROCARBONS IN WETLAND SEDIMENTS CONTAINING *SPARTINA PATENS*

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It is the glory of God to conceal a matter;
to search out a matter is the glory of kings.

- Proverbs 25:2

This work is dedicated to

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To the advancement of environmental science.

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CHAPTER 1

INTRODUCTION

The amount of petroleum hydrocarbons (PHC) released into marine environments worldwide is estimated to be more than 570,000 tons per year (National Research Council, 2003). This includes crude oil as well as refined petroleum, like diesel fuel and gasoline. Crude oil and lower boiling point (i.e., semi-volatile) forms of refinery products such as hydraulic oil, lubricating oils, fuel oil and heating oil can contain significant amounts of polycyclic aromatic hydrocarbons (PAH), which persist in the environment and are generally considered to be the more toxic components of PHC. The U.S. Department of Health and Human Services, National Toxicology Program's, December 2002 Report on Carcinogens, Tenth Edition (USDOH, 2002) identifies PAHs as a general class of compounds, and the following individual PAHs as reasonably anticipated to be human carcinogens:

Benzo[*a*]anthracene
Benzo[*b*]fluoranthene
Benzo[*j*]fluoranthene
Benzo[*k*]fluoranthene
Benzo[*a*]pyrene
Dibenz[*a,h*]anthracene

While PHC usually contains a mixture of organic constituents including lighter, volatile hydrocarbon fractions, the heavier, less volatile fractions and PAH make up much of the petroleum residue that persists in PHC-impacted soil and sediments (Carman et al., 2000). The widespread use of petroleum as heating and motor fuel has contributed to the presence of PHC and PAH in many surface water bodies and associated sediments

via storm drainage from roadways and industrial areas. In addition, oil spills associated with oceanic petroleum exploration, and refining and shipping of petroleum in and around coastal areas, have resulted in exposure of estuarine marshes to PHC and PAH (Coates et al., 1997; Lin and Mendelssohn, 1998).

Coastal wetlands and surface waters (e.g., estuaries) are identified as environmentally sensitive areas by federal and state governmental agencies like the United States Environmental Protection Agency (USEPA) and the New Jersey Department of Environmental Protection (NJDEP). Estuaries are the mixing zones where salty ocean water coalesces with fresh river water in coastal areas and are important links to a complex food web that include endangered species, and to commercially important marine fisheries. According to the National Safety Council (NSC, 1998), estuaries represent the habitat for over 75% of the fish caught annually by commercial operators in the U.S. In 2001, fisheries in the continental US alone produced over $3.3 Billion in revenue (NMFS 2002). Many port cities and shipping facilities are located in or near estuaries, facilitating pathways for migration of PHC and PAH to these areas from commercial/industrial areas and urban centers via direct discharges, spills and storm water runoff.

In the U.S., state and federal laws and regulations require cleanup or "remediation" of oil spills and related residues in marsh and wetland areas (Federal Clean Water Act; Comprehensive Environmental Remediation, Compensation and Liabilities Act, etc). Remedial strategies that have historically been used for cleanup of PHC in marsh ecosystems include low-pressure flushing, removal of oiled vegetation, application of oil sorbents and vacuuming (Mattson et al., 1977; Hoff et al., 1993). For many sites,

cleanup action is not recommended since the remedial activities themselves can cause more wetland damage than the PHC being removed (Mearns, 1993).

Phytoremediation - the use of vascular plants for treatment of contaminated soil and sediment - is an emerging remedial technology that has potential for reducing PHC in wetland sediments without the deleterious effects associated with other approaches (USEPA, 2000). This approach has potential as a bioremediation technology applicable to PHC spill cleanup and can be used alone or in combination with other remedial measures. The use of plants to treat oiled wetland soils and sediments may be feasible for large areas of surface sediments that contain relatively immobile contaminants (Cunningham et al., 1995), such as residual PHC and PAH.

Phytoremediation is a process by which higher plants degrade, extract, contain or immobilize contaminants from soil and water (USEPA, 2000). The idea of treating waste materials using plants is not new; wastewater treatment systems that incorporate constructed wetlands, reed beds or floating plant systems have been operating for many years (Cunningham et al., 1995). Plants possess a general ability to enhance the degradation rate of PHC and PAHs in soil and sediment. As an example, one study demonstrated significant degradation of pyrene, with reductions of up to 74% of pyrene in 28 days in all of 9 different plant species tested (Liste and Alexander, 1999). Of the different phytoremediation processes described below, rhizodegradation is most applicable to sediments containing PHC and PAH.

There are several branches of phytoremediation identified by the USEPA (2000), including phytoextraction, rhizofiltration, phytovolatization, rhizodegradation and phytodegradation, and phytostabilization. The areas of phytoremediation that are

amenable to treatment of organic contaminants in soils and sediments include rhizodegradation, phytodegradation and, to a lesser extent, phytostabilization. Rhizodegradation refers to the microbial breakdown of organic contaminants in the root zone (rhizosphere) soil and sediment. This process uses the natural ability of plants to manipulate the biological, chemical and physical characteristics of the rhizosphere for reducing organic contaminant concentrations in soil and sediment (USEPA, 2000). Field and greenhouse studies completed in recent years indicate that several wetland plant species have a good potential for use in rhizodegradation of petroleum-related organics in soil and sediment (Cunningham et al., 1995; Lin et al., 1998). Phytodegradation involves the transformation of organic compounds by plant-generated enzymes within the plant tissue and in the rhizosphere via root exudates. Phytostabilization uses the biological activity in the rhizosphere to bind organic contaminants, which can reduce bioavailability and prevent contaminant leaching. However, phytostabilization of organic contaminants is not considered as important as degradation, since the former is potentially reversible and the latter is not.

Therefore, the focus of this review is on rhizosphere degradation of the less soluble, higher molecular weight PHC residues and PAHs that typically adsorb to soil and sediment particles, and tends to persist in the environment. Some advantages of phytoremediation for organic contaminants are:

- in-situ, on-site technology
- decreased handling and transportation of contaminated sediment
- lowered costs
- reduced future disposal liabilities
- more environmentally friendly than physicochemical technologies
- good potential for dual use as a remedial measure and restoration for ecologically-sensitive areas (i.e., wetlands mitigation)

Some disadvantages include:

- limited to shallow sediments (up to several feet, depending on plant species)
- regulatory acceptance more difficult due to emerging technology status
- long-term remediation time compared with other technologies (e.g., excavation and disposal)
- treatability studies are complicated by long time requirements, lack of standard measurement/monitoring parameters, multiple confounding environmental variables (e.g., precipitation patterns, sediment-contaminant heterogeneity, nutrient variations)
- potential increased exposure to receptors
- high contaminant concentrations may impede degradation and/or may be toxic to plants

Projections of the U.S. domestic market for phytoremediation technology range as high as $370 million from 1998 to 2005; costs for full-scale application of phytoremediation at contaminated sites are estimated to be 50% to 80% less than conventional alternatives, like excavation and disposal (Rock and Sayre, 1998). Assuming these estimates are accurate, the use of plants for cleanup of contaminated sites could save $370 million to $1.48 billion in remediation costs, not to mention other benefits associated with establishing plant communities, like added wildlife habitat and aesthetic value.

Although some forms of phytoremediation have been selected as part of a remedial approach at a number of sites, the database required for general regulatory acceptance is limited due to a lack of research (USEPA, 2000). Thus, for phytoremediation to be more broadly applied and optimized, the fundamental processes must be further investigated.

The USEPA, National Science Foundation and US Department of Defense have all expressed interest in continued research into the fundamental mechanisms and interactions between microorganisms, plants and contaminants in sediment and soils that

result in degradation of hazardous substances and petroleum. The results of this research are intended to further the scientific understanding of fundamental mechanisms involved in phytoremediation and support its future use as an accepted remedial technology.

1.1 Attenuation Mechanisms

Plants may degrade organic contaminants by several methods, including direct uptake or release of enzymatic root exudates (i.e., phytodegradation) and enhancement of rhizo-microbial activity (i.e., rhizodegradation) (Anderson et al., 1993; USEPA, 2000). Phytodegradation mechanisms include plant enzymes or enzyme systems that act on contaminants within the plant tissue after they have been taken into the plant through the roots, or in the rhizosphere via plant enzymes exuded from the roots (Dec and Bollag 1994; Korte et al, 2000). Phytodegradation does not include plant-mediated microbial degradation, which is referred to as rhizodegradation. It should be noted that, while it is important to provide clear definitions for these terms, in practice it may be difficult to separate the activity of plant enzymes present in root exudates from those associated with microbial degradation sequences that mineralize complex organic contaminants.

Phytodegradation Mechanisms

After being absorbed by the plant, organic compounds can be stored without transformation, transformed by metabolic activity or degraded (Hathaway, 1989). According to Nakajima et al. (1996) higher plants can transform organic molecules into glycosyl conjugates, and enzymatic activity in plant leaves may also convert pyrene to 1-hydroxypyrene. Two substances, *B*-O-glucoside and *B*-O-glucuronide, which form

conjugates with pyrene, have been identified in the leaves of several species of woody plants. Cytochrome P-450, which can oxidize PAHs into smaller molecules like phenols and quinone, has also been identified in the tissue of higher plants (Nakajima et al., 1996). Positive correlations have been established between the presence and activities of plant enzyme isolates and transformations of organic contaminants in sediment; associated enzymes include dehalogenase, nitroreductase, peroxidase, laccase and nitrilase. (Schnoor et al., 1995). The presence and activity of these enzymes suggests that enzymes released into sediments by decay of plant material may contribute to degradation of organic pollutants in sediment (Cunningham et al., 1995). Studies incorporating mass balance analyses show that some plant enzymes can break down organic pollutants and incorporate the degradation products into plant and soil organic material, while other enzymes mineralize organic contaminants (Schnoor et al., 1995).

One limitation of enzyme activity in the sediment is that subsurface conditions such as low pH, elevated metals concentrations and bacterial toxins can inactivate or destroy plant exudate enzymes. However, exudate enzymes are protected by the presence and activity of the plant roots, which can neutralize pH and chelate metals (Schnoor et al., 1995). According to the USEPA (2000), phytodegradation of organics associated with uptake of contaminants may be limited to the more soluble fractions of PHC. The uptake of higher molecular weight PHC and PAH compounds is likely limited by low solubility, partitioning to sediment particles, and effects of weathering (aging) on bioavailability (Schnoor et al., 1985).

There are some potential advantages to phytodegradation of PHC and PAH in sediments by plant enzymes released via root exudation or decaying plant material (e.g., plant degradation of organics that are toxic to microorganisms). However, the referenced literature indicates that microbial activity in the rhizosphere likely accounts for the majority of degradation of the larger, less mobile organic compounds (i.e., PHC and PAH) in soil and sediment.

Rhizodegradation Mechanisms

Rhizodegradation exploits plant rhizosphere microbial communities, which can accelerate the rate of PHC degradation (Anderson and Coats, 1994). This type of phytoremediation is most amenable to sites with contaminants in the surface and shallow soils and sediment, within reach of plant root systems (USEPA, 2000). Bioremediation of higher molecular weight PAH and PHC compounds in non-vegetated media has sometimes proven unsuccessful (Wilson et al., 1993) and sometimes requires improvement (Romantschuk et al., 2000; Yerushalmi et al., 2003). According to Sandmann (1984) and Walton (1990), microbial activity and degradation of recalcitrant organic substances, such as PAH, is greater in rhizosphere soil as compared to non-vegetated soil (i.e., bulk soil). Plants release dissolved organic carbon from their roots in the form of sugars, alcohols and acids, which can be good substrates for microorganism growth (Moser and Haselwandter, 1983). Plant exudates may also include enzymes that may catalyze important steps in the sequence of organic contaminant degradation. PHC degradation in the rhizosphere can occur from increased microbial activity fostered by increases of plant root exudates, which may be positively correlated with production of

biomass (Sandmann and Loos, 1984; Walton and Anderson, 1990). In addition, increased microbial numbers and activity can also be enhanced by the use of plant fertilizers (Lin et al., 1998).

Degradation of organic contaminants in the rhizosphere relies on plants and associated microbiota, which are limited by availability of moisture, oxygen and nutrients, and are also affected by other conditions including pH, salinity and pollutants. These physicochemical conditions are to some extent controlled by plant root exudates to favor relatively active synergistic microbial assemblages. A factor that may contribute to increased organics degradation in the rhizosphere may be that plant root systems exhibit a tremendous surface area (Atlas and Bartha, 1998). As an example, the length of the roots of a typical wheat plant can be 200 meters, with more than 6 m^2 of surface area. In addition, plant root exudates containing organic substrates and nutrients enhance microbial populations in the rhizosphere. Typical rhizosphere microbial numbers are 2 to 5 times higher than populations in non-vegetated soil, although it is not uncommon to find microbial numbers 100 times above those in non-vegetated soil (Gray and Parkinson 1968). Some important rhizosphere microorganisms include bacteria like pseudomonads and actinomycetes, and fungi (e.g., mycorrhizae), the latter of which establish very close mutualistic relationships with plant root systems that enhance performance of both species (Smith and Read 1997).

Effective degradation of PHC and PAH in the rhizosphere may depend upon root growth and density in contaminated sediment. Depending on the type, age and concentration of the contaminants, roots may grow into contaminated zones or they may avoid them. In addition, contaminants may cause root stress and death at higher

concentrations (Pezeshki, 2000). Thus, a possible limitation of rhizodegradation is the potential for new root growth to avoid higher contaminant concentrations (Schnoor et al., 1995), which could result in non-uniform degradation of PHC in some sediment. However, this limitation may be overcome by the plants response to stress. Typical plant responses to rhizosphere stressors, such as the presence of organic pollutants, include enhancement of existing metabolic pathways or development of new pathways (Pletsch et al., 1999), both of which can lead to stimulation of plant-mediated contaminant decay in the root zone.

1.2 Microbial Processes

Naturally occurring microbial processes in the environment provide an important function in the bioavailability of nutrients, carbon and oxygen in soil and sediments. Biogeochemical cycling of nutrients (e.g., nitrogen, phosphorus, sulfur), release of carbon through the degradation of organic matter, and oxidation/reduction (redox) conditions in the subsurface are all profoundly influenced by microbial activity. As described above, the rhizosphere of most plants typically contains an abundance of microorganisms that is more vigorous and diverse than that in non-vegetated soil and sediment due to the activity of the plant roots. Thus, (1) the mechanism of rhizodegradation is a function of the microbial processes that result in PHC and PAH degradation in root zone sediments; and (2) laboratory microbial biodegradability test data can serve as indicators of bioavailability and phytodegradation potential (Cunningham et al., 1995).

Effects of Plant Roots on Microbial Populations

Bacteria can colonize 4% to 10% of root surfaces in sediment, and the rhizosphere generally contains an abundance of culturable microorganisms, both in numbers and diversity, with typical communities of $5x10^6$ bacteria, $9x10^5$ actinomycetes and $2x10^3$ fungi per dry gram of soil (Foth, 1991; Shimp et al., 1993). Plant roots supply oxygen to the rhizosphere and release exudates through the roots, which can encourage degradation of organic contaminants by co-metabolic microbial processes (Joner and Leyval 2003). These processes may include direct transformation of the contaminant by plant enzymes and/or stimulation of microbial diversity, populations and activity. Oxygen is transported to the rhizosphere in many wetland plants via the aerenchyma system (Armstrong, 1978; Armstrong, 1979; San-Jensen et al., 1982; Bedford et al., 1991). The supply of oxygen to the rhizosphere through the roots can enhance aerobic degradation of organic compounds. In tidal marshes, more oxic subsurface conditions may be enhanced by plant transpiration (Dacey, 1984).

PHC Degradation

Naturally occurring microbial assemblages have the capacity to degrade PHC. Petroleum hydrocarbons are amenable to treatment by microbiological degradation and PHC-degrading microorganisms are naturally present in the subsurface (i.e., soil and sediment) environment (Borden, 1994). In addition, large influx of PHC can stimulate an immediate increase of hydrocarbon degrading microbial populations in sediment, especially where populations have a history of PHC exposure (Li et al., 1990; Lin and Mendelssohn, 1996; Hayes et al., 1999). Microbial communities that are exposed to PHC

exhibit genetic changes and selection that result in sediments that contain elevated numbers of bacteria that have the ability to degrade hydrocarbons (Atlas and Bartha, 1998; Hayes et al., 1999).

Experiments conducted by Carman et al. (1996) identified microbial degradation of PAH in salt marsh sediments obtained from non-vegetated estuarine mud flats surrounded by *Spartina alterniflora* marsh. Sediments treated with diesel fuel exhibited degradation half-lives of 4.5 days for sediments historically exposed to petroleum hydrocarbons and 137 days for ambient sediments with no prior exposure history. Based on the results of microbial analysis of the sediment treatments, microbial numbers were similar regardless of PHC exposure history, but previously exposed sediments exhibited a significantly greater capacity to degrade PAH. Also, the degradation rate of ^{14}C-labeled phenanthrene increased with increased addition of diesel fuel. The study conducted by Carman et al. (1996) was limited in that it only considered the upper 1 cm of sediment, which is typically an oxic environment where microbial degradation occurs aerobically. In addition, ^{14}C-acetate was added to treatments, which could have contributed to the PAH decay by enhancing co-metabolic processes similar to those induced by plant exudates in the rhizosphere. Despite these apparent limitations, the study confirms PHC and PAH degradation by indigenous marsh sediment microbiota.

Earlier investigations of aquatic sediments describe the decay of organic compounds by microbial consortia (Nedwell, 1984). More recent studies using molecular methods have identified salt marsh plant rhizosphere organisms that can degrade PAH (Daane et al., 2001).

Contaminant Biodegradability

Biodegradability of PHC and PAH varies depending on several factors including the size and type of the organic molecule (Cerniglia, 1993). Short-chain alkanes tend to be toxic to microorganisms, but evaporate quickly due to their high vapor pressure and low boiling point. Long-chain alkanes and polycyclic molecules generally have lower solubility, low vapor pressure and high soil/water partitioning coefficients. These characteristics result in resistance to biodegradation and persistence in soils and sediments (Carmichael et al., 1997).

Weathered PHC and PAH compounds are resistant to decay in natural environmental media such as soil, water and sediment and tend to persist as oil spill residue after more volatile and degradable hydrocarbon fractions have degraded (Alexander, 2000). In general, lower molecular weight (MW) PAH's (i.e., 2-3-rings) degrade faster than higher MW PAH's (≥ 4-ring) (Cerniglia, 1993; Atlas and Bartha, 1998; Kanaly et al., 2000). However, generalizations about PAH structure-biodegradability relationships that may apply to aerobic environments should not be assumed for anaerobic conditions without further research (Alexander, 1999).

The majority of published studies that report biodegradation of PAHs focus on lower molecular weight (MW) PAHs such as naphthalene and phenantherene (Schneider et al., 1996; Karthikeyan and Bhandari, 2001). In some of the literature, degradation of lower MW PAHs is used as a basis for general conclusions about PAH degradation in the environment (Gibson and Subramanian, 1984; Cerniglia and Heitkamp, 1989; Shuttleworth and Cerniglia, 1995). However, since lower MW PAHs are generally more biodegradable than the higher MW PAHs, the results of studies using low MW PAHs

cannot be used to accurately predict biodegradation of the more recalcitrant, high MW PAH compounds.

Research conducted in the last several years provides further insight regarding biodegradation of the higher MW PAHs (Kanaly and Haryama, 2000). Transformation and/or mineralization of 4-ring PAHs [e.g., fluoranthene (MW 190), pyrene (MW 216), chrysene (MW 228) and benzo(a)anthracene (MW 228)] as primary substrate or co-metabolism has been reported by several different researchers (Weissenfels et al., 1990; Weissenfels et al., 1991; Mueller et al., 1990; Mueller et al., 1989; Ye et al., 1996; Kelley et al., 1991; Kelley et al., 1991; Kelley et al., 1993; Grosser et al., 1991). Biodegradation of 5- and 6-ring PAHs, including ring cleavage and mineralization, has been reported for several compounds, such as benzo(a)pyrene, benzo(b)fluoranthene and dibenz(a,h)anthracene (Kanaly and Haryama, 2000).

According to Karthikeyan and Bhandari (2001), many of the published PAH degradation studies evaluate aerobic conditions, while relatively few address PAH transformations in microaerobic and anaerobic environments, which are common in saturated soil and sediment. Several publications indicate PAHs are transformed and/or mineralized in ground water, soil and sediment under nitrate- or sulfate-reducing conditions (Mihelcic et al., 1988; Coates et al., 1997; Hayes et al., 1999; Chang et al., 2002; Meckenstock et al., 2004) and under iron-reducing conditions (Kanaly and Haryama, 2000). While some of the studies apparently assumed redox conditions from microbial consortia or the oxidation state of major ion species, Ma and Love (2001) successfully used ORP measurements to monitor experimental sequencing batch reactor conditions in anaerobic degradation of monoaromatic compounds.

Enzyme systems associated with the biodegradation of PAHs include dioxygenases and monooxygenases (Kanaly and Haryama, 2000). Pyrene, benzo(a)anthracene and benzo(a)pyrene ring cleavage by *Mycobacterium* sp. strain RJGII-135 isolated from coal gas plant soils via dioxygenase enzymatic pathways has been reported (Schneider et al., 1996).

Microbial Co-metabolism

A general limitation of microbial degradation of PHC and PAH is the potential for incomplete degradation of the parent substance (Smith et al., 1999). Decay of a contaminant used as a primary substrate by a single microbial population may result in the transformation of the parent compound to another toxic intermediate that is resistant to further decay. Also, in the absence of another carbon source, microbial populations may crash before the contaminant of concern is degraded below the target concentration.

Co-metabolism refers to the degradation of organic compounds by a heterogeneous assemblage of microorganisms, where the compound(s) are sequentially transformed through a series of degradation steps that would not be possible for a homogeneous microbial population (Atlas and Bartha, 1998). Co-metabolic degradation has the potential to overcome the limitations of incomplete degradation of organic contaminants by the processes of a diverse microbial consortium using something other than the contaminant as primary substrate (McCarty and Semprini, 1994). Thus, co-metabolism can result in mineralization of complex organic compounds (Alexander, 1999), rather than transformation of organic substances to recalcitrant intermediates. For example, Kanaly et al. (2000) identified co-metabolic mineralization of benzo(a)pyrene

with diesel fuel by microbial consortia from the taxonomic class *Proteobacteria*, and genera *Mycobacterium* and *Sphingobacterium*.

The presence of lower molecular weight aromatic compounds can cause induction of the enzymes that degrade PAH (Atlas and Bartha, 1998). This suggests that mechanisms of co-metabolism may involve enzyme activation by the presence of other organic constituents associated with root exudates, such as acetate or mono-benzene ring structures cleaved from PAH during transformation. Co-metabolism appears as a significant decay process identified by many researchers investigating biodegradation of high MW PAHs (Kanaly and Haryama, 2000).

The potential advantage of rhizosphere microbial assemblages for co-metabolic degradation of PHC and PAH is the naturally high level of microbial activity and community diversity fostered by the plant roots, and the constant influx of degradable carbon from root exudates and degradable root biomass as primary substrate. In addition, many plants establish symbiotic associations with mycorrhizae, which are fungi that grow in and around the plant roots (Smith and Read, 1997). Mycorrhizae exhibit specialized enzymatic pathways for degradation of organic contaminants that cannot be metabolized by bacteria alone (Schnoor et al., 1995).

1.3 Physicochemical Effects

Geochemical conditions in sediments being considered for treatment via phytoremediation can have a major affect on PHC and PAH degradation mechanisms. Plants can tolerate higher organic contaminant concentrations than many microorganisms (Schnoor et al., 1995), providing the potential opportunity for development of microbial

communities that may not otherwise grow in contaminated bulk soils or sediment. The root systems of vascular plants also exhibit mechanisms to prevent phytotoxicity from some inorganic contaminants, such as dissolved metals (Grosse, 1997; Jaffe et al., 2002). This is especially important when evaluating the feasibility of using plants to treat or stabilize soils that contain mixed organic and metallic wastes. Factors that control the contaminant degradation kinetics include (1) the capability of the microbial consortia to degrade the material, (2) the chemical properties of the substance being degraded, and (3) environmental conditions such as pH, redox potential, and the presence of available oxygen and nutrients (Atlas and Bartha, 1998). However, these factors are interrelated, either directly by abiotic processes, or indirectly by shifts in equilibrium due to microbial activity. For example, microbial degradation of PHC consumes oxygen and creates reducing conditions in the subsurface (Borden, 1994). Some inorganics (e.g., divalent metals) become mobilized in reducing environments (Jorgensen, 1996). Thus, the mobilization of metals is a secondary, abiotic effect caused by the microbial decay of PHC. If the leached metal happens to be toxic to the microbial population/community degrading the PHC, the process may be inhibited.

Effects of PHC in Sediment

Petroleum hydrocarbons can penetrate sediments and cause acute and chronic damage to plants, including plant mortality (Krebs and Tanner, 1981; Ferrell et al., 1984; Alexander and Webb, 1987; Lin and Mendelssohn, 1996). Oil coating of leaves can block the flow of oxygen to the roots and increase root stress, which can be especially problematic in wetland plants (Armstrong, 1978). Root stress may also result from oil

coating the sediment surface, limiting oxygen exchange at the sediment surface and contributing to more hypoxic conditions at shallower sediment depths (Pezeshki et al., 2000). The presence of PHC in soils and sediments can also cause root damage and plant mortality from direct toxicity, or indirectly from anoxic conditions and generation of H_2S gas resulting from biodegradation (Atlas and Bartha, 1998). However, these conditions may apply more to the initial impacts of oil spills due to the nature of the oil to coat the surface and block natural movement of air and pore water. Sediments that contain weathered PHC and PAH from historical sources, even at relatively high concentrations (e.g., 10 - 15%), do not exhibit the severe phytotoxic effects as those observed in areas subjected to fresh oil spills (Lin and Mendelssohn, 1998).

Nitrogen cycling and availability of usable forms of nitrogen is of major importance for plant survival and growth. According to Carman et al. (2000), PHC in sediment causes NH_4^+ efflux from sediment due to several potential co-acting mechanisms, including increased microbial activity in response to the carbon present in PHC, release of N from complex compounds by PAH degraders, and microbial co-metabolism of refractory organic detritus stimulated by the presence of PHC. Increases in salt marsh sediment microalgae populations and increased growth of wetland macrophytes in PHC-contaminated sediments appear to be responses to the nitrogen made available by microbial degradation of PHC and PAH (Carman et al., 2000). The increase in release of nitrogen is likely a short-term condition since PHC does not contain nitrogen.

Effects of Iron and Sulfur in Sediment

The abundance of iron and sulfate in sediment is controlled primarily by the site location. Iron is often found at high concentrations as part of the mineral content in many types of sediment due to its presence in most parent material of surface geologic formations (i.e., underlying bedrock). Marine and estuarine environments are provided an abundant supply of sulfur, which is normally present at significant concentrations in seawater in the form of sulfate (SO_4). The presence of both iron and sulfur in sediment can affect the microbial degradation pathway of PAH (Coates et al., 1996; Chang et al., 2002). Under anaerobic conditions, which are common in wetland sediments below a depth of about 5 cm, benzene can be mineralized by iron reducing microorganisms in the presence of chelated ferric iron, and sulfate reducers can completely oxidize aromatics (i.e., benzene) and PAH (i.e., naphthalene and phenanthrene) (Atlas and Bartha, 1998).

Contaminant Bioavailability

The effectiveness of rhizodegradation depends on the bioavailability of the contaminant(s), which is related to several factors including chemodynamic partitioning and the type of sediment present (e.g., clay content, organic matter) (Cunningham et al., 1995). PHC and related compounds, like PAH, adsorb to sediment particles, which may render them less toxic but may cause them to be less vulnerable to biodegradation (Atlas and Bartha, 1998). According to Kukkonen et al. (2003), desorption kinetics of pyrene efflux from sediment are closely related to the type of organic matter present. A recent study by Jonker et al. (2003) indicates that sorption kinetics of higher MW PAHs are

similar for natural organic carbon and petroleum hydrocarbons derived from oil contamination.

Alexander (2000) suggested that weathering or aging of some organic compounds, such as PAH, can make them less bioavailable for degradation by microorganisms. Experiments conducted by Lin and Mendelssohn (1998) indicates that fresh (i.e., unweathered) oil is more toxic to some wetland plants than weathered oil. Although the detailed mechanisms of contaminant weathering after release to the environment are not completely understood, there is evidence that indicates it may be associated with sorption to fine particles and organic carbon in the sediment (Alexander, 2000). Decreases in microbial degradation rates of PAH in non-vegetated soil over time have been used as evidence of lowered availability of PAH due to aging (Carmichael et al., 1997; Alexander, 2000). The frequent failure of bioremediation to achieve cleanup goals may be due to the presence of contaminants that have become unavailable to microorganisms via the process of weathering.

The rate of microbial degradation of organic contaminants can be faster than the rates of abiotic desorption (Carmichael et al., 1997). However, some microorganisms can produce surfactants that desorb organic compounds from soil (Scheibenbogen et al., 1994). Weathered PAH in sediment likely requires biologically-catalyzed desorption from sediment particles by surfactants or plant root exudates to make them bioavailable for attack by microorganisms. Lin and Mendelssohn (1998) demonstrated degradation of weathered PHC and PAH in rhizosphere sediments, indicating that root exudates and associated microbial assemblages, and/or plant exudate enzymes, may be catalyzing desorption, causing these compounds to become vulnerable to microbial degradation. The

experiments involved investigation of PHC impacts to established plants and transplanting *S. alterniflora* and *S. patens* in PHC-contaminated salt marsh sediments, to evaluate plant survival, PHC degradation rates and effects of fertilizer. The microcosm study used 40 sods, each with a 20 cm diameter. Controls were maintained with no PHC added and three concentration treatments, medium (~2.5%), high (~5%) and very high (~40%) were made by adding varying amounts of Louisiana crude oil to the sod pots. Most of the original plants died in all but the lowest treatment soon after addition of oil. However, after two years, transplanted *S. alterniflora* and *S. patens* survived in all treatments and biomass was significantly increased by adding fertilizer in all but the highest concentration sods. During the one-year period after transplanting, PHC concentrations decreased by 28.8% in unfertilized sediments and 58.5% in fertilized treatments, regardless of concentration. These studies demonstrate that establishing and fertilizing plants in sediments containing weathered oil has potential as a remedial alternative for PHC and PAH-contaminated marsh sediments.

Rates of Rhizodegradation

PHC and PAH degradation is controlled by several physicochemical factors, such as availability of nutrients, contaminants desorption rate and contaminant solubility. In general, rates of hydrocarbon microbial degradation will be sub-optimal if the contaminated media is nutrient limited (Venosa et al., 1996). Increased microbial numbers and activity, which is responsible for co-metabolic degradation of PHC in the rhizosphere, may also be enhanced by the use of plant fertilizers (Lin et al., 1998). PHC and PAH degradation rates may be limited by contaminant/sediment desorption kinetics

(Carmichael et al., 1997). PHC decay rates may also be affected by sediment type, such as particle size distribution, clay and non-petroleum organic carbon content, and availability of nutrients. Soils that contain high amounts of clay tend to exhibit slower PHC degradation rates as compared to soils with lower clay content (Apitz et al., 1996) because petroleum binds tightly to the clay particles, making it less bioavailable. Phytoremediation requires more time than other conventional cleanup technologies (e.g., excavation) to achieve remedial standards due, in part to the low solubility and high Log K_{ow} (i.e., octanol-water partition coefficient) of PHC and PAH (Schnoor et al., 1995).

Another physical effect associated with rhizodegradation involves mass transfer of water in the subsurface. Plants may induce movement of dissolved contaminants into and through the rhizosphere by the action of evapotranspiration (Ferro et al., 1994). According to Liste and Alexander (1999), this phenomenon may also occur with more soluble PAH, which can be drawn into the rhizosphere where their concentrations are reduced to below detectable levels. The PAH desorption kinetics would likely be a limiting factor in this process.

Experiments conducted by Venosa et al. (1996) confirmed the assumption that biodegradation of PAH in salt marsh sediments follows first-order reaction rate kinetics. Data generated by their study was evaluated using a variation of the following first-order rate equation:

$$C_t = C_o e^{-kt} \qquad (1.1)$$

Where

C_t = Contaminant concentration at time t
C_o = Contaminant concentration at time t = 0
k = first-order rate constant (k/t)

Degradation rates estimated from simultaneous laboratory respirometer tests support those generated from field plot experiments that describe first-order rate kinetics for degradation of PAH in sediment (Venosa et al., 1996). Reductions of 80 mg/l of weathered PHC per day (6-month average) were measured in vegetated salt marsh sediments (see Chapter 2). This is significantly more than the biodegradation estimates of 1 to 30 µg/l of crude oil per day in seawater (Atlas and Bartha, 1998).

Hopane has been identified as a conservative internal standard for evaluating biodegradation of PAHs (Butler et al., 1991; Douglas et al., 1994) using the following calculation:

$$\% \text{ Biodegradation (for time t)} = [1 - C_t/C_0 \times H_0/H_t] \times 100\% \qquad (1.2)$$

Where

C_0 = initial compound concentration (ppm)
C_t = compound concentration (ppm) at time t
H_0 = initial hopane concentration (ppm)
H_t = hopane concentration (ppm) at time t

However, Huesemann et al. (2003) found that hopane is not always conserved and indiscriminate use of hopane as a biomarker to normalize PAH data for evaluation of biodegradation can generate erroneous results. The study identified benzo(a)pyrene as more stable than hopane in some batch experiments, and suggests use of recalcitrant PAHs rather than hopane as biomarkers for data normalization in evaluating the more degradable PAHs. While the use of biomarkers for evaluating biodegradation in this way seems promising, identifying a universally acceptable internal standard will require more research.

Abiotic Advantages of Phytoremediation

According to Schnoor et al. (1995), physicochemical advantages of phytoremediation at contaminated sites include:

1) increase of sediment organic carbon, which enhances microbial activity and retards subsurface contaminant migration;

2) stabilization of soil by plant roots, which can decrease uncontrolled mass transport of contaminants away from impacted areas by reducing wind-blown dust and water erosion; and

3) removal of sediment pore water via evapotranspiration, which can decrease the rate and volume of water infiltration from surface precipitation to ground water.

1.4 Phytoenzymology

Exposure Mechanisms

Plants exchange liquids, gases and dissolved chemicals between plant tissue (e.g., roots, leaves) and the surrounding soil, water, and air. After being absorbed by the plant, organic compounds can be stored without transformation, transformed by metabolic activity, or degraded (Cunningham et al., 1995).

Plants release exudates to the soil from the roots that may include high concentrations of dissolved organic carbon in the form of sugars, alcohols and organic acids, which can be good substrates for microorganism growth (Schnoor et al., 1995). Plant exudates also include enzymes that may catalyze important steps in the sequence of organic contaminant degradation, or increase their solubility and uptake by plant roots.

According to the USEPA (2000), phytodegradation of organics associated with uptake of contaminants may be limited to the more soluble fractions of PHC. The uptake

of higher molecular weight PHC and PAH compounds is likely limited by low solubility, partitioning to sediment particles and effects of weathering (aging) on bioavailability.

Weathered PAH in sediment likely requires biologically-catalyzed desorption from sediment particles by surfactants or plant root exudate enzymes to make them bioavailable for transport into plant roots or subject to attack by microorganisms. Lin et al. (1998) demonstrated degradation of weathered PHC and PAH in rhizosphere sediments, indicating that root exudates and/or associated microbial assemblages, may be catalyzing desorption, causing these compounds to become more soluble and vulnerable to microbial degradation or plant uptake. Organic compounds can also penetrate leaves through the stomata and through the cuticle, although the waxy cuticles of some plants can resist absorption (Korte et al., 2000).

According to Shimabukuro and Walsh (1979), xenobiotic metabolism by plants is a function of the degree of penetration of the chemical into the plant internal cells and tissue via the primary exposure routes of absorption through the roots and leaves. Once inside the plant, absorbed hydrocarbons and their transformation intermediates can be translocated from leaves to roots and roots to leaves, respectively (Korte et al., 2000). Earlier research demonstrated that organic xenobiotics absorbed by leaves are excreted by plant root systems (Chandler et al., 1974). Absorption, translocation and metabolism of alkanes and arenes in solution have been reported for several plant species (Korte et al., 2000).

Classes of Plant Enzymes and the "Green Liver" Concept

Sandermann (1977) reported that plants, like animals, could metabolize many different types of organic xenobiotics, including PAH. Plant enzymes active in biotransformations of organic xenobiotics identified by Cole (1983) include hydroxylases (as mixed function oxidases) and cytochrome P_{450}. Early studies of plant enzymatic metabolism of organic pesticides identified peroxidases, mixed function oxidases and glutathione-S-transfersases among others (Lamoureux and Frear, 1979), which have more recently been identified as important plant enzymes associated with metabolism of PHCs and PAH. Cole (1983) provides a review of the more prevalent classes of Phase I oxidation found in plants that are integral to the degradation of organic xenobiotics, including β-oxidation, ring hydroxylation, alkyl hydroxylation, sulfoxidation, and oxidative desulfuration. Although Cole (1983) focuses on pesticide metabolism, it notes the oxidation of benzo(a)pyrene to polar metabolites (quinone isomers) in pea leaf and soybean microsomal cell suspension cultures (Figure 1.1).

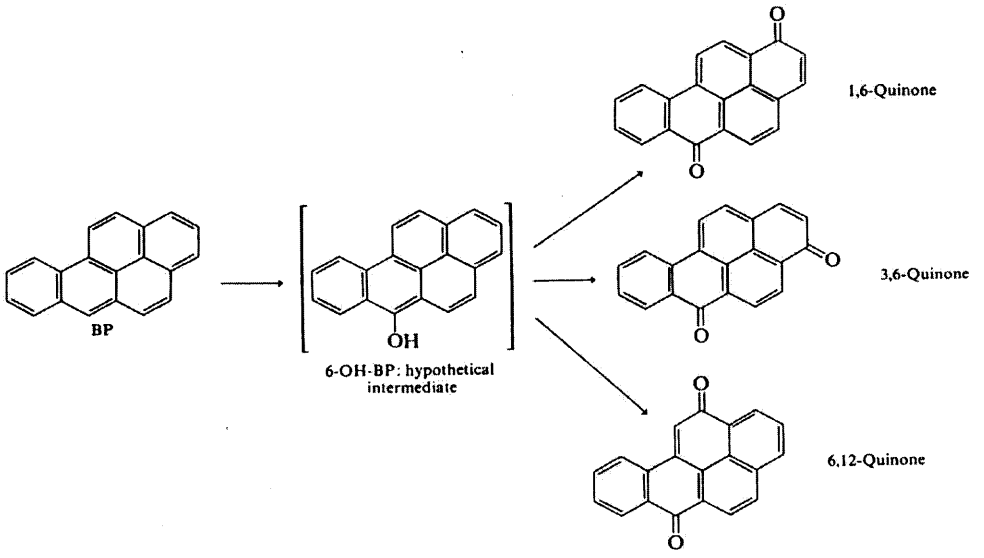


Figure 1.1 Microsomal oxidation of benzo(a)pyrene (Cole, 1983).

There are stark similarities between plant and animal enzymes and enzyme systems, examples being mixed function oxidases and cytochrome P_{450}, which are found in both plants and animals (Cole, 1983). At least two major enzymatic systems common to animal liver function, cytochrome P_{450} monooxygenases and glutathione transferases, were identified in plants as early as the mid-1970's (Sandermann, 1977).

Based on the similarity of the enzymatic pathways of plants and animal liver metabolism, Sandermann (1977) introduced the "green liver"concept, suggesting that plant function is analogous to liver function with regard to pollutant metabolism/assimilation on a global scale. More recent research by Sandermann (1992) indicates some enzymes found in both plants and animals exhibit similar molecular masses in addition to functional similarities. Examples include cytochromes P_{450} (~55

kDa), NADPH-cytochrome P_{450} reductase (~80 kDa) and subunits of glutathione transferases (~25 kDa per subunit).

The relatively recent advances in DNA sequencing provide additional tools for comparing plant and animal enzymes. According to Sandermann (1992), cDNA sequences mapped for several enzymes of different plant species were similar to those of mammalian, insect and microbial enzymes. An example includes homologous glutathione transferase protein sequences found in maize, rats, humans and *E. coli.*

In the early 1980's, the ability of plants to metabolize xenobiotics was considered limited compared with other organisms, i.e., animals and microorganisms (Cole, 1983). However, some of the most current investigations of plant genome sequencing and other biochemical research indicates that plant DNA harbors the capacity to produce an especially large diversity of enzymes that metabolize a wide spectrum of organic xenobiotics (Schaffner et al., 2002). Based on review of the entire gene code for one plant (*Arabidopsis thaliana*), the capacity of plant enzymes to degrade organics may even exceed that of rhizosphere microorganisms. Plants also possess the ability to grow in media that are too contaminated for microbial growth, which may be indicative of their more diverse enzymatic potential.

Research conducted by Sashwati et al. (1996) documents the activity of the Halliwell-Asada pathway enzymes (i.e., ascorbate peroxidase, superoxide dismutase and glutathione reductase) in plants. The fact that these enzymes are also prominent in animals underscores the stark similarities in the biochemistry of living systems and lends credence to the "green liver" concept of xenobiotic metabolism by plants.

In order to advance phytoremediation as a usable technology for PHC and PAH, a knowledge of the biotransformation mechanisms involved in organic contaminant degradation by plants and the fate of related enzymatic metabolites is necessary.

Transformation Mechanisms

Most organic xenobiotics studied induce enzymatic activity in plant cells, and their inductive effect is generally higher than endogenous metabolites (Korte et al., 2000). One study of plant enzymes obtained from inducer solutions exhibited an ability to transform xenobiotic substrate that was chemically unlike the inducer species, indicating a non-specific monooxygenase activity wider than that of analogous animal enzyme systems.

Some notable xenobiotic oxidative degradation processes involving plant enzymes include hydroxylation and hydrolysis (Korte et al., 2000). A common sequence of exogenous alkane oxidation by plants includes initial hydroxylation of alkyl groups and formation of intermediates, which can be completely mineralized to carbon dioxide. Initial enzymatic oxidative degradation of organic xenobiotics in plants includes enzymatic activation of atmospheric oxygen (O_2) to singlet oxygen (superoxide; $O^{\cdot}$) and insertion of singlet oxygen into the substrate molecule (monooxygenation), resulting in hydroxylation, as illustrated in Figure 1.2.

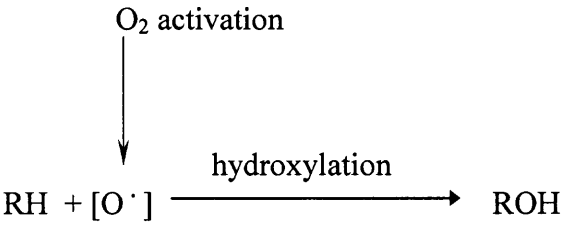


Figure 1.2 Schematic diagram of monooxygenation of hydrocarbon (Korte et al., 2000).

Enzymes identified by Korte et al. (2000) as using previously activated superoxide include catalase and peroxidase; NADH-dependent monooxygenases, which activate oxygen, include ascorbatoxidase and phenoloxidases. Hydroxylation of benzene, naphthalene and benzo(a)pyrene by microsomal monooxygenase in plants has been demonstrated (Trenck and Sandermann, 1980). Enzymatic oxidation of benzo(a)pyrene yields quinone isomers, which may be followed by ring cleavage (Cole, 1983; Kirso et al., 1983; Warshawsky et al., 1983).

In a recent study by Chroma et al (2002), plant cell cultures were treated with a PAH mixture, including acenaphthalene, anthracene, phenanthrene and pyrene, to identify enzymatic transformation of these substrates; peroxidase activity was positively correlated with PAH transformation. Also, peroxidase and cytochrome P_{450} inhibitors significantly decreased plant cell metabolism of PAH, indicating involvement of both enzyme systems. Plant tripeptides, including glutathione and homoglutathione, form conjugates with hydroxyl groups of xenobiotics, such as hydroxylated benzo(a)pyrene (Jansen et al., 1977). Some of these reactions are catalyzed by glutathione-S-transferase.

Enzyme systems important to xenobiotic metabolism in plants can be affected by intracellular distribution of enzymes. For example, microsomal cytochrome P_{450} reductase has been identified in both membrane-bound and soluble form in soybean roots (Cole, 1983). The *in vivo* activity of cytochrome P_{450} monooxygenases and reductases may be somewhat limited due to localization of these enzymes in the cell membrane (Strobel et al., 1989). For example, chloroplasts, found within the photosynthetic parts of higher plants (e.g., leaf, stem) have been shown to contain robust enzyme systems with the capacity for complete xenobiotic degradation from hydroxylation to aromatic ring cleavage. Enzymes identified in chloroplasts include peroxidase and catalase. Chloroplast enzyme systems also exhibit light-dependent and age-dependent activities (Korte et al., 2000).

According to Korte et al. (2000), hydroxylation is also suspected in the plant metabolism of cyclic and aromatic hydrocarbons, such as cyclohexane, benzene, phenol, benzo(a)pyrene, benzo(a)anthracene and dibenzo(a)anthracene, where the ring structures are cleaved and aliphatic products are formed. This is based on research observations indicating significant increases of hydroxylase activity associated with phenoxyacetic acid formation, the latter of which is important in the deep oxidation of aromatic rings in plants. Aromatic ring hydroxylation is immediately followed by glycosylation (Korte et al, 2000). Nakajima et al. (1996) demonstrated that higher plants can transform organic molecules into glycosyl conjugates, and enzymatic activity in plant leaves may also convert pyrene to 1-hydroxypyrene. Two substances, β-O-glucoside and β-O-glucuronide, which form conjugates with pyrene, were identified in the leaves of several species of woody plants. Cytochrome P_{450}, which can oxidize PAHs into quinone or

smaller molecules (e.g., phenols), has also been identified in the tissue of higher plants (Nakajima et al., 1996).

Several research studies using ^{14}C-labeled substrates have been published that describe phytooxidation of short- and long-chain alkanes, cycloalkanes and aromatic hydrocarbons, and incorporation of the substrate carbon molecules into endogenous metabolites, such as carbonic and amino acids (Korte et al., 2000). Muconic acid and fumaric acid production are also associated with benzene and phenol ring cleavage by plant enzymatic oxidation (Korte et al., 2000).

Recent experiments with lucerne (*Medicago sativa* L.) demonstrated that oxydoreductase activities in the roots increased in the presence of arbuscular mycorrhizal fungi (AM), and anthracene (Criquet et al., 2000). The study identifies peroxidases as the dominant oxidative enzyme species in lucerne roots, which were induced by AM and anthracene. Laccase was also detected but activities were inhibited by AM, and not induced by anthracene.

Fate of Transformation Products

Plants can translocate organic pollutants between different plant tissues, where they may be transpired, transformed or mineralized by enzymes (Macek et al., 2000). Products of transformation may be stored in vacuoles or used in cell structural polymers (e.g., lignin).

According to Sandermann (1992), metabolism of xenobiotics by plants is similar to animal liver function, including substrate transformation and conjugation reactions. However, while animals can excrete polar metabolites, plants may compartmentalize them in vacuoles or incorporate them into the cell wall structure (e.g., lignin).

Studies of mass balance analyses show some plant enzymes can break down organic pollutants and incorporate the degradation products into plant and soil organic material, while other enzymes mineralize organic contaminants (Schnoor et al., 1995). According to Cole (1983), quinones derived from oxidation of benzo(a)pyrene can form polymers that resemble lignin in the presence of peroxidase. Benzo(a)pyrene and dissolved benzo(a)pyrene metabolites were found associated with lignin and in suspension cultures of parsley and soybean (Mumma and Davidonis, 1983).

Higher plants can uptake and accumulate more soluble hydrocarbons from soil and water. Korte et al. (2000) described hyperaccumulation of benzo(a)pyrene in English ryegrass (*Lolium perenne*) to concentrations three orders of magnitude (x1,000) above water concentrations in hydroponic studies. However, the applicability of these results to weathered PAH in sediment is uncertain due to their low solubility and affinity for sediment clay and organic matter.

Transformation and conjugation of lipophilic organics like PHC and PAH by plant enzymes produce polar metabolites that are generally more soluble than the parent compounds, thus allowing their translocation within plant tissue and excretion from roots and leaves (Korte et al., 2000). Xenobiotic conjugates can be stored as soluble or insoluble compounds that may be available to the food chain upon plant tissue decay or digestion (Sandermann, 1992). According to Cole (1983), evidence from research

suggests that plant storage of xenobiotic conjugates may be released into the gut of animals upon ingestion of previously contaminated vegetation (Cole, 1983).

Organic xenobiotics can affect changes to plant cell structure and metabolism (Korte et al., 2000). Although plant cells can assimilate low concentrations of PAHs without the cells incurring structural damage, exposure to elevated concentrations of PAH can adversely affect the nuclear membrane, interrupt DNA synthesis, and cause loss of mitochondrial content, leading to cell destruction. Thus, plants are expected to exhibit physiological manifestations of stress consistent with the damage mechanism caused by exposure to higher PAH concentrations.

Reaction Kinetics and Induction

The biodegradation of organic contaminants is essentially driven by enzyme activity. Enzyme kinetics can be described by reaction mechanisms and thermodynamic equilibrium. Enzymatic transformation of PHC and PAH is generally expected to follow zero-order and first-order reaction kinetics. Zero-order reactions are limited by the rate constant (k). First-order reactions are a function of the rate constant (k) and substrate concentration. Temperature and pH are key factors affecting enzyme activity. Reaction energy, inhibition and induction are also considered in evaluation of enzyme activity and kinetics.

Factors effecting xenobiotic transformation in plant tissue cultures include age, incubation period, cell type/source, culture medium, etc. (Mumma and Davidonis, 1983). An example of age-dependency includes enzyme levels in older rice plants cells that were measured at 2 to 4 times greater than enzyme levels in younger rice plant cell cultures is

an example of age-dependent metabolism. An example of age and cell type factors is found in chloroplast enzyme systems that exhibit light-dependent and age-dependent activities (Korte et al., 2000).

The transformation of organic contaminants by plant enzymes is fast compared to their rate of desorption from soils matrix; however, PHC and PAH degradation rates are limited by contaminant-soil desorption kinetics (Carmichael et al., 1997). For example, soils that contain high amounts of clay tend to exhibit slower PHC degradation rates as compared to soils with lower clay content (Apitz, 1996). Thus, PHC decay rates in rhizosphere soils are likely affected by soil structure and mineralogy, such as particle size distribution, percent clay, organic carbon content and availability of nutrients.

Sashwati et al. (1996) completed an investigation of PAH bioaccumulation and enzymatic response to PAH in aquatic moss (*Fontinalis antipyretica*) on Lake Kallavesi, Finland. The research hypothesis was based on the premise that there is a measurable enzymatic response in plants to limit concentrations of strong oxidizers, such as hydrogen peroxide, and free radicales of hydroxide and oxygen (i.e., superoxide), which may be generated by PAH. The study documented significant increases in moss enzymatic activities (induction) of antioxidant enzymes such as peroxidase, ascorbate peroxidase and superoxide dismutase [SOD] in response to low-level (ppt-range) PAH in surface water. For example, SOD activity in moss exposed to higher PAH concentrations was up to 80% higher than SOD activities in moss exposed to low PAH concentrations. In addition, a strong correlation was observed between PAH concentrations and activities of the noted enzymes.

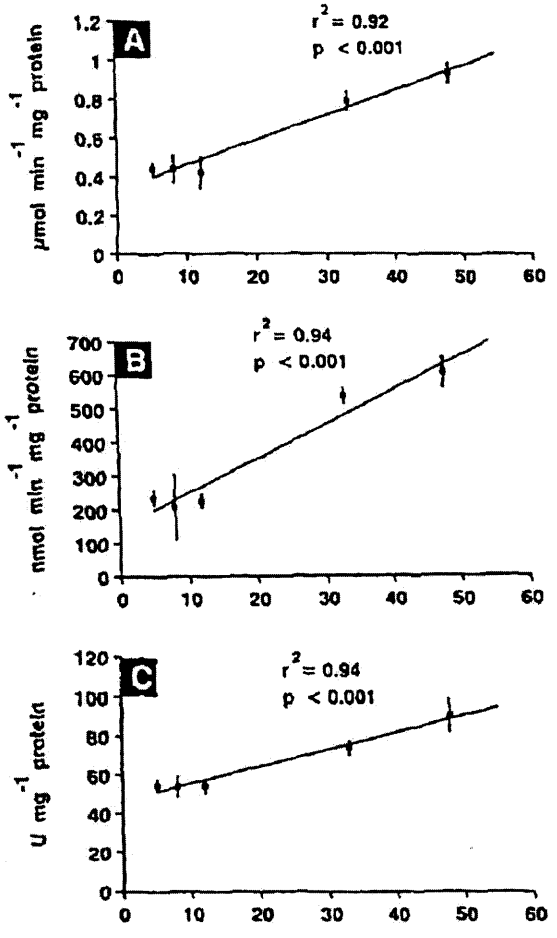


Figure 1.3 Relationship between the levels (ng/g dry weight) of total PAHs (mean; n = 4) content in moss tissue and activities (mean ± SD; n = 12) of peroxidase (A); ascorbate peroxidase (B); and superoxide dismutase (C) transplanted at five sites on Lake Kallavesi, Finland (Sashwati et al., 1996).

A notable limitation of enzyme activity in the rhizosphere is that unfavorable subsurface conditions such as low pH, elevated metals concentrations and bacterial toxins can inactivate or destroy plant exudate enzymes. However, exudate enzymes can also be protected by the presence and activity of the plant roots, which can control soil pH and chelated metals in the root zone (Schnoor et al., 1995).

According to Korte et al. (2000) plants differ in their xenobiotic assimilation capacity; examples include maple leaves (*Acer campestre*) that absorb benzene in air at 100 times that of alder (*Alnus barbata*) and 1000 times that of white mulberry (*Morus alba*).

Use and Limitations of Enzyme Study Data

Enzymatic experiments with xenobiotics were commonly performed by application of substrate chemicals to the roots or by injection into the stems and fruits of whole plants. However, the difficulties associated with accounting for non-enzymatic factors, such as root and leaf absorption, translocation, etc. complicate investigation of enzyme activities when using whole plant studies (Shimabukuro and Walsh, 1979).

The use of *in-vitro* plant tissue assays and plant cell culture assays to investigate phytometabolism and phytoenzyme system function is becoming more common. *In-vitro* techniques that employ isolated leaves, roots and cells represent lower-cost methods for xenobiotic metabolic investigations. When using excised leaves for *in-vitro* assays, absorption and translocation of organic xenobiotics is very rapid and occurs through the cut edge of the exposed tissue (Shimabukuro and Walsh, 1979). Mumma and Hamilton (1979) suggested that pathways of xenobiotic metabolism within whole plants could be approximated with data from investigations using plant tissue cultures comprised of undifferentiated plant cell cultures (i.e., callus cultures). According to Macek (2000), plant tissue culture assays have several advantages over conventional whole plant and hydroponic designs, including:

1) Only standard laboratory conditions required
2) Independent of weather/climate
3) More rapid growth
4) Allow focus on plant enzymes without interference from microorganisms
5) Lower cost

However, there are limitations and uncertainties associated with extrapolating results from excised organs to whole plants (Shimabukuro and Walsh, 1979). According to Mumma and Davidonis (1983), plant tissue cultures can be used to study the fate of xenobiotics in plant tissue, compare inter-specific metabolic kinetics, determine enzyme levels, assess biological activity, evaluate phytotoxicity and mass-produce metabolites. But the study methods used do not account for whole plant physical/structural effects and the data they generate cannot be quantitatively extrapolated to represent whole plant metabolism. Differences in the *in-vitro* and *in-vivo* fate of plant xenobiotic metabolites may be due to plant physiology and compartmentalization of enzyme systems within the plant cell and tissue structure (Cole, 1983).

1.5 Regulatory Acceptance

Phytoremediation is evaluated by regulators in the same manner as any other treatment technology and must be demonstrated to be protective of human health and the environment (Rock and Sayre, 1998). Proven technologies are more readily accepted by regulatory agencies, whereas emerging technologies like phytoremediation undergo a more rigorous approval process. Regulators often view phytoremediation as a final "polishing" step in a chain of treatment technologies for cleanup of most sites (Schnoor et al., 1995, USEPA, 2000). However, regulatory approval of phytoremediation is

encumbered by a lack of data due to the limited number of related publications in peer-reviewed literature and relative newness of the technology (Rock and Sayer, 1998).

According to Rock and Sayer (1998), items that should be addressed prior to regulatory acceptance may include:

1) Demonstration of efficacy by bench-scale (laboratory) and/or pilot-scale (field) testing using the specific plants and contaminated media from the site.

2) Proper containment of contaminated soils and sediment for the interim period of time required to establish the desired plant population.

3) Monitoring of contaminant degradation and fate during implementation.

4) Contingency plan for site remediation using an alternate technology if full-scale phytoremediation proves ineffective.

Plant selection criteria for phytoremediation should consider plant response and potential toxic effect of the contaminant(s) because they can vary between species. For example, some marsh macrophytes, like the salt meadow grass, *Spartina patens* and the fresh water marsh herbaceous plantain, *Sagittaria lancifolia*, exhibit greater tolerance of exposure to PHC than other species (Hester, 1998 and Lin et al., 1996).

The USEPA established the Remediation Technology Development Forum (RTDF) in 1992 to bring together public and private entities to advance emerging remedial technologies (Rock and Sayre, 1998). "Action Teams" formed under the RTDF focus on specific topics, such as phytoremediation of organics and contaminated sediment remediation. Objectives of the RTDF include identification of data gaps that prevent or slow application of promising technologies and exchange of information between researcher, governmental agencies and application engineers. Some of the

information needed to aid in acceptance of phytoremediation as a proven technology includes:

1) Potential to achieve cleanup goals;
2) Fate of parent compounds and products of incomplete degradation;
3) Elucidation of degradation mechanisms;
4) Potential adverse effects on plants;
5) Rates of degradation.

A better understanding of the relationships between plants, root exudate enzymes and degradation mechanisms of specific contaminants is needed for phytoremediation to mature as an accepted cleanup technology (Schnoor et al., 1995).

There are several areas of research that should be explored to support the general acceptance of phytoremediation as a proven cleanup technology for PHC and PAH-contaminated sediments. Field-oriented research of the mechanisms associated with rhizodegradation of organic compounds is not always feasible due to analytical limitations, incomplete mass balance data, gaps in contaminant fate information and unknown potential impacts of the contaminants on plant metabolism (Cunningham et al., 1995). According to Schnoor et al. (1995), another area for future development of phytoremediation technology involves isolating plant enzyme systems that will degrade specific pollutants. An active area of laboratory research involves enhancing contaminant rhizodegradation rates by manipulating the interface between roots and microorganisms (Cunningham et al., 1995). Re-establishment of indigenous marsh plant species such as *Spartina alterinflora* and *Spartina patens* can be an effective means of reducing PHC concentrations in wetland sediments (Lin et al., 1998). However, the

application of phytoremediation as a remedial measure is limited in part by the lack of available data evaluating its use to restore PHC-contaminated wetlands (USEPA, 2000). Additional published fundamental research of the mechanisms involved in phytoremediation is necessary to provide regulators with the basic information for acceptance of this technology (Rock and Sayre, 1998; USEPA, 2000).

1.6 Research Objectives

The purpose of this research is to evaluate the feasibility of using phytoremediation to aid in the restoration of an oil-contaminated former salt marsh area at a refinery located on a tidal creek near the coast of New Jersey. The research began as a field investigation to determine if native salt marsh plants (*S. patens*) would grow and survive for one growing season in sediments containing high concentrations of oil. The field investigation is described in Chapter 2 and concluded that indicated decreases in petroleum hydrocarbons and polycyclic aromatic hydrocarbons had occurred based on limited sample collection and analysis. The field study also included an evaluation of the potential for uptake and translocation of metals from the sediment. The data and experience from the field study were used to design a more detailed greenhouse investigation using plants grown at the site during the field study. This greenhouse study (Chapter 3) evaluates biodegradation of PHC and 16 PAH compounds in the rhizosphere of *S. patens* over two growing seasons at two depths using replicate analysis of sediment samples collected at the beginning and end of two growing seasons (four sample events). Concomitant monitoring of plant biomass, microbial density, nutrients, pH and ORP was also included as part of the investigation. The data was used to: (1) evaluate differences of PHC and

PAH concentrations in rhizosphere and bulk sediments, (2) contrast microbial densities in rhizosphere and bulk sediments containing PHC and PAH, and (3) compare rhizosphere physicochemical conditions in rhizosphere and bulk sediment.

CHAPTER 2

FIELD STUDY

2.1 Abstract

The native salt meadow grass *Spartina patens* was grown in petroleum-contaminated sediments located in a stormwater basin at an oil refinery for one growing season during a preliminary field study to evaluate the potential feasibility for use of phytoremediation as an alternative to treat the basin sediments. The primary objective of the Field Study was to determine whether *S. patens* would grow in the sludge and sediment present within the basin and to identify the potential for use of *S. patens* to decrease organic and inorganic contaminants in the sediment. The basin is located in an area that was formerly a salt marsh, converted into a stormwater detention basin to treat refinery stormwater runoff prior to discharge to the local creek. The basin was in operation for over 30 years prior to closure. During the period of operation, an oily sludge sediment layer accumulated that did not support plant growth based on field observations. The field study included four 1-square meter test plots each planted with 100 *S. patens* seedlings in two different areas of the basin with both fertilized and unfertilized treatments. Sediment samples were collected from in and around the plots and analyzed for total petroleum hydrocarbons (TPHC), volatile and semi-volatile organic compounds (SVOC), pesticides and target analyte list (TAL) metals. In addition, plant tissue samples were analyzed for metals. Significant plant mortality (100%) was observed and plant growth was insignificant in sediments containing over 12% TPHC. The plants grown in sediments containing from 6.3% to 11.9% TPHC exhibited better than 80% survival and growth

was significant. Sediment samples within the plots where plants survived exhibited decreases in TPHC (24%), total SVOC (45-51%), and PAH (43-51%) compared to bulk sediment from around the plots. Phytoextraction coefficients for metals were less than 1, with the exception of manganese (1.37). However, metals concentrations were generally below remedial action levels.

2.2 Introduction

A preliminary study was conducted in the field ("Field Study") from May 17, 2000 through November 21, 2000 within the North Field Basin (NFB) area at the Chevron Refinery, located in Perth Amboy, New Jersey (Figure 2.1). The objectives of this study were to (1) determine if the plants would grow in the NFB sludge and sediment under field conditions, (2) evaluate the effects of fertilization and, (3) evaluate potential for PHC and PAH contaminant reduction in the root-zone sediments, and (4) assess the uptake and translocation of metal contaminants. *S. patens* was selected for the study based on the following:

- The NFB area was being considered for restoration as a tidally flowed salt marsh.
- *S. patens* is a native salt marsh species and its presence represents ecologically higher value wetlands.
- Allocation of biomass in *S. patens* favors growth of a diffuse root system.
- Published research indicated that *S. patens* possess a good potential to degrade PHC and PAH in the rhizosphere, and to extract metals from the root zone via translocation to the leaves.
- *S. patens* can survive in sediment and soil containing relatively high concentrations of PHC.

Figure 2.1 Field study refinery site location on Perth Amboy, NJ-NY 7.5 minute series topographic quadrangle (1956; photorevised 1981).

Phytoremediation is a process by which plants degrade, extract, contain or immobilize contaminants from soil and water (USEPA, 2000). As described in Chapter 1, there are several mechanisms by which plants may facilitate degradation or removal of contaminants from soil and sediment; the two examined in this study include rhizodegradation and phytoextraction. Rhizodegradation is the breakdown of organic

contaminants in soil, enhanced by microbial activity in the root zone. Phytoextraction is the uptake of contaminants by plant roots and translocation within the plants. Field and greenhouse studies completed by others indicate that wetland plants, including *S. patens* exhibit potential for use in rhizodegradation of petroleum-related organics and phytoextraction of metals from soil and sediment (Hester, 1998; Lin and Mendelssohn, 1998).

Sediment and plant tissue samples collected and analyzed as part of this study provided the data needed to (1) document the presence and concentrations of potential contaminants in the NFB sludge/sediments within the test plots, (2) provide a basis to compare the chemistry the test plots, (3) indicate reduction of sediments petroleum hydrocarbon concentrations in the plant root zone (rhizosphere), and (4) evaluate potential metals translocation from the sediments to plant tissue. The field observations and laboratory data were used as a preliminary screening tool to draw conclusions and provide recommendations regarding the potential feasibility of using *S. patens* for remediation in the NFB area.

2.3 Physical Setting

2.3.1 Site Description and History

The Chevron Refinery is a 368-acre facility located in Perth Amboy, Middlesex County, New Jersey, adjacent to the Arthur Kill and Woodbridge Creek, which are tidal, estuarine surface waters (Figure 2.1). The site has been operated as a petroleum refinery since 1946. Products handled for many years at the refinery included liquefied petroleum gas,

gasoline, fuel oil, asphalt and sulfur. In 1983, the refinery reduced its operations and became primarily an asphalt refinery and product terminal.

In certain areas of the refinery soils and sediments are impacted with petroleum refining wastes and oil sludge that include elevated levels of heavy metals, heavy-end PHC and PAH from refinery operations and historical wastewater management practices conducted at the refinery. One such area, the North Field Basin (NFB), is an 11-acre area previously used by Chevron to detain stormwater from the refinery over a period of approximately 40 years, from about 1960 to around 2000.

Prior to 1960, the current NFB area was a tidal salt marsh, which is present in the 1960 historical aerial photograph (Figure 2.2). In the early 1960's a containment dyke was constructed to facilitate stormwater management for the refinery. The installation of the dyke essentially stopped the flow of brackish water through the area. Thus, the NFB has been a freshwater regime due to the influx of stormwater for over 40 years.

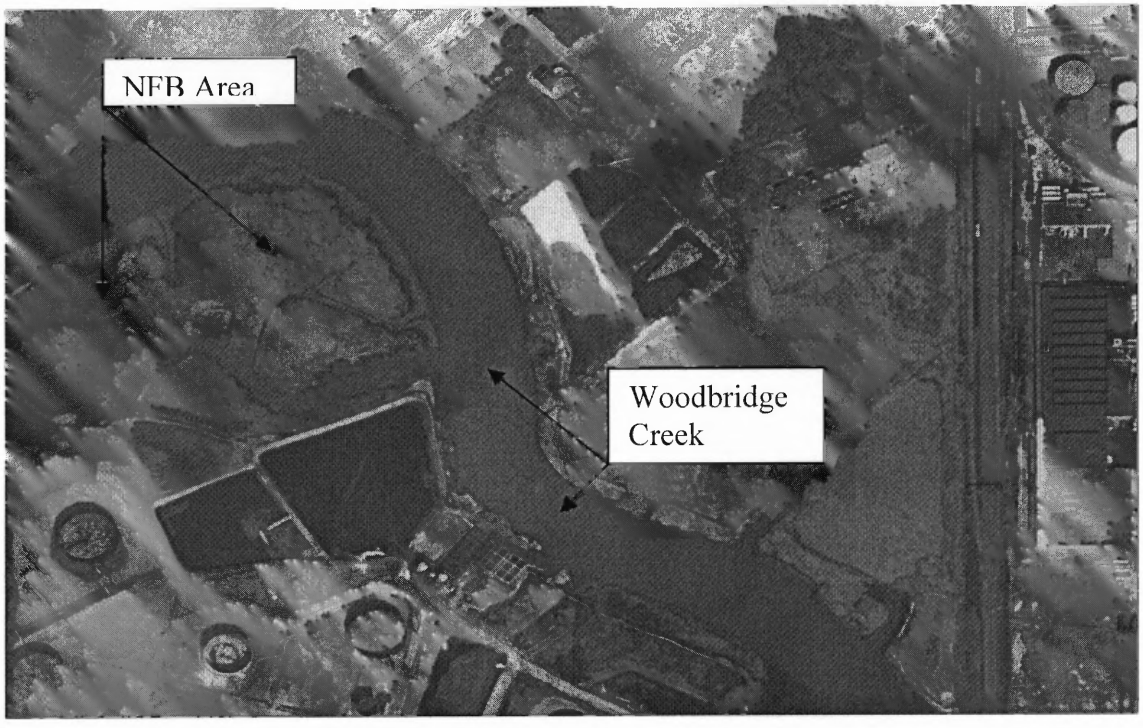


Figure 2.2 Aerial photograph of Field Study area prior to North Field Basin (NFB) construction – March 1959.

According to the USGS Perth Amboy, NJ-NY Topographic Quadrangle for Perth Amboy (Figure 2.1), the overall topography of the site is relatively flat and the surface elevation of the basin is near mean sea level. The NFB area is bounded to the north by Spa Spring Creek and to the east by Woodbridge Creek - both are tidal, estuarine water bodies that support natural communities of *Spartina alterniflora* and *Spartina patens*.

The New Jersey Department of Environmental Protection's (NJDEP's) GIS Land Use/Land Cover database (1995/1997) identifies the NFB area as an artificial lake (Figure 2.3). Portions of the banks along Woodbridge Creek near the site are identified as tidal herbaceous wetlands.

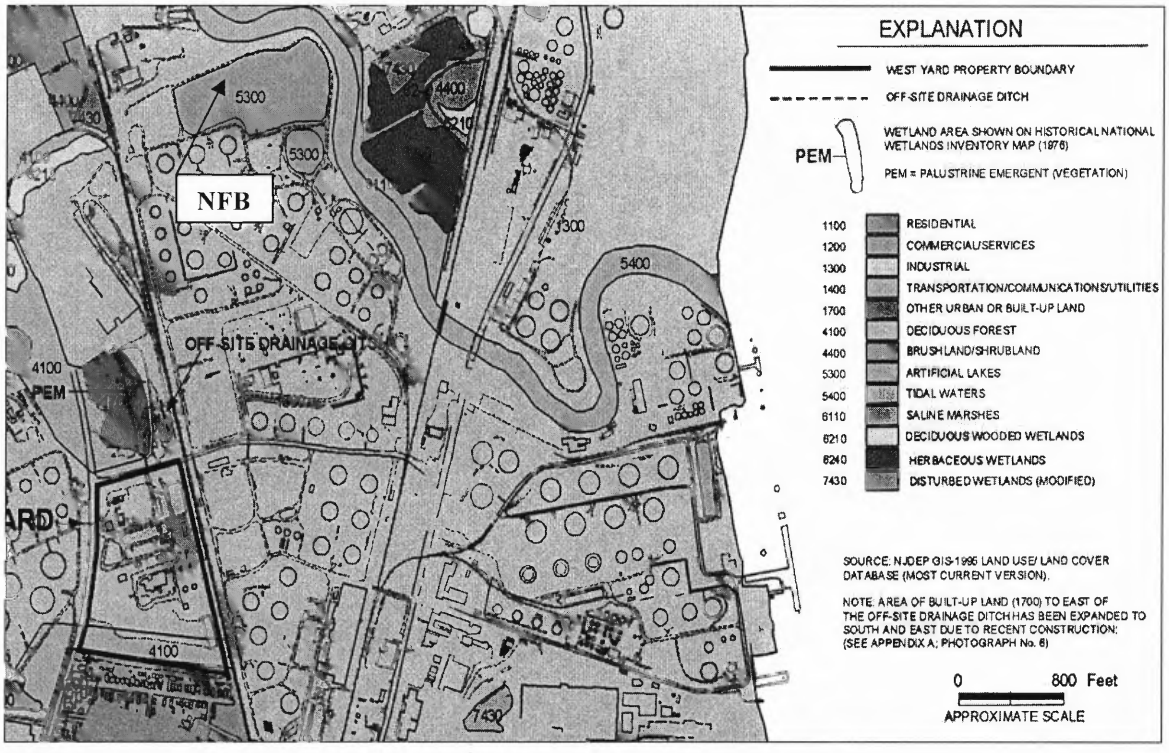


Figure 2.3 NJDEP GIS Land Use/Land Cover map of the North Field Basin (NFB) field study area.

The NFB no longer receives stormwater from the refinery; however, stormwater from direct precipitation enters the basin during storm events. Chevron maintains the water level within the basin at approximately 5 feet or more below the top of the surrounding containment dyke. The lowered water level has resulted in a 20 to 50-foot wide perimeter of exposed bottom sediments within the basin.

2.3.2 Study Area Conditions

Most of the oily sludge and sediments that had accumulated in the basin were recently removed as part of an effort to treat this area prior to commencement of this study. A layer of residual oily sludge and sediment remains in the upper one to two feet within the

basin. Initial observations of sediments and subsurface conditions in the North Field Basin directly prior to planting *S. patens* identified two areas where test plots (Plot A and Plot B) were established (Figure 2.4). Plot A was located in the northwest part of the basin in an area where the oily sludge was nearly 2-feet thick. Plot B was established near the southwest corner of the basin where oily material was limited to the first few inches of sediment.

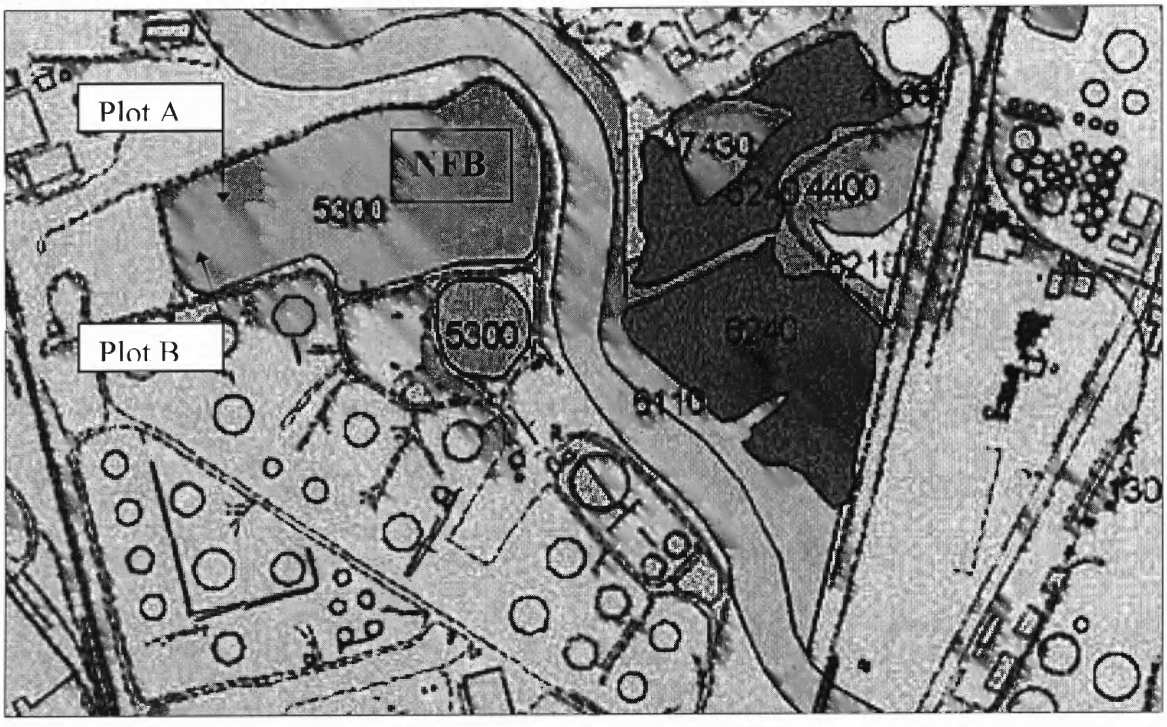


Figure 2.4 Location of field study Plots A and B in the North Field Basin (NFB).

The sediment in both areas was moist or saturated immediately beneath the surface and an oily sheen with petroleum odor was observed in all plots. Plot A contained about 2 inches of black silt and sludge at the surface, and one inch of brown fine-to-medium sand with oily silt. Oily sediment was present from 3 to 18 inches below surface grade (bg), which included saturated dark-gray fine sand and silt. Saturated, oily gray-

brown, silty clay was present from 18 to 24 inches bg. In the Plot B area there was a 0.5-inch surface of silty sludge underlain by 1.5 feet of brownish gray, fine-to-medium sand and silt, with some fine to medium gravel. Photographs of the test plots are included in Appendix A.

2.4 Materials and Methods

2.4.1 Study Design

On May 17, 2000, the two test plot areas (Plots A and B) were established by planting 400 *S. patens* plants in test plots within the sludge and sediments exposed within the NFB (Figure 2-4). Pinelands Nursery of Columbus, New Jersey supplied the plants, which were delivered on May 15, 2000. Each Plot contained two, 1-square meter (m^2) sub-plots identified as A-1 and A-2, and B-1 and B-2 (Figure 2-5). One hundred plants were planted per each of the four sub-plots (total 400 plants) in rows spaced every 10-cm on center in both directions. This spacing allowed the plants room to grow and facilitated removal of individuals with sufficiently large sediment cores needed for future greenhouse studies. At the time of planting, the plants were approximately 10 centimeters tall and each included a 2-inch diameter plug of potting soil.

As with most planting, turning or tilling the ground surface is typically done to facilitate establishing plant roots and overall planting success. Tilling would likely be required at the study site if phytoremediation were to be implemented. Thus, prior to planting, the plots were manually tilled to a depth of approximately 12 inches in a manner intended to mimic large scale tilling of the entire 11-acre basin to facilitate scale-up of the study results later.

Sub-plots A-1 and B-1 were fertilized with Professional Turf and Landscape, Greens Keeper homogenous, granular fertilizer containing a 20:8:8 percent ratio of nitrogen, phosphate and potassium, respectively. The fertilizer was applied at a rate of 50 g/m^2 per month beginning May 17, 2000 through August 2000. Sub-plots A-2 and B-2 were not fertilized during most of the test period; however, 50 g of fertilizer was added to sub-plot B-2 in late August 2000.

2.4.2 Field Sampling and Analytical Methods

During the study, average plant stem and leaf length was measured monthly (May 2000 through November 2000) as an indicator of growth. Plant growth was evaluated by taking periodic measurements of the stem and leaf length from the ground surface during the study period. The length of the stem and leaves were measured from three plants per sub-plot, which were averaged to produce one measurement of standard growth for that monthly period. Additional observations were noted regarding general condition of the plots, such as yellowing leaves, presence of rhizomes and production of seeds.

On November 21, 2000, sediment and plant tissue samples were collected from both Plots A and B. Most of the sampling was focused on Plot B, since the plants in Plot A did not survive. Sediment samples were collected from the 0-0.5-foot depth interval in Plots A and B, and outside Plot B. Two sediment samples were obtained from subplots A-1 and A-2 (one sample per subplot), four samples were collected from sub-plots B-1 and B-2 (two samples per subplot), and three control samples were collected from outside sub-plots B-1 and B-2 (total 9 samples). The sample analytical results and plant growth and survival are discussed in Section 2.4.

Samples of plant leaves and plant roots were obtained from Plot B only. Four samples of plant material (2 leaf samples and 2 root samples) were collected from the plants in sub-plots B-1 and B-2 (total 8 samples). The leaf and root samples were collected from six plants that were sacrificed from within sub-plots B-1 and B-2. Plant tissue samples were combined in the field as two, 3-into-1 composite samples per sub-plot. In order to ensure random sampling, three plants were taken from a different area of each sub-plot around their corresponding sediment samples (Figure 2.5).

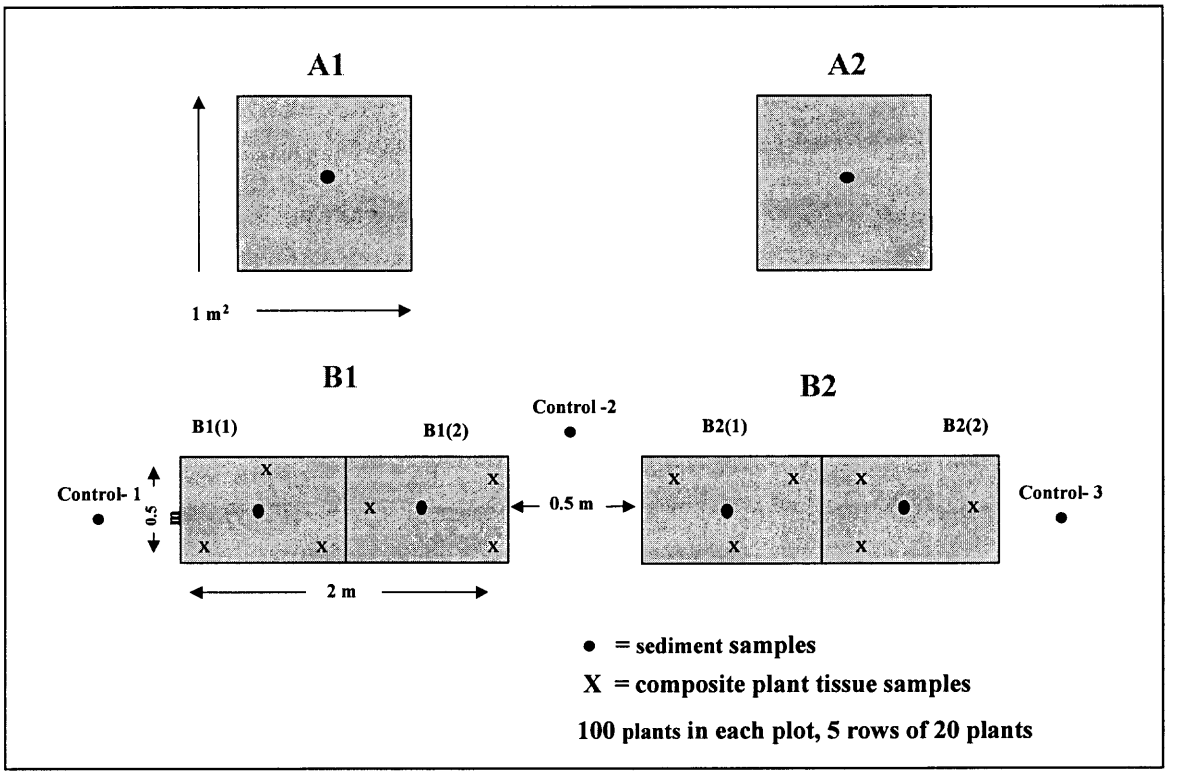


Figure 2.5 Schematic of field study sample locations – Plots A and B.

Each sediment sample included a 300 ml glass jar, an 80 ml glass jar and a 5g Encore ™ sampler. Plant tissue composite samples were collected in 300 ml glass jars. The sediment samples were analyzed for the following parameters:

Parameter	Analytical Method
Total Petroleum Hydrocarbons (PHC)	EPA 418.1 M (modified)
Target Analyte List Metals (TAL Metals)	SW-846/6010B & 7471A
TCL volatile organic compounds (VOCs)	SW-846/8260B
TCL semi-volatile organic compounds (SVOCs)	SW-846/8270C & 3550B
TCL Pesticides	SW-846/8081 & 3550B

Notes: TCL = Target Compound List
SVOCs include the PAHs.

The individual compounds and elements included in the sample analysis are listed in Appendix B. The plant tissue samples were analyzed for TAL metals only.

2.5 Field Study Results

The small number of samples collected and analyzed in this study limits the statistical significance of the laboratory data. However, due to the preliminary nature of this study, the data are being used only as an indication of potential effects associated with contaminant-sediment-plant interactions.

During the preliminary study, survival of *S. patens* in Plot A was short lived and there was no significant growth observed. The plants in Plot B exhibited good growth and survival. The sediment analytical results indicate that the primary contaminants of concern are PHC and the base neutral (BN) fraction of semi-volatile organic compounds, which include polycyclic aromatic hydrocarbons (PAH). As described below, a

comparison of the sediment data from the controls and Plot B indicate that the PHC, BN and PAH concentrations were all reduced in the root zone of the plants within Plot B.

In addition, the data indicate that metals are generally not a concern, since only a few metals were present at relatively low concentrations in the sediment. The results of the sediment and plant tissue analyses for the most frequently detected metals that are a potential concern indicate that uptake of metals by *S. patens* may occur, but metal accumulation in plant shoots and leaves did not appear to be significant.

2.5.1 Plant Survival and Growth

Photographs of the study plots and plants are provided in Appendix A. The plants in Plot A survived for a few weeks, but leaves and shoots of more than 50% of the individuals began to wither and die by early June 2000. These plants showed no appreciable growth throughout the study period. Based on field observations made in August 2000, the roots did not extend out from the original plugs into the surrounding sediment.

S. patens in Plot B exhibited continual healthy growth for both sub-plots B1 and B2. The plants in sub-plot B1 (fertilized) grew from the initial length of approximately 51 centimeters (cm) to about 114 cm in average length. Plant growth in sub-plot B2 (unfertilized) demonstrated an increase in average stem and leaf length from about 51 cm to 91 cm. A comparison of the sub-plots shows that B1 grew significantly more than B2. In addition, sub-plot B1 appeared more lush than B2, as evidenced by more dense leaf mass and an abundance of more lengthy rhizomes in B1. The increased growth in sub-plot B1 is attributable to the addition of fertilizer.

Some plant mortality was noted in both plots, along the topographically lower edge of the plots. Most of the 20 plants in the first row of each sub-plot (40 plants total) located nearest to the edge of the ponded basin water did not survive and/or demonstrated little to no growth. This effect was observed in both sub-plots, and is assumed to be associated with the physical site conditions, such as the sediment elevation and proximity to saturated sediment in the lower end of the study plot.

The success of rhizodegradation depends on root penetration into the surrounding media - in this case the bulk sediments containing PHC and PAHs. *S. patens* root growth into surrounding sediments is expected under natural conditions, but was not assumed in this case since the effect of the contaminated sediments on root growth was uncertain. In contrast to the plants in Plot A, hairy root growth from plants throughout both sub-plots B1 and B2 extended well beyond the original root plugs, into the surrounding contaminated bulk sediments (Appendix A). This is significant since the extent of root growth is one of the main limitations to be considered in evaluation of phytoremediation studies.

2.5.2 Analytical Results

The laboratory analytical results are included in Appendix B. In general, the results were similar to existing data from prior sample analysis of the NFB sediments collected by others (unpublished data; not shown). A summary of the organics and metals analytical results is provided below; all concentrations are expressed as dry weight basis.

2.5.2.1 Organic Compounds. Based on the analytical results the organic compounds that are of most concern due to elevated concentrations in sediments are PHC and BNs; VOCs and pesticides were for the most part not detected in the sediment (Appendix B). The majority of the elevated BNs comprise targeted PAH and tentatively identified compounds (TICs).

Petroleum Hydrocarbons (PHC)

The average and range of PHC concentrations in sediment samples from within the study plots and around the plots (control sediments) are presented on Table 2.1. The average concentrations indicates that the average PHC concentration in Plot A is about 80% higher than the average PHC concentrations around Plot B (i.e., the controls were collected around Plot B). Also, the average PHC concentration of the sediment within sub-plot B-1 is 24% lower than the average PHC concentration in the surrounding sediment. The average PHC concentration of sub-plot B-2 is higher than the average for the Controls. However, this may be attributable to the location of Control-2, which was collected further upslope than Control-1 and Control-3, and between sub-plots B-1 and B-2. Therefore, Control-2 is considered an outlier since it may represent a soil area where concentrations were below average for the area due to the distance away from the contaminant source and higher elevation on the bank.

Table 2.1 Summary of PHC Concentrations in sediment

Sample No.	Average (ppm)	Range (ppm)
Plot A	179,000	120,000 to 238,000
Plot B-1	75,600	62,500 to 88,700
Plot B-2	106,500	105,000 to 108,000
Controls	99,633	66,900 to 119,000

ppm = parts per million.

Total Base Neutral Compounds (BNs)

The targeted BNs detected account for less than 6% of the total BNs reported in sediment samples and the average ratio of targeted to non-targeted BNs is 4.1% (SD=0.09, n=9). Nearly 95% or more of the total BNs are represented by TICs. The targeted BNs are primarily PAHs; bis (2 ethylhexyl) phthalate was detected in all of the samples at concentrations below concern ranging from 2.5 to 8.16 ppm.

The average and range of total targeted and non-targeted BN concentrations in the study plots and control sediments are included in Table 2.2. The average BN concentrations in sediment indicate that the BN concentration in Plot A is about twice the average BN concentration of the control area around Plot B. The average BN concentrations in the sediment within sub-plots B-1 and B-2 are about 51% and 46% lower than the average BN concentration in the controls, respectively. Most of the decrease in total BNs is attributable to reductions in the non-targeted compound concentrations. However, similar reductions in targeted PAHs were identified, as described below.

Table 2.2 Summary of total Targeted and Non-targeted Base Neutral Compounds (BNs) in sediment

Sample No.	Average (ppm)	Range (ppm)
Plot A	3,333	1,842 to 4,824
Plot B-1	816	675 to 957
Plot B-2	904	734 to 1,074
Controls	1,682	833 to 2,234

Polycyclic Aromatic Hydrocarbons (PAHs)

The average and range of total PAH concentrations in the study plots and control sediments are presented in Table 2.3. The average PAH concentrations in sediment indicate that the PAH concentration in Plot A was more than 2.5 times the average PAH concentration of the control area around Plot B. The average PAH concentrations in the sediment within sub-plots B-1 and B-2 were about 51% and 43% lower than the average PAH concentration in the controls, respectively. In addition, similar reductions were demonstrated for all six of the most prevalent individual PAHs detected in sub-plots B-1 and B-2. The detection frequency of the other five PAHs was too low to evaluate decreases in concentration.

The individual PAHs detected in the sediment samples are listed in the order of detection frequency in Table 2.4. The first six individual PAHs listed were the most commonly detected and all were in the low ppm range. Most individual PAH concentrations were under 10 ppm; the highest individual concentration was pyrene at 56 ppm in sub-plot A-1. The suite of PAHs is generally consistent among the sediment samples. The one notable exception is sub-plot A-1, which contained only chrysene and pyrene.

Table 2.3 Summary of Total Polycyclic Aromatic Hydrocarbons (PAHs) in sediment

Sample No.	Average (ppm)	Range (ppm)
A	85	83 to 88
B-1	33	28 to 39
B-2	38	27 to 49
Controls	67	27 to 75

Table 2.4 Detection frequencies of individual Polycyclic Aromatic Hydrocarbons (PAHs)

Targeted PAHs	No. Detected	No. Analyzed
Chrysene	9	9
Pyrene	9	9
Benzo(a)pyrene	8	9
Benzo(g,h,i)perylene	8	9
Benzo(a)anthracene	7	9
Benzo(b)fluoranthene	6	9
Phenanthrene	3	9
Benzo(k)fluoranthene	2	9
Acenaphthene	1	9
Indeno(1,2,3-cd)pyrene	1	9
2-Methylnaphthalene	1	9

Only those PAHs detected are presented.

2.5.2.2 Metals. Most of the 22 TAL metals analyzed are not considered to be a concern for this study due to either their low concentrations, infrequent detection or their high natural concentrations in typical background sediments (Appendix B). However, chromium (Cr), copper (Cu), lead (Pb), manganese (Mn) and zinc (Zn) are discussed further because they were present in all of the sediment and plant tissue samples. The concentration ranges for each of these metals detected in plant leaves, roots and

sediments from the plots and control locations are presented in Table 2.5. No plant tissue data is provided for Plot A or controls since no plant growth occurred in these areas.

Table 2.5 Summary of selected metals data from Field Study Plot sediments and Plant Tissue samples

		Concentration Range (ppm)				
Location	**Media**	**Chromium**	**Copper**	**Lead**	**Manganese**	**Zinc**
A-1and A-2	Sediment	101-251	202-360	83.4-114	62.5-101	221-460
B-1	Leaves	0.97-1.3	4.8-5.5	3.1-8	87.6-120	21.7-24.2
	Roots	2.8-7	8.6-15.7	11.5-31	11.6-20.9	16.5-28.3
	Sediment	70.1-105	95.5-96.7	126-277	61.4-90.5	84.9-99.9
B-2	Leaves	2.2-7.1	9.5-21.2	5.1-13.2	27.5-40.8	22.6-33.6
	Roots	4.8-9.8	15-21.5	19.5-92.3	10.2-28.5	6.9-26
	Sediment	74.4-89.2	112-159	222-297	85.4-103	94.8-134
Controls	Sediment	50.5-83	111-127	159-337	96-130	97.4-146

The sediment concentrations of the selected metals are highly variable; however, Plot A sediments appear to contain the highest concentrations with the exception of lead. In general, metal concentrations were highest in the sediments, lower in the roots and lowest in the leaves with the exception manganese and zinc. The plant leaves contained Cr, Cu, Pb and Zn at concentrations in the low ppm range, and below the sediment concentrations. However, manganese concentrations were greater in leaves than in sediments and roots. The phytoextraction coefficient, which is the ratio of the metal concentration in the leaves and shoots, divided by the metal concentration in sediment, can be used as an indication of metals uptake (Nanda Kumar et al., 1995; USEPA 2000). Phytoextraction coefficients for the five metals listed were calculated for sub-plots B-1 and B-2 (Table 2.6).

Table 2.6 Phytoextraction Coefficients for selected metals detected in Plots B-1 and B-2

Metal	B-1	B-2
Chromium	0.01	0.57
Copper	0.31	0.11
Lead	0.03	0.04
Manganese	1.37	0.37
Zinc	0.12	0.25

The phytoextraction coefficients calculated for the field study are low, mostly below 1, indicating that there is little uptake of Cr, Cu, Pb, Mn and Zn from the sediments into the plant leaves and shoots.

2.6 Discussion

The results of this preliminary study confirm that *S. patens* can survive and grow in the NFB sludge and sediment containing high concentrations of PHC (up to 12%) and PAH (≈50 ppm), where the contaminated sediment thickness is less than a few inches near the sediment surface. In addition, the hairy roots from plants throughout Plot B extended into the surrounding contaminated sediment. In contrast, all of the plants died within 60-days in Plot A, where the sediment concentrations of PHC in the sediment ranged from approximately 12% to 20% PHC. There was no extension of roots into the surrounding sediments in Plot A and root growth appeared limited to the original 2-inch root plugs.

The concentration of PHC and PAH in sediment samples collected from within Plot B were up to 24% and 51% lower than those in bulk sediment samples obtained adjacent to the test plot, respectively. In both sub-plots where the plants survived (Plots B-1 and B-2) there was an apparent decrease in PHC and PAH concentrations regardless

of fertilization. Comparisons of plant growth and sediment quality data from the fertilized and unfertilized sub-plots (i.e., Plots B-1 and B-2, respectively) indicate that the addition of fertilizer increased plant growth and appears to have contributed to a slightly larger reduction of organic contaminants. These data are consistent with results from a similar study reported by Lin and Mendelssohn (1998).

A comparison of results from sediment and plant tissue analyses indicates a low degree of translocation of selected metals from the sediment to the shoots and leaves by the plants, demonstrated by low phytoextraction coefficients (Nanda Kumar et al., 1995; USEPA, 2000). However, the data indicate that metals are generally not a concern, since only a few metals are present at relatively low concentrations in the sediment, and accumulation in plant tissue is relatively low. Therefore, additional analysis for metals in the NFB sediment is not warranted.

The small number of samples collected and the lack of initial samples from the study plots prior to establishment of the plants limit the usability of the data generated by this study. Although the data set is too small to establish statistical significance, the results are useful within the context of their intended purpose as a screening tool in a phased approach of the technology feasibility review process. In order to overcome these limitations and generate a more robust data set for the next phase in the remedial action selection decision process, a long-term greenhouse study was conducted over the next two consecutive growing seasons (i.e., 2001 and 2002) using the sediments and plants from the field study plots. The greenhouse study is described in Chapter 3.

CHAPTER 3

GREENHOUSE STUDY

3.1 Abstract

Biodegradation of total petroleum hydrocarbons (TPHC) and 16 priority pollutant polycyclic aromatic hydrocarbon (PAH) compounds was evaluated in sediments from a stormwater basin that was a former salt marsh at an active oil refinery. *Spartina patens* were grown in basin sediments in a greenhouse study over two consecutive growing seasons. TPHC and PAH concentrations were monitored in planted (i.e., vegetated) and bulk sediments, along with microbial density, nutrients, pH, oxidation-reduction potential (ORP) and root biomass at two depths (2.5 and 7.5 cm). The sediments contained on average 6.5% to 9.5% TPHC and 56 to 124 parts per million (ppm) total PAH. The plants exhibited a 95% survival rate and added from 1% to 2% root biomass to the sediment. Microbial densities in planted sediments were significantly higher than in bulk sediments ($p<0.05$). Oxidizing conditions were prevalent in all treatments at both depths; however, reducing conditions developed in the planted sediments at 7.5 cm. TPHC was 35% to 37% lower in the planted vs. bulk sediment at the end of the study. Planted sediment PAH concentrations were generally lower than in bulk sediments. Low MW PAHs (2 and 3-rings) were either absent or at relatively low concentrations in all treatments. Phenanthrene concentrations in bulk sediments did not change, but were reduced by 95% in planted sediments ($p<0.01$), with half-life estimates of 141 to 165 days in the planted sediments at the 2.5 cm depth. Temporal changes in high MW PAHs (4 to 6-rings) were not observed over the greenhouse study period. Differences in PAH

concentrations between planted and bulk sediments exhibited a decreasing trend with PAH molecular weight. Evaluation of differences in planted and bulk sediment PAH concentrations suggests lower MW PAHs degrade better under oxidizing conditions, and higher MW PAHs under reducing conditions in the planted sediments. Comparison of planted and bulk sediment PAH concentrations links depth and redox conditions to decreases in PAH as a function of PAH MW. Therefore, it was concluded that spacio-temporal variations and cycling of redox conditions should be considered to properly evaluate biodegradation of PAH.

3.2 Introduction

Biodegradation studies of organic contaminants such as PHC and PAHs have focused largely on microbial activity for potential use in remediation of contaminated sites (Van Hamme et al., 2003). More recently, vascular plants have also been evaluated for use in bioremediation (USEPA, 2000). A long-term greenhouse experiment was conducted at the Rutgers University experimental greenhouse in Newark, New Jersey using *Spartina patens* grown in sediment as a continuation of the field study described in Chapter 2. The study evaluates the potential for using a plant species native to the local tidal marshes, and associated microorganism interactions in the rhizosphere, to degrade PHC and PAH in contaminated sediment. The results will be used in part to evaluate phytoremediation as a component of the remedial alternative selected for treatment of PHC and PAH in the sediments at the field study site described in Chapter 2.

Phytoremediation is described in the literature as a promising technology, but one that needs further research to be considered as a proven remedial alternative (Rock and Sayre, 1998). Many published biodegradation studies have investigated the microbial mechanisms of degradation for a few low MW PAHs (Karthikeyan et al., 2001). While such fundamental research is certainly important, it does not answer the ultimate question of whether bioremediation using plants will be effective at a site as evaluated in standard remedial engineering terms, such as giving consideration to all of the contaminants of concern, implementability, short and long-term effectiveness, etc. For example, research describing degradation of phenenthrene and naphthalene does not provide a basis for evaluating a site contaminated with a mixture of several PAHs, including higher molecular weight (MW) PAHs like benzo(a)pyrene, chrysene and benzo(g,h,i)perylene. Unfortunately, sites that are contaminated with only one or two PAHs are rare. Based on the author's experience investigating hundreds of contaminated sites, in the northeast United States, it is estimated that most PAH-contaminated sites contain a mixture of PAH compounds consistent with the source of PAH from crude oil or petroleum products, which are inherently mixtures of hydrocarbon compounds. At sites with older historical contamination, the lower MW PAHs tend to be absent and the higher MW PAHs that are resistant to natural degradation remain as the primary contaminants of concern (Kanaly et al., 2000a). When petroleum contamination is the focus of a remedial investigation and feasibility study, a complete list of PAHs are typically evaluated, which usually includes the 16 PAHs identified in the USEPA Target Compound List (TCL), or a similar list.

This study evaluates biodegradation of PHC and 16 PAH compounds in the root zone (rhizosphere and surrounding sediment) of *S. patens* over two growing seasons at two depths by investigating:

1) Differences of PHC and PAH concentrations in planted and bulk sediments;
2) Contrast of microbial densities in planted and bulk sediments containing PHC and PAH; and
3) Physicochemical conditions in planted sediment compared to bulk sediment.

The study design includes several elements necessary for the results and conclusions to be useful as a treatability study for remedial alternative decisions under USEPA and NJDEP regulations and oversight. Sample analyses for PHC and PAH was completed by a New Jersey certified laboratory using USEPA analytical methods (described below). Also, the Remedial Action Workplan for the site proposes to restore the North Field Basin (NFB), i.e., the study area, to a tidally flowed salt marsh. Thus, the greenhouse study was conducted using salt water in anticipation of future site conditions. It should be noted that the field study was conducted under fresh water conditions due to the presence of the perimeter containment berm around the field study area. Thus, the only source of water in the basin was storm water. The potential effects of the change to salt water are considered a necessary part of the test procedure to support data usability.

Another treatability element of the study design involves the heterogeneity of the test matrix. The classic challenge for environmental chemical analysis is to identify and quantify low (dilute) concentrations of an analyte in environmental media, such as soil or water. Water samples are easily manipulated to provide a homogenous matrix, which is not the case with soil and sediments. For pure research, a relatively homogeneous soil

may be obtained or manufactured in the laboratory. Since it is either technically infeasible or cost-prohibitive to completely homogenize the large volume of soil or sediment at the site, bench test results based on such homogenized soils are unreliable for engineering scale-up. As described in Chapter 2, in order to maintain the study data usability for NFB site, the test plots were manually tilled to emulate the method of tilling that would be practicable for the entire 11-acres.

Indigenous microbial communities found in petroleum-impacted soils and sediments can contain microorganisms that exhibit an inherent capacity to use PHC and PAH as a source of carbon and energy (Stapleton et al., 2000). However, biodegradation by indigenous microbiota without enhancement may not result in appreciable decay of PHC and PAH in severely contaminated sediments (Norris, 1994). In this case, the surface of the entire 11-acre study site has appeared black, oily and completely devoid of any form of vegetation for years, indicating an obvious lack of any reasonable degree of contaminant degradation by natural processes. Stimulation of indigenous microbial populations and their associated metabolic activity by replanting with the appropriate plant species can result in microbial degradation of the recalcitrant organic contaminants at such sites (Lin et al., 1998). Compared to bulk soil and sediment, rhizosphere microbial communities are more dense, more diverse, and generally more metabolically active due to phyto-microbial interactions (Schnoor et al., 1995; Atlas and Bartha, 1998).

The study design anticipates reverting the NFB area to a tidally-flowed salt marsh with *Spartina patens*, a salt-tolerant, perennial grass, that exhibits a diffuse root system and is native species to the area of the Field Study site. Therefore, it is expected to

dominate less salt-tolerant, invasive species, such as *Phragmites communis,* once re-established in the study site area.

The study design omitted the seed germination step and anticipated planting of seedlings in the NFB sediments because the seed germination may be inhibited by the elevated PHC concentrations in the surface sediments at the site, as is indicated by the conspicuous lack of plant growth in the vicinity of the field plots described in Chapter 2.

As a perennial macrophyte, *S. patens* allocates significant metabolic resources to the roots, suggesting increased root biomass and associated increases in root exudates, which are important for sustaining more robust microbial activity in the rhizosphere. The diffuse root system represents a large root surface area for increased contact with interstitial space in the sediment that is expected to provide efficient delivery of exudates and organic carbon throughout the matrix. *S. patens* also demonstrates a good potential for rhizodegradation of PHC and PAH in salt marsh sediments based on published literature (Hester et al., 1998; Lin et al., 1998) and the Field Study (Chapter 2). Growth of *S. patens* in the contaminated sediments is expected to:

- Add root biomass and associated root exudates;
- Increase aeration of the sediments in the rhizosphere and surrounding sediment via roots;
- Increase microbial density;
- Develop closely associated aerobic and anaerobic microbial niches; and
- Decrease PHC and PAH concentrations in planted vs. bulk sediment.

The root system naturally fosters microbial activity through (1) the continual addition of root biomass and root turnover that adds natural organic carbon to the rhizosphere sediments; (2) extending oxidizing conditions deeper into planted sediments; and (3) supplying bioavailable forms of carbon and controlling pH via root exudates. The plant-microbial relationship is mutualistic, with the microorganisms providing biogeochemical cycling of nutrients for uptake by plant roots. These aspects of root function represent distinct advantages for microbial consortia in planted sediments over those in bulk sediments for biodegradation of organic contaminants.

This research presents a method of evaluating the effectiveness of using plant root growth to stimulate microbial decay of weathered PHC and PAHs in sediments from historical discharges. Monitoring replicate treatments of sediments with and without plants under controlled conditions allows identification of anticipated cumulative effects of the rhizosphere on microbial activity and decay of PHC and PAHs. By maintaining test conditions such as temperature, watering regime, light-cycles and nutrient addition the same for all of the treatments, the results from time-series monitoring PHC and PAH concentrations, and microbial density data, can be used to support conclusions regarding rhizosphere effects on the biodegradation of these parameters.

Biogeochemical conditions important to microbial degradation of organics vary with depth in sediment, where reducing conditions can dominate within a few centimeters of the surface (Atlas and Bartha, 1998). Knowledge of the capacity of plants to deepen the oxic zone in wetland sediments via plant root activity has been used to model subsurface redox conditions in wetland sediments (Jaffe et al., 2002). Therefore, the

greenhouse study includes monitoring of target and indicator parameters at two depths (2.5 and 7.5 cm) in all treatments to evaluate affects of different redox conditions.

The suspected mechanism for the predicted rhizodegradation is primarily via co-metabolism of PHC and PAH by indigenous microbiota stimulated from root growth and the associated conditions generated in and around the rhizosphere of *S. patens*. Root exudates may in themselves have capacity for decay of organic contaminants, but distinguishing between plant-derived enzymatic effects and microbial degradation is beyond the scope of this study.

Recently published studies have described some success with rhizodegradation of PHC and/or PAH using several plant species (Liste and Alexander, 2000). However, relatively few include *S. patens* and none were found that investigate all 16 USEPA Target Compound List PAHs in bulk sediments and planted sediments under variable redox conditions.

The research methods were selected to incorporate the current state-of-art in phytoremediation study design, including:

- Use of native plants to maximize data usability for future field applications;
- Greenhouse setting to provide controlled conditions and limit variables that may otherwise confound the interpretation of results;
- Use of actual contaminated site sediments with established plants to provide a more realistic representation of subsurface biogeochemical conditions and effects of weathering; and
- Simultaneous review of conditions in planted and bulk sediments to evaluate the effects of plant roots and associated changes to microbial densities on PHC and PAH concentrations.

The study methods, including plant pots, sample collection and pore water analysis, are consistent with those used in an earlier study with *S. patens* in uncontaminated sediments at Rutgers University (Burke, 2001).

3.3 Materials and Methods

Native salt marsh plants (*Spartina patens*) procured from the Field Study that was conducted in the North Field Basin at the Chevron Refinery in Perth Amboy New Jersey during 2000 (Chapter 2) were grown in the Rutgers University experimental greenhouse using contaminated sediment obtained from within the NFB. The experiment used actual site sediments contaminated with elevated concentrations of PHC and PAH from historical sources at the site. The results of the Field Study demonstrate that the selected plant species will grow at the site in the contaminated sediment and indicate a potential for reduction of PHC and PAH within the sediment in and around the rhizosphere of *S. patens*. However, the Field Study data are limited to a small number of samples and the plant viability past one growing season was not evaluated.

Experimental Set-Up

Thirty sediment cores were obtained for use in this experiment from in and around Plot B located in the North Field Basin (NFB) study site at the Perth Amboy Refinery described under the Field Study in Chapter 2. The sediments in Plot B, and the cores taken for the study, are generally representative of sediment conditions in the 11-acre NFB area, including the presence of black, oily sludge visible in the sediment matrix that contains elevated concentrations of PHC and PAHs. Each core was removed as an

undisturbed plug approximately 10x10x10cm (approx. 1 Kg each), placed in 10x10x10cm square polyethylene planting pots with flow-through holes in the base. The pots were inserted into 2.5-liter polyethylene pails filled with 5-g/L artificial seawater (Instant Ocean sea salt/ laboratory deionized water) and transported to the Rutgers experimental greenhouse located in Newark, New Jersey.

Of the 30 cores used for this study, 20 included *S. patens* (one plant per core) and 10 cores contained bulk sediment without plants or plant roots. Also, the core depth of 10 cm corresponds to the approximate maximum root depth achieved by the plants after the first growing season (i.e., May 2000 through October 2000).

During the greenhouse study growing seasons (i.e., March through September of 2001 and 2002), the treatment pots were staged together on a bench in the greenhouse in random order and were rotated periodically to minimize any potential effects due to bench location. Greenhouse ambient conditions provided natural light and temperature regulation at 25±5 ^{0}C. The pots were maintained together in a cold storage refrigerator adjacent to the greenhouse at 2.8±1.7 ^{0}C during cold weather periods of the study (i.e., late October 2001/2002 through February 2001/2002).

The water level in the bowls was maintained for the duration of the study within a few centimeters from the sediment surface in the pots for all treatments (with and w/o plants) by periodic addition of 5 g/L artificial seawater and weekly exchange of the water in the treatment bowls with freshly prepared 5 g/L artificial seawater. Throughout the study, each batch of artificial seawater was amended with potassium phosphate monobasic (KH_2PO_4) and anhydrous ammonium chloride (NH_4Cl), which were added to the artificial seawater as nutrients at 7.25 mg/L and 14.75 mg/L, respectively.

Sample Collection, Measurements and Analytical Parameters

A total of 240 sediment samples were collected for this study; 60 samples from each of four sampling events over two growing seasons. Initial sediment samples were collected in the Spring 2001 (April 4 - 6, 2001) from two depth intervals in each pot (1.5 - 3.5 cm and 6.5 – 8.5 cm). The vertical centers of each interval are 2.5 cm and 7.5 cm, respectfully. Thus, the sample depths are referred to as 2.5 and 7.5 cm. Additional sediment samples were collected at the same two depths from all 30 pots in Fall 2001 (October 9 – 11, 2001), Spring 2002 (March 4 – 6, 2002) and Fall 2002 (September 3 – 5, 2002). The sediment samples from both depths were obtained using a 1.5-cm diameter cork corer and a stainless steel spatula.

Upon removal of the corer, oxidation-reduction (redox) potential (ORP), pH and temperature were measured at each sample depth using a calibrated Oakton Series 100 pH/mV meter (Cole-Parmer Instrument Co., Vernon Hills, IL). The pH measurements are temperature-corrected by the meter using a temperature-corrected combination electrode. The ORP measurements represent relative millivolts (mV) measured with a silver/silver chloride electrode that is not temperature or pH corrected. ORP measurements are not normally temperature-compensated because data are usually obtained at relatively contant temperatures and temperature compensation would require detailed knowledge of the isopotential points for all redox-reacting species present in the samples (ORP Theory – Phoenix Electrode Co. [phoenixelectrode.com]). Correction of ORP data for pH requires establishing a linear relationship between redox and pH measurements (Zhang et al., 1999). However, the relationship between pH and ORP

measurements is not necessarily linear, since the contribution of H^+ ions to the electron activity may represent only a faction of the total electron activity contribution by other dissolved species. This is expected to be especially true for more complex matrices, such as the pore water in grossly contaminated salt marsh sediments encountered in this study. In fact, there is poor correlation between ORP and pH measurements based on the study data described below. The ORP probe manufacturer does not recommend temperature compensation for ORP measurements. In addition, ORP measurements using a standard mV meter and ORP probe are acceptable for making relative comparisons of site-specific redox conditions for evaluating environmental conditions and biodegradation of chlorinated organics in ground water (USEPA, 1998). The ORP data generated in this study is considered comparable because the same meter and probe was used for all readings for the study duration, the accuracy of the ORP probe was confirmed for each sampling event, and the samples are from the same source and were all subject to the same physical conditions for the study period, including temperature.

Each sediment sample core from each of the two sample depths were split into four 1-gram sub-samples and one 5-gram sub-sample, and immediately placed on ice in a dark cooler. Of the 1-gram portions, one sub-sample was retained for root mass determinations, one sub-sample was fixed with 4% paraformaldahyde in phosphate buffered saline (PBS) (Hahn et al., 1992) placed in 96% ethanol and stored at $-20^{\circ}C$ for later molecular microbial analysis by total cell count using 4',6-diamidino-2-phenylindole (DAPI) staining and epifluorescence microscopy, and two sub-samples were placed in $-80^{\circ}C$ cold storage. The 5-gram sub-samples were collected for analysis of percent solids,

PHC and PAH using industry standard laboratory protocols: USEPA Method 418.1 for PHC; and USEPA SW-846 Methods 8270C and 3550B for PAH.

Root biomass was determined gravimetrically as a percentage of the total sediment sample weight on a dry-weight basis. Roots were physically separated from sediment samples, washed with de-ionized water and dried at 90 ^{o}C for 4 days in pre-weighed aluminum tins. The remaining sediment and wash water was also dried at 90 ^{o}C for 4 days in pre-weighed aluminum tins. Dried roots and sediments were weighed separately. Determination of the percent root mass was made by dividing the root dry weight by the sediment plus root dry weight (i.e., total dry sample weight) and multiplying by 100.

Leaf and shoot biomass was determined gravimetrically on a dry-weight basis, twice for all 20 pots (i.e., once after each growing season). At the end of each of the greenhouse study growing periods (i.e., Fall 2001 and Fall 2002) the aerial portions of the all 20 study specimens were cut at approximately 3 cm above the sediment surface in each pot, placed in pre-weighed paper bags, dried at 90 ^{o}C for 4-days and weighed.

Ammonia nitrogen, sulfate and total sulfide were monitored in pore water for all 30 treatments at two depths (2.5 and 7.5 cm) for all four sediment sampling events. Pore water samples were obtained using polyacrylamide gel probes and a back-equilibration procedure (Krom et al., 1994), with modifications as described in Burke (2001) to obtain a one-milliliter deionized water extract for aqueous analysis.

Two composite samples were collected for grain size (sieve) analysis, one from planted sediments (with plants) and one from bulk sediments (without plants). Each composite sample was prepared by collecting six discrete 100-gram sediment samples

from existing test pots. Three discrete samples from the pots with plants were combined into one composite sample, and three discrete samples from the pots w/o plants were combined into another composite sample (equal weight basis).

Collection of samples for metals analysis was omitted from the greenhouse study; however, metals analytical data from sediment samples obtained from planted and bulk sediments (Plot B) described as part of the field study (Chapter 2) are included in the following discussion of analytical results to provide a comparison of baseline sediment conditions in planted and bulk test sediments.

Sample Analysis

Microbial numbers were determined in sediment samples from two depths (2.5 and 7.5 cm) for eight selected pots, four bulk sediments and four containing *S. patens*. Enumeration was performed by direct visual counting of DAPI-stained microorganisms using a Nikon high resolution inverted Eclipse TE200 microscope equipped with a 100-watt high-pressure mercury bulb and filter set UV-2B (EX330-380, DM400, BA435) for DAPI epifluorescence at a magnification of 1000x. Microorganisms were counted in 20 random fields, each covering an area of 0.01 mm^2 from samples distributed over slide wells of 53 mm^2 each. Slides were prepared by dispersing 50 µl of the sediment sample suspended in ethanol in 950 µl of 0.1% sodium pyrophosphate. After mild sonication for 20 sec., 10 µl of the diluted sample suspension was spotted onto gelatin-coated glass slides and completely dried at 42 ^{o}C for about 15 minutes. Each slide well was treated with a 0.01% DAPI solution and slides were incubated in the dark, under a petri dish cover for 20 minutes. The slides were then rinsed with de-ionized water before and after

a 10-minute wash in hybridization buffer (0.9 M NaCl, 20mM Tris/HCl, 5 mM EDTA, 0.01% SDS) and air dried at room temperature. Upon dryness, the slides were mounted with Citifluor anti-fadant solution and cover slides, and immediately processed.

The PAH analyses were performed using a Hewlett Packard 5890/5972 gas chromatograph/mass spectrometer (GCMS) in selective-ion mode (SIM) to accommodate low sample volumes (~1 g) while maintaining low detection levels (<0.3 mg/kg) and minimizing matrix interference. The GC was operated with a 30-meter DB-5 column (0.25mm I.D., 0.25mm stationary phase) and a step-temperature program that had an initial temperature of 45 ^{o}C, increased to 130 ^{o}C at 20 ^{o}C per minute, increased from 130 ^{o}C to 250 ^{o}C at 10 ^{o}C per minute, and increased to a final temperature of 310 ^{o}C at 15 ^{o}C per minute, which was held for 10 minutes.

The targeted PAHs analyzed for this evaluation included:

	PAH	CAS No.	MW	Rings
1	Naphthalene	91-20-3	128	2
2	Acenaphthylene	208-96-8	152	2
3	Acenaphthene	83.32-9	154	2
4	Fluorene	86-73.7	166	3
5	Anthracene	120-12-7	178	3
6	Phenanthrene	85-01-8	178	3
7	Fluoranthene	206-44-0	190	4
8	Pyrene	129-00-0	216	4
9	Benzo(a)anthracene	56-55-3	228	4
10	Chrysene	218-01-9	228	4
11	Benzo(a)pyrene	50-32-8	252	5
12	Benzo(b)fluoranthene	205-99-2	252	5
13	Benzo(k)fluoranthene	207-08-9	252	5
14	Benzo(g,h,i)perylene	191-24-2	276	6
15	Indeno(1,2,3.cd)pyrene	193.39-5	276	6
16	Dibenzo(a,h)anthracene	53.70-3	278	5

All samples were spiked with internal standards and surrogates for PAH analysis. Internal standards added to sample extracts after extraction and prior to injection into the GC included acenaphthene-d10, phenanthrene-d10, chrysene-d12 and perylene-d12. Surrogates added to the samples prior to extraction included nitrobenzene-d5, 2-fluorobiphenyl and *p*-terphenyl-d14 ("terphenyl"). PAH data were corrected based on terphenyl surrogate recovery data as a measure of instrument response using a method adapted from Swartz et al. (2003). The basis, background and description of the procedure used for data correction are included in Appendix C.

Back-equilibration aqueous extracts were analyzed colorimetrically by UV/Visual spectrophotometry for ammonia and total sulfide, and photometrically for sulfate by a barium chloride turbidity method, as described by Burlage et al. (1998).

The grain size (sieve) analysis of two composite samples was performed using ASTM D422-63.

Statistical Analysis

Statistical analyses of the study data were performed using StatTools software (Professional Edition, version 1.0.1; Palisade Corporation 2003). Data are reported as summary statistics, including the arithmetic mean plus/minus (±) the standard deviation of the mean. One-way analysis of variance (ANOVA) and Tukey confidence interval tests were performed for comparison of sample means at both depth intervals within treatments, with P-values and F-ratios calculated per treatment using a statistical significance level of 95%. Statistical significance was evaluated between treatments at corresponding sample depths using two-tailed t-tests; the resulting summary statistics are

reported at either the 95% or 99% significance level. The assumption of normal (Gaussian) distribution was checked using Lilliefors Test, which is based on a comparison of the normal and empirical cumulative distribution (cdf) function. For time-series trends and linear regression analysis of the data in this study, a regression coefficient (R^2) of ≥ 0.8 is considered significant.

3.4 Greenhouse Study Results

3.4.1 Plant Survival and Biomass

Nineteen of the 20 plants survived through the entire greenhouse study period (i.e., two growing seasons), which represents a 95% rate of survival. Leaf and shoot biomass production was fairly consistent for the two growing seasons; with average dry weights of 6.5±3.7 grams measured in the Fall 2001 and 6.1±2.5 grams measured in the Fall 2002. The average and range for each period appear to be similar between growing seasons.

The mean root biomass (Table 3.1 and Figure 3.1) at 2.5 cm ranged from 0.8±0.6% to 2.6±3.6% and at 7.5 cm mean root biomass ranged from 1±0.9% to 2.3±4.9% (ANOVA P=0.2722). Like the aerial biomass production, root biomass data are consistent for the study period, with no significant differences between depths. The biomass data and observations made during sample collection indicate generally consistent, stable plant growth and function. The root biomass data demonstrate root penetration and relatively even growth distribution through the sediment matrix. The presence of the root biomass represents addition of about 1–2% natural organic carbon to the root-zone sediment, and unquantified amounts of organic root exudates. The addition of such a significant and diffuse input of organic carbon in contaminated sediment has

important implications for microbial density and diversity, biogeochemical conditions, and contaminant sorption, bioavailability and degradation.

Table 3.1 Summary Statistics for Root Biomass at two depths

One Variable Summary	Spring 01 2.5cm Data Set #1	Fall 01 2.5cm Data Set #2	Spring 02 2.5cm Data Set #3	Fall 02 2.5cm Data Set #4
Mean	2.552	1.884	0.839	1.980
Variance	12.970	3.931	0.438	4.574
Std. Dev.	3.601	1.983	0.662	2.139
Minimum	0.001	0.102	0.089	0.001
Maximum	9.766	9.005	2.866	7.680
Range	9.765	8.903	2.778	7.679
Count	20	20	20	20

One Variable Summary	Spring 01 7.5cm Data Set #5	Fall 01 7.5cm Data Set #6	Spring 02 7.5cm Data Set #7	Fall 02 7.5cm Data Set #8
Mean	1.048	1.339	2.319	1.065
Variance	3.047	2.309	23.976	0.886
Std. Dev.	1.745	1.520	4.896	0.941
Minimum	0.001	0.102	0.001	0.001
Maximum	6.964	6.358	20.560	3.807
Range	6.963	6.256	20.559	3.806
Count	20	20	20	20

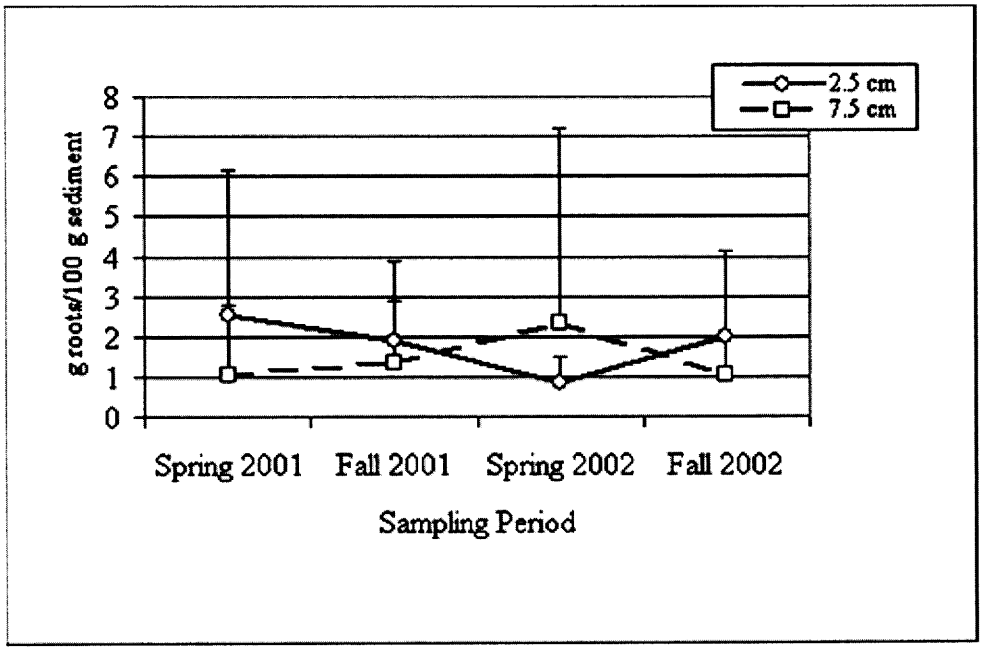


Figure 3.1 Summary of average in-situ root biomass in sediment.

3.4.2 Grain Size Distribution and Metals

The percent gravel, sand and silt/clay/colloids in the two composite samples is presented in Figure 3.2a and a particle size distribution curve is given as Figure 3.2b. Based on the analytical results, the grain size distribution in the bulk and planted sediments is similar. The samples contain 23.8 and 13.8% gravel, 73.3% and 84.4% sand, and 2.9% and 1.9% fines (silt/clay), respectively. Given the particle size data and gradation, the estimated sediment porosity ranges from 25% to 30% (Driscoll, 1986).

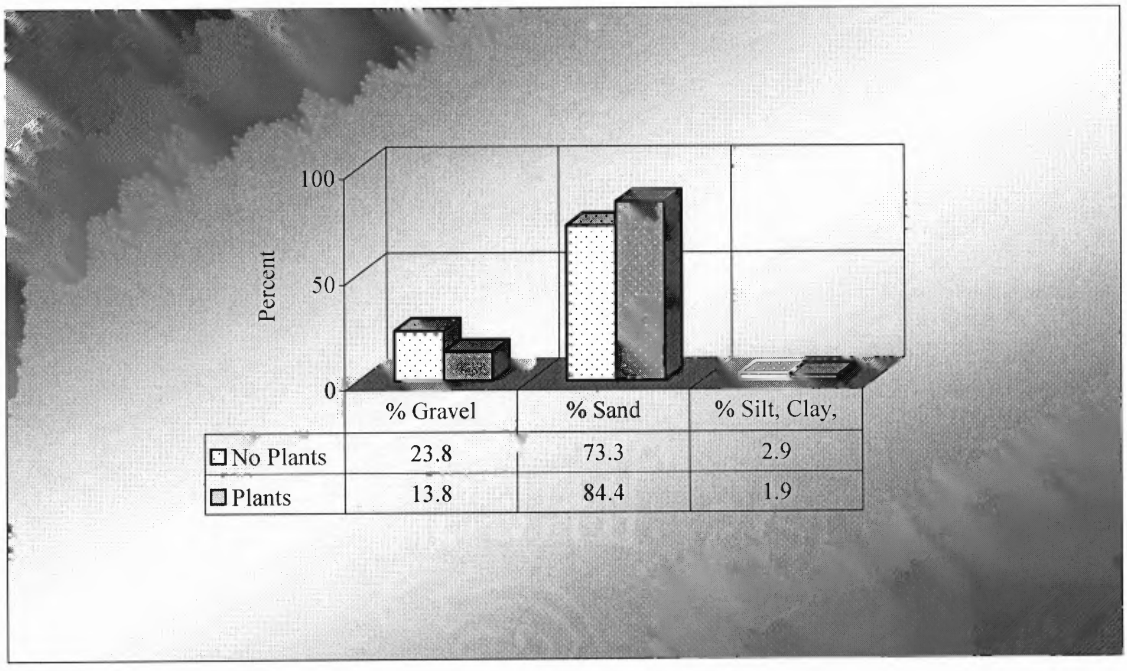


Figure 3.2a Percent gravel, sand and silt, clay and colloids in planted and bulk sediments.

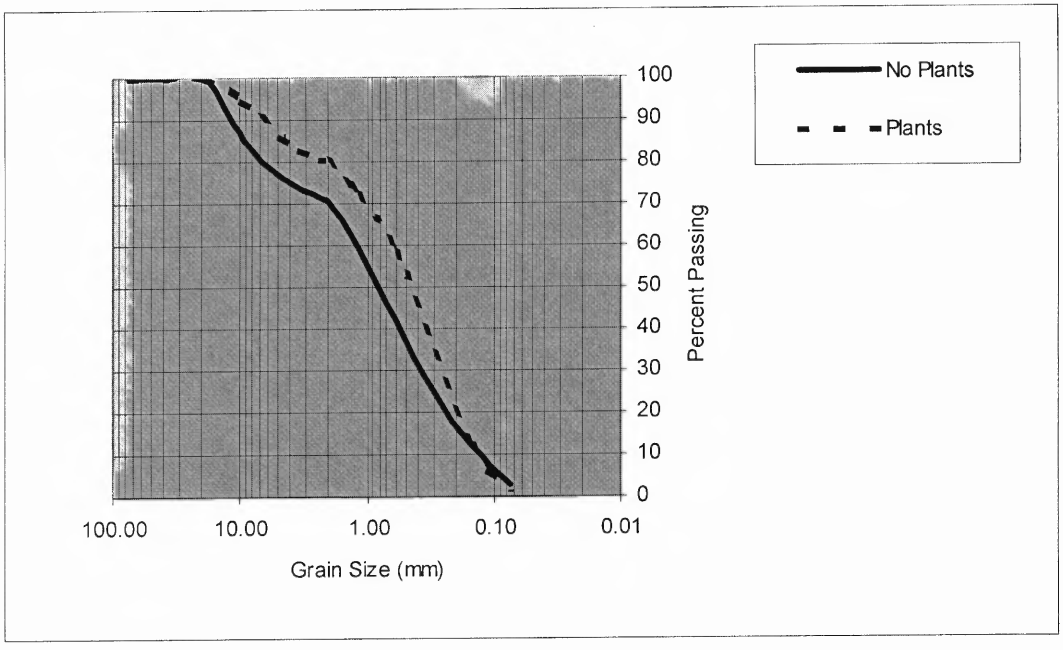


Figure 3.2b Particle size distribution in planted and bulk sediment composite samples.

During the field study (Chapter 2), seven discrete sediment samples were collected from the upper 15 cm in and around the Plot B area. Three samples were obtained from outside the study plot (bulk sediment) and four samples were collected from within the plot area (planted sediment). The samples were analyzed for total TAL Metals, which includes aluminum, antimony, arsenic, barium, beryllium, cadmium, calcium, chromium, cobalt, copper, iron, lead, magnesium, manganese, mercury, nickel, potassium, selenium, silver, sodium, thallium, vanadium, and zinc. Based on t-tests comparing the sample means for each of the 23 TAL metals (Table 3.2), there is no significant difference in the planted and bulk sediment metal concentrations ($P>0.05$).

Table 3.2 Summary Statistics for TAL Metals in samples obtained from the upper 15 cm of Vegetated and Bulk Sediments collected during the Field Study (November 2000)

	Plants		No Plants		
TAL Metals (ppm)	Mean (n=4)	SD	Mean (n=3)	SD	p-Value
Aluminum	6558	1489	7337	320	0.4234
Antimony	2.5	1.0	1.9	0.1	0.3120
Arsenic	9.4	2.5	9.7	1.0	0.8294
Barium	49.1	7.7	46.9	8.7	0.7409
Beryllium	0.7	0.0	0.7	0.1	0.9602
Cadmium	0.7	0.0	0.7	0.1	0.9602
Calcium	1404	452	1510	252	0.7332
Chromium	84.7	15.8	61.6	18.5	0.1352
Cobalt	7.1	0.5	7.1	0.8	0.9602
Copper	115.8	29.8	120.0	8.2	0.8252
Iron	20250	5256	24633	252	0.1945
Lead	230.5	76.5	245.0	89.2	0.8257
Magnesium	1738	227	1857	372	0.6182
Manganese	85.1	17.4	114.7	17.2	0.0759
Mercury	0.4	0.2	0.2	0.1	0.2869
Nickel	21.7	3.7	24.5	3.1	0.3448
Potassium	845	253	925	101	0.6324
Selenium	1.7	0.2	1.6	0.4	0.7560
Silver	1.6	0.4	1.4	0.2	0.4849
Sodium	708	45	710	82	0.9602
Thallium	1.4	0.1	1.4	0.2	0.7771
Vanadium	59.7	2.6	59.0	5.3	0.8280
Zinc	103.4	21.3	122.8	24.4	0.3120

p-Value represents two-sample, two-tailed t-test of means.

3.4.3 Ammonia Nitrogen, Sulfate and Sulfide

Summary statistics for the ammonia nitrogen, sulfate and total sulfide data are included in Table 3.3 and presented on Figures 3.3a, 3.3b and 3.3c, respectively.

Table 3.3 Ammonia, Sulfate and Total Sulfide in Vegetated and Bulk Sediment Pore Water

COMPOUND	Treatment	Depth	Spring 2001		Fall 2001		Spring 2002		Fall 2002		ANOVA	
			Mean	+/- SD	Mean	+/- SD	Mean	+/- SD	Mean	+/- SD	p-Value	F-ratio
Ammonia (as Nitrogen)	No Plants	2.5 cm	3.95	3.35	3.55	3.69	2.06	1.13	2.07	0.77		
		7.5 cm	2.35	1.05	1.49	2.93	2.21	0.70	2.52	1.19	0.2170	1.4
	Plants	2.5 cm	3.14	3.11	**5.15**	3.72	2.56	0.70	1.65	0.57		
		7.5 cm	2.56	2.71	3.18	3.59	2.41	0.71	1.62 *	0.44	0.0002	4.4
Sulfate	No Plants	2.5 cm	1118	226	802	303	**2327** *	1135	719	408		
		7.5 cm	1263	312	700	216	**1823** *	715	565	211	0.0000	13.4
	Plants	2.5 cm	1139	310	712	190	910	141	**586**	242		
		7.5 cm	1130	532	**443** *	102	988	219	**479**	225	0.0000	21.0
Sulfide	No Plants	2.5 cm	0.05	0.02	0.03 *	0.03	0.02	0.03	0.01	0.03		
		7.5 cm	**0.11**	0.05	0.04 *	0.03	0.01	0.02	0.01	0.04	0.0000	9.6
	Plants	2.5 cm	**0.07** *	0.03	0.00	0.01	0.01	0.02	0.00	0.01		
		7.5 cm	**0.10**	0.04	0.01	0.01	0.01	0.02	0.01	0.02	0.0000	53.1

Notes: (1) Sample mean and standard deviation (SD) data are in mg/L.
(2) For "No Plants" treatments, n=10; F-critical=2.08
(3) For "Plants" treatments, n=20; F-critical=2.14
(4) **Bold** mean values are significant at $p<0.05$ within treatments based on one-way ANOVA and Tukey confidence interval analysis.
(5) Shaded mean values indicate significance at $p<0.05$ between depths within treatments.
(6) Single asterisk (*) indicates significant difference of means at $p<0.05$ between treatments at similar depths (t-test).
(7) Double asterisk (**) indicates significant difference of means at $p<0.01$ between treatments at similar depths (t-test).

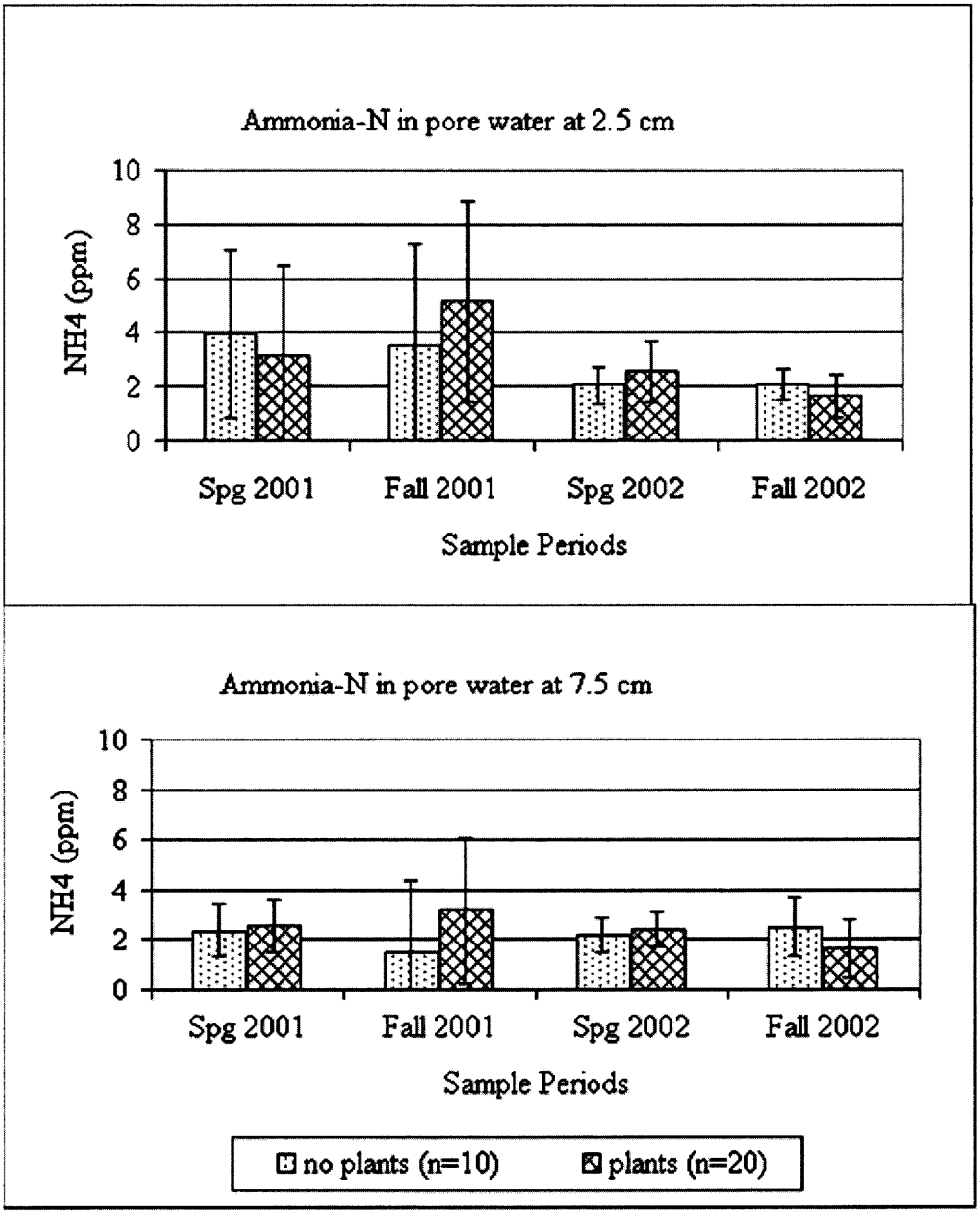


Figure 3.3a Ammonia nitrogen in pore water at 2.5 cm and 7.5 cm.

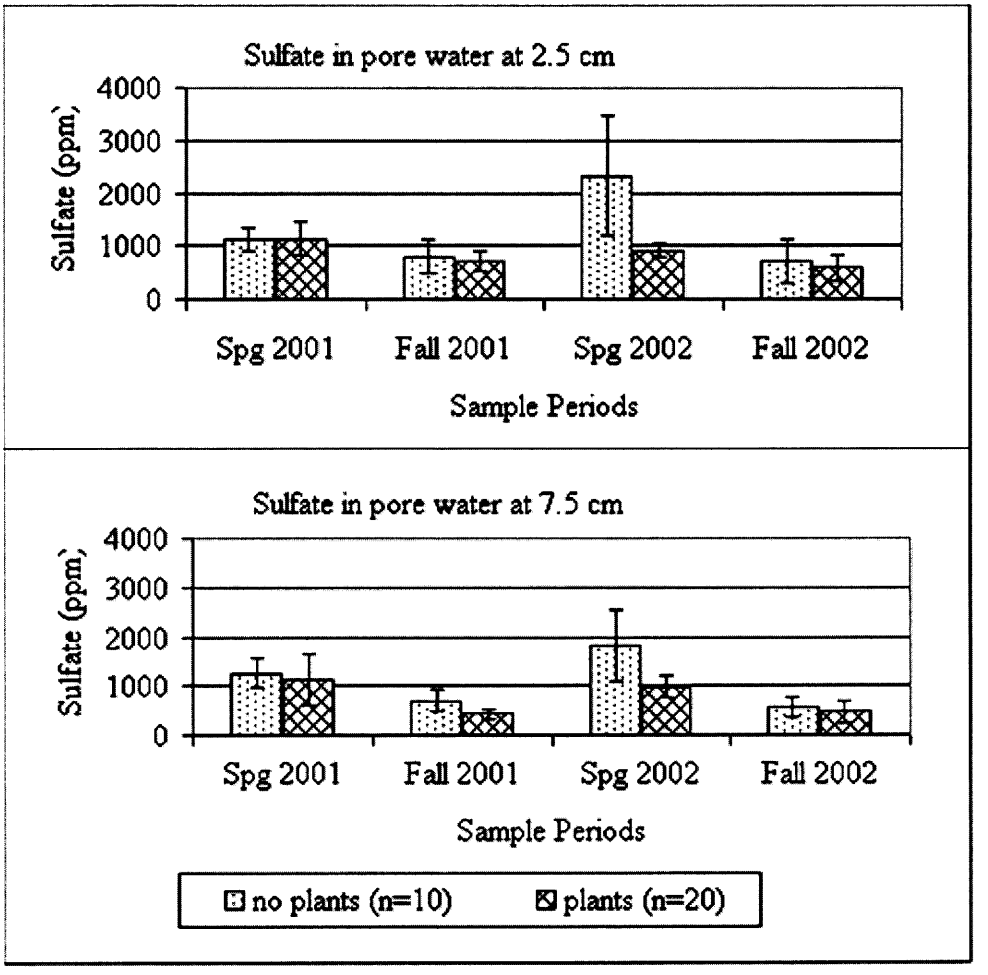


Figure 3.3b Sulfate in pore water at two depths.

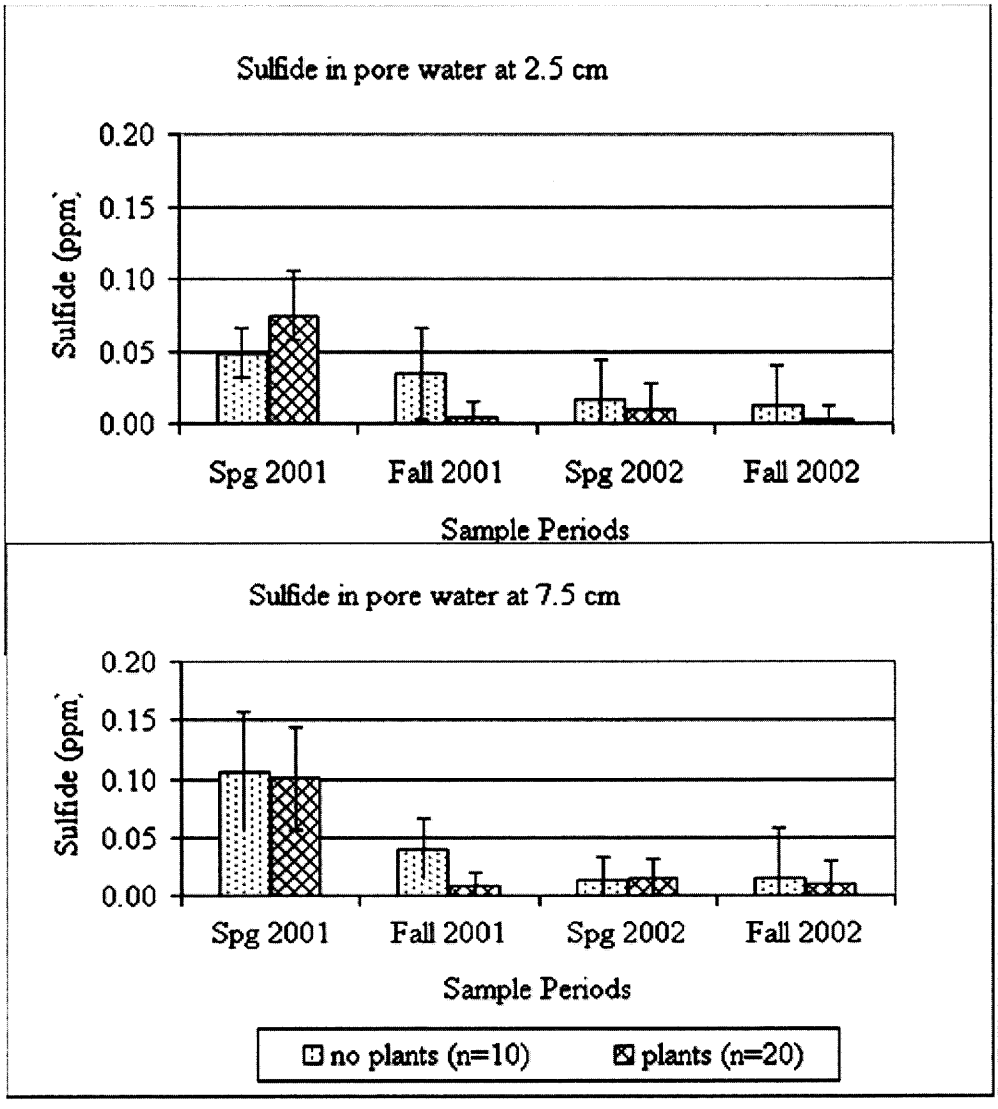


Figure 3-3c Total sulfide in pore water at 2.5 cm and 7.5 cm.

Average ammonia nitrogen concentrations in bulk sediment pore water (Figure 3.3a) ranged from 2.1±1.1 mg/l to 3.9±3.4 mg/l at the 2.5 cm depth, and from 1.5±2.9 mg/l to 2.5±1.2 mg/l at the 7.5 cm depth (ANOVA P=0.217). In the planted treatments, the average ammonia nitrogen concentrations in planted sediment pore water ranged from 1.7±0.6 mg/l to 5.2±3.7 mg/l at the 2.5 cm depth, and from 1.6±0.4 mg/l to 3.2±3.6 mg/l

at the 7.5 cm depth (ANOVA P=0.0002). The statistical significance in the planted treatment is due solely to anomalously high ammonia concentrations in the Fall 2001 at 2.5 cm; the data for both treatments and both depths are not otherwise significantly different. Despite the elevated concentrations in the one sample even, the overall similarity of ammonia concentrations is due to the consistent addition of ammonia via routine watering.

The mean sulfate concentrations in bulk sediment pore water ranged from 719±408 mg/l to 2327±1135 mg/l at the 2.5 cm depth, and from 565±211 mg/l to 1823±715 mg/l at the 7.5 cm depth (ANOVA P<0.0001). In the planted treatments, the average sulfate concentrations in planted sediment pore water ranged from 1139±310 mg/l to 586±242 mg/l at the 2.5 cm depth, and from 1130±532 mg/l to 443±102 mg/l at the 7.5 cm depth (ANOVA P<0.0001). The statistical significance noted for sulfate in the bulk sediments is due solely to anomalously high sulfate concentrations in the Spring 2002 at both depths, and for planted sediment in the Fall 2001 at 7.5 cm and in the Fall 2002 at both depths. The overall similarity in sulfate concentrations is the result of routine watering with reconstituted salt water that naturally contains sulfate. The data demonstrate an even distribution of sulfate through the test sediment matrix for use by microbial sulfate reducers at concentrations that are not limiting.

Total sulfide was initially detected the bulk and planted sediment pore water at mean concentrations ranging from 0.05±0.02 mg/l to 0.11±0.05 mg/l in samples collected during the Spring 2001 sampling event. However, mean total sulfide concentrations stabilized just above the limit of detection, at about 0.02 mg/l in all test sediments for Fall 2001, Spring 2002 and Fall 2002 data (ANOVA P<0.0001).

3.4.4 Temperature, pH and ORP

Summary statistics for temperature, pH and ORP data are included in Table 3.4 and presented in Figures 3.4a, 3.4b and 3.4c, respectively. The mean temperature measured in bulk sediment ranged from 22.5±0.4 C to 27.8±2 C at the 2.5 cm depth, and from 22.7±0.9 C to 27.9±1.9 C at the 7.5 cm depth. In the planted treatments, the average temperature in planted sediment ranged from 22.0±1.8 C to 27.4±2 C at the 2.5 cm depth, and from 22.1±2 C to 27.2±1.8 C at the 7.5 cm depth. Temperature was virtually the same for all depths and replicates for each sample event, indicating the test treatments were maintained at a reasonably constant temperature in the greenhouse. The small variation in temperature between the four sample dates follows a seasonal pattern that corresponds to outdoor conditions, i.e., cooler in the spring and warmer in the fall.

Table 3.4 Oxidation-reduction (ORP), pH and Temperature in Vegetated and Bulk Sediments

PARAMETER	Treatment	Depth	Spring 2001		Fall 2001		Spring 2002		Fall 2002		ANOVA	
			Mean	+/- SD	Mean	+/- SD	Mean	+/- SD	Mean	+/- SD	p-Value	F-ratio
ORP (mV)	No Plants	2.5 cm	417	29	**255**	37	362	38	**205**	78		
		7.5 cm	344	31	**110**	115	**212**	148	**50**	132	<0.0001	20.3
	Plants	2.5 cm	233 *	108	135 *	106	195 *	116	**64** *	115		
		7.5 cm	138 *	139	**-63** *	126	101	177	**-48** *	99	<0.0001	14.3
pH (su)	No Plants	2.5 cm	3.13	0.47	3.21	0.21	3.42	0.22	3.65	0.38		
		7.5 cm	2.96	0.18	**4.07**	0.94	**4.40**	0.74	**4.97**	0.97	<0.0001	13.6
	Plants	2.5 cm	4.39 *	0.74	4.75 *	0.86	4.73 *	0.70	**5.43** *	1.04		
		7.5 cm	4.81 *	0.74	5.44 *	0.69	5.28 *	0.70	**5.88** *	0.66	<0.0001	8.2
Temperature (C)	No Plants	2.5 cm	**22.5**	0.4	25.1	2.2	**22.7**	1.7	**27.8**	2.0		
		7.5 cm	**22.7**	0.9	25.4	2.2	**22.7**	1.7	**27.9**	1.9	<0.0001	17.2
	Plants	2.5 cm	**22.0**	1.8	24.3	2.1	23.0	2.1	**27.4**	2.0		
		7.5 cm	**22.1**	2.0	24.6	2.3	23.0	2.1	**27.2**	1.8	<0.0001	22.0

Notes: (1) Sample mean and standard deviation (SD) data are in millivolts (mV), standard units (su) and celsius (C).
(2) For "No Plants" treatments, n=10; F-critical=2.08
(3) For "Plants" treatments, n=20; F-critical=2.14
(4) **Bold** mean values are significant at $p<0.05$ within treatments based on one-way ANOVA and Tukey confidence interval analysis.
(5) Shaded mean values indicate significance at $p<0.05$ between depths within treatments.
(6) Single asterisk (*) indicates significant difference of means at $p<0.05$ between treatments at similar depths (t-test).
(7) Double asterisk (**) indicates significant difference of means at $p<0.01$ between treatments at similar depths (t-test).

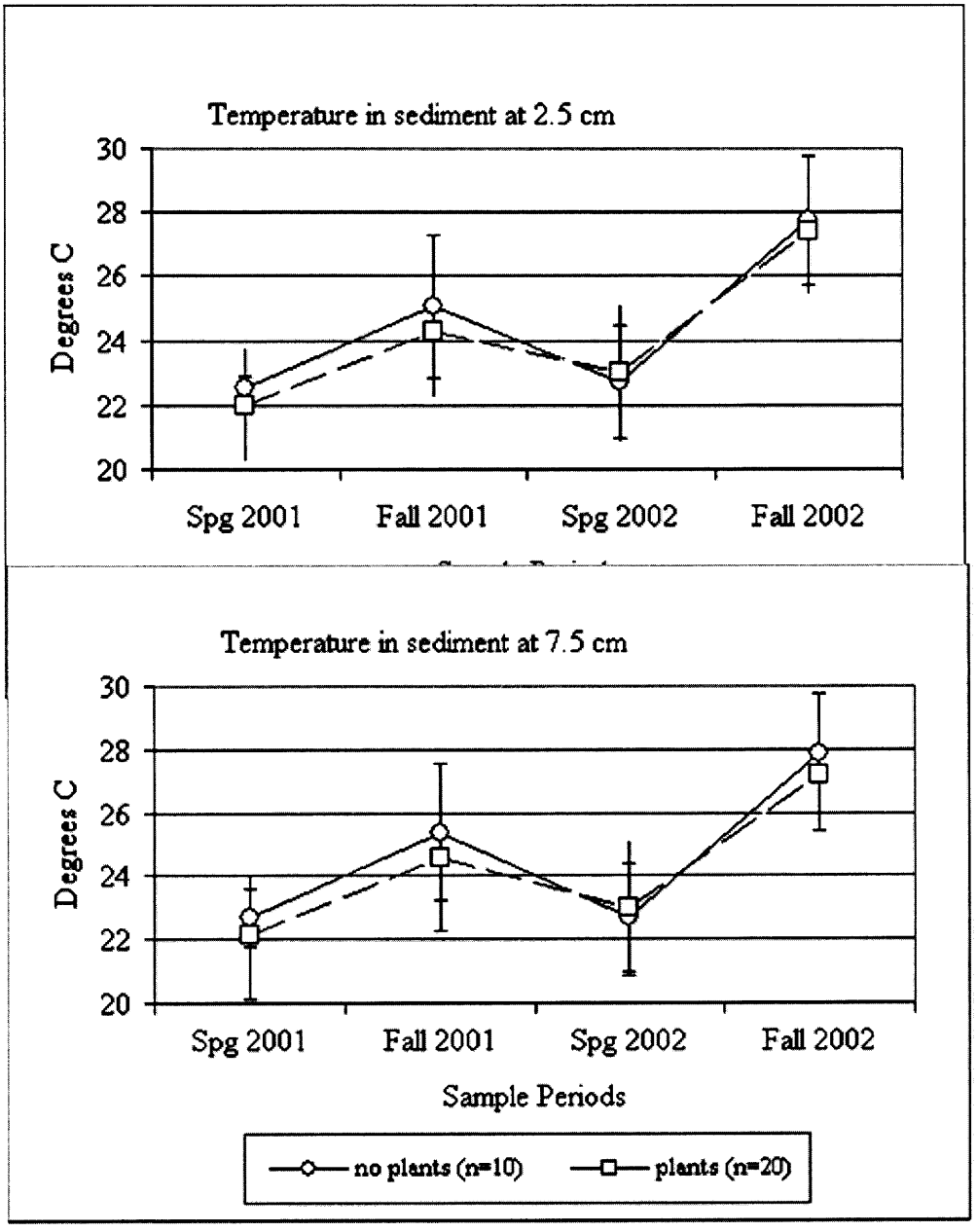


Figure 3-4a Temperature in test treatments at 2.5 cm and 7.5 cm.

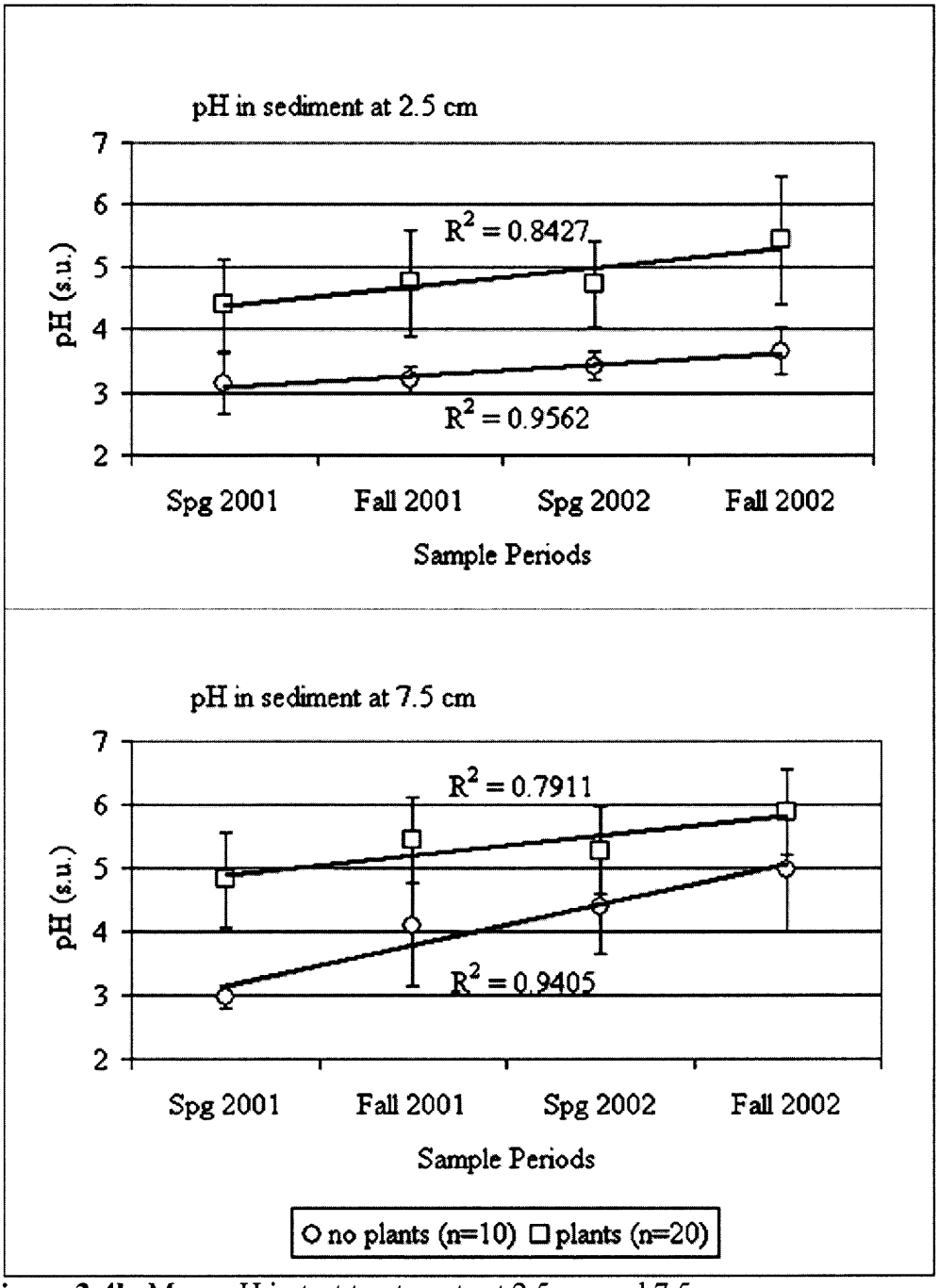


Figure 3-4b Mean pH in test treatments at 2.5 cm and 7.5 cm.

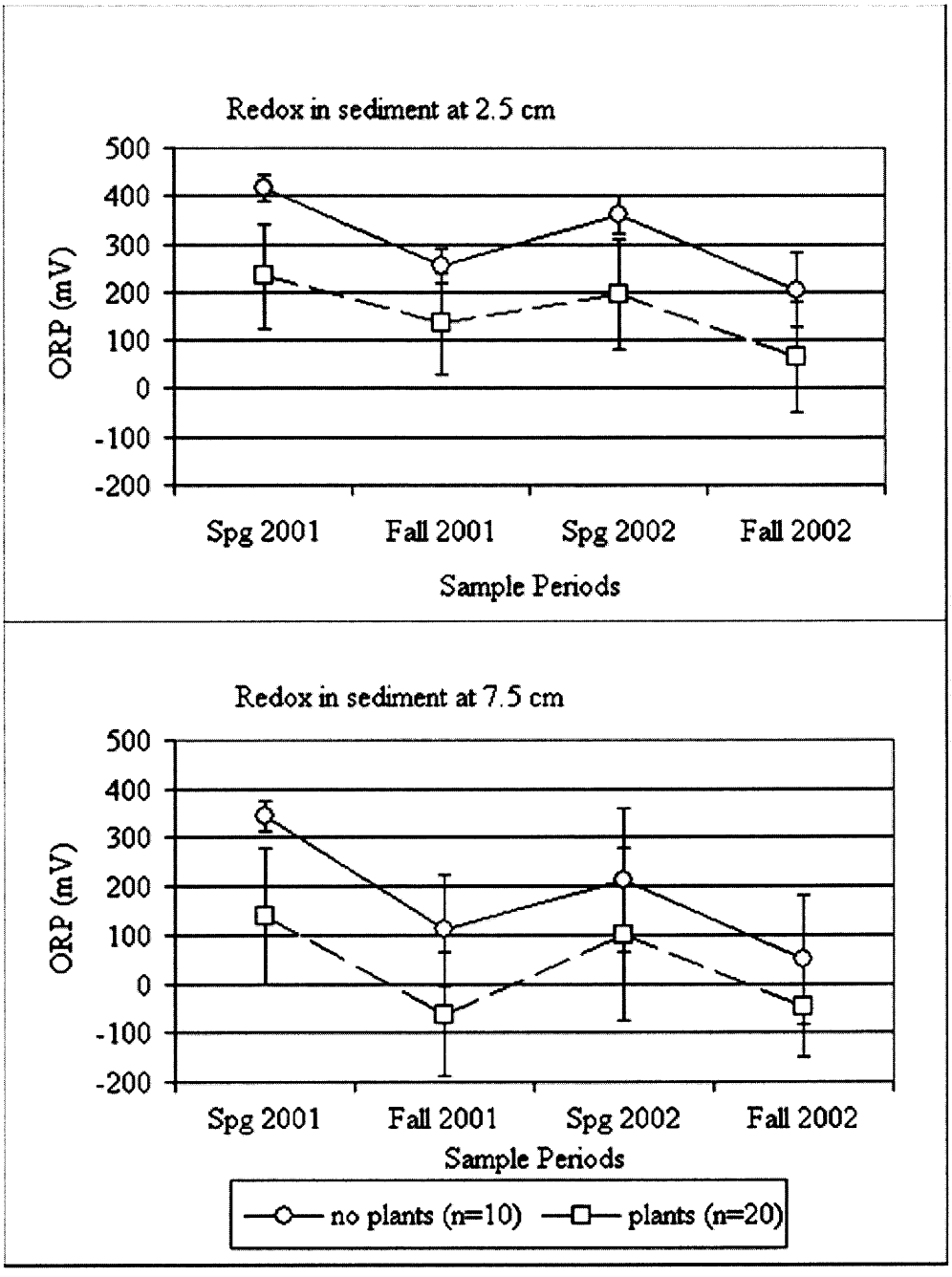


Figure 3.4c Oxidation-reduction potential (ORP) in test treatments at 2.5 cm and 7.5 cm.

Test sediments for both treatments were acidic and exhibited marked change over the test period. The mean pH (standard pH units [su]) in bulk sediment ranged from 3.13±0.47 to 3.65±0.38 at the 2.5 cm depth, and from 2.96±0.18 to 4.97±0.97 at the 7.5 cm depth (ANOVA $P<0.0001$). In the planted treatments, the pH averaged from 4.39±0.74 to 5.43±1.0 at the 2.5 cm depth, and from 4.81±0.74 to 5.88±0.66 at the 7.5 cm depth (ANOVA $P<0.0001$). In both treatments and at both depths pH exhibits an increasing trend for the study period; trends are nearly linear ($R^2 = 0.7911$ to 0.9562). The pH data in the planted sediment at both depths for all four sampling events are significantly higher ($P<0.01$) than those in the bulk sediments. The pH conditions in the bulk sediments are low enough to be expected to hinder microbial/enzyme activity (Norris, 1994). The higher pH values measured in the planted sediments are indicative of the positive influence of root exudates and represent improved conditions (vs. bulk sediments) for microbial growth and biodegradation.

Average ORP measurements in bulk sediment pore water ranged from 205±78 mV to 417±29 mV at the 2.5 cm depth, and from 50±132 mV to 344±31 mg/l at the 7.5 cm depth (ANOVA $P<0.0001$). In the planted treatments, the average ORP in planted sediment pore water ranged from 64±115 mV to 233±108 mV at the 2.5 cm depth, and from -63±126 mV to 138±139 mV at the 7.5 cm depth (ANOVA $P<0.0001$). The statistical significance in the bulk sediment ORP measurements appears to be from seasonal low ORP in the Fall 2001 and Fall 2002, which is likely a function of diffusion and solubility of atmospheric oxygen and modest seasonal temperature variation in the greenhouse. The presence of even low concentrations of dissolved oxygen (DO) drives the redox in a positive direction; even low amounts of DO will generally cause ORP to be

above zero. Since DO solubility is inversely proportional to temperature, the slight decreases in ORP measured in the bulk sediment treatment replicates during Fall 2001 and Fall 2002 are more likely related to increases in greenhouse temperature than to microbial activity based on low, unchanging microbial densities described below. The pronounced ORP changes in bulk sediments at the 2.5 cm depth is evidence of re-oxygenation by surface air diffusion; the more pronounced negative ORP measurements in the planted treatments are indicative of microbial activity causing more reducing conditions at the deeper sample interval (i.e., 7.5 cm). The temporal pattern of changes in the planted sediment ORP data suggests that redox conditions oscillate from aerobic to microaerobic in the upper 3 cm, and from microaerobic to anaerobic (reducing) at around 7.5 cm.

3.4.5 Microbial Density

The mean microbial density (DAPI-stained cells/g sediment [dry weight basis]) in bulk sediment (Table 3.5 and Figure 3.5a) ranged from $7.5\pm8.8\text{x}10^6$ cells/g to $5.2\pm8.0\text{x}10^7$ cells/g at the 2.5 cm depth (ANOVA P=0.727), and from $1.4\pm0.86\text{x}10^7$ cells/g to $5.0\pm2.1\text{x}10^7$ cells/g at the 7.5 cm depth (ANOVA P=0.287). In the planted treatments, microbial densities averaged from $6.7\pm7.7\text{x}10^7$ cells/g to $3.5\pm2.9\text{x}10^8$ cells/g at the 2.5 cm depth (ANOVA P=0.155), and from $1.2\pm1.9\text{x}10^8$ cells/g to $1.1\pm0.57\text{x}10^8$ cells/g at the 7.5 cm depth (ANOVA P=0.993). The average microbial densities at 2.5 cm increased linearly in the planted sediment ($R^2 = 0.9675$) by approximately 4.2 times (i.e., $5.5\text{x}10^6$ cells/g/day), and decreased linearly in the bulk sediments ($R^2 = 0.944$) (Figure 3.5b). The microbial density data in the planted sediment at 2.5 cm for the last two sample events are significantly higher than those in the bulk sediments (Spring 2002, P=0.0361 and Fall

2002, P=0.0276). In contrast, microbial densities measured in the planted treatment at 7.5 cm exhibit no trend and remain at about $1x10^8$ cells/g for the test duration (Figure 3.5c). The microbial densities in the bulk sediment treatment at 7.5 cm are similar to those found in bulk sediment at 2.5 cm (i.e., approx. $1x10^7$ cells/g), and exhibit a near-linear downward trend ($R^2 = 0.8403$) over the study period (Figure 3.5c).

Table 3.5 Microbial Density in Vegetated and Bulk Sediments

Microbial Density	Treatment	Depth	Spring 2001		Fall 2001		Spring 2002		Fall 2002		ANOVA	
			Mean	+/- SD	Mean	+/- SD	Mean	+/- SD	Mean	+/- SD	p-Value	F-ratio
DAPI Count (Cells/ g soil)	No Plants	2.5 cm	5.2E+07	8.0E+07	4.5E+07	7.1E+07	1.8E+07	2.5E+07	7.5E+06	8.8E+06	0.727	0.445
		7.5 cm	5.0E+07	2.1E+07	3.4E+07	4.6E+07	9.8E+06	1.0E+07	1.4E+07	8.6E+06	0.287	1.502
	Plants	2.5 cm	6.7E+07	7.7E+07	1.4E+08	1.7E+08	2.1E+08 *	1.7E+08	3.5E+08 *	2.9E+08	0.155	1.998
		7.5 cm	1.2E+08	1.9E+08	1.3E+08	1.4E+08	1.3E+08 *	1.2E+08	1.1E+08 *	5.7E+07	0.993	0.030

Notes: (1) Sample mean and standard deviation (SD) data are in cells per gram soil, dry weight basis.
(2) For "No Plants" treatments, n=3; F-critical = 4.07
(3) For "Plants" treatments, n=5; F-critical = 3.24
(4) **Bold** mean values are significant at $p<0.05$ based on one-way ANOVA and Tukey confidence interval analysis.
(5) Single asterisk (*) indicates significant difference of means at $p<0.05$ between treatments at similar depths (t-test).

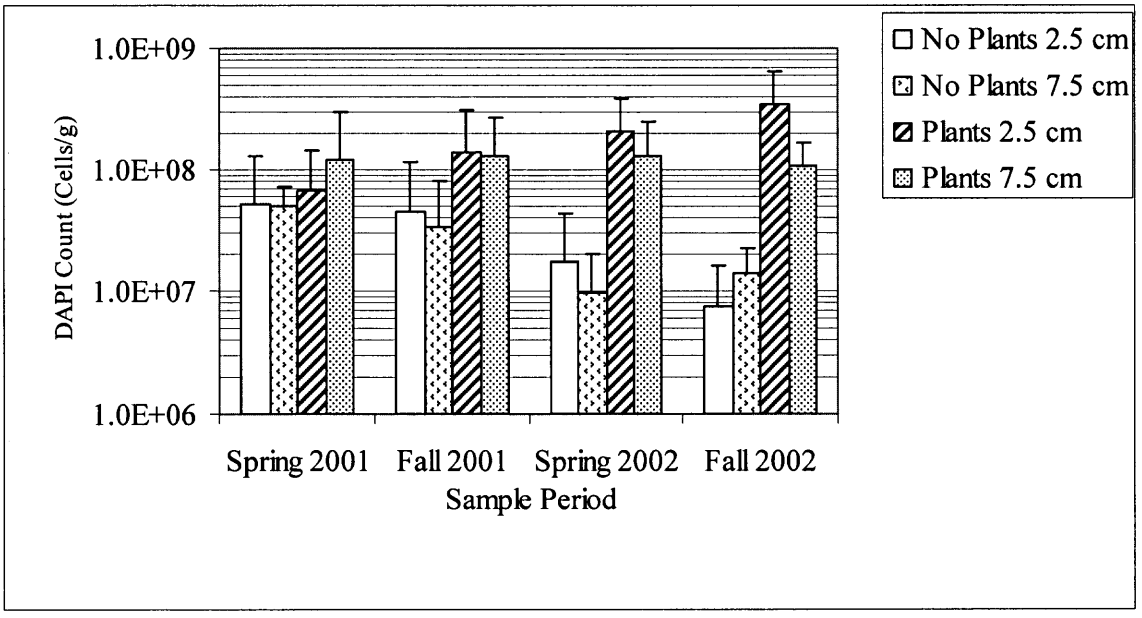


Figure 3.5a Microbial density in planted and bulk sediment at 2.5 and 7.5 cm.

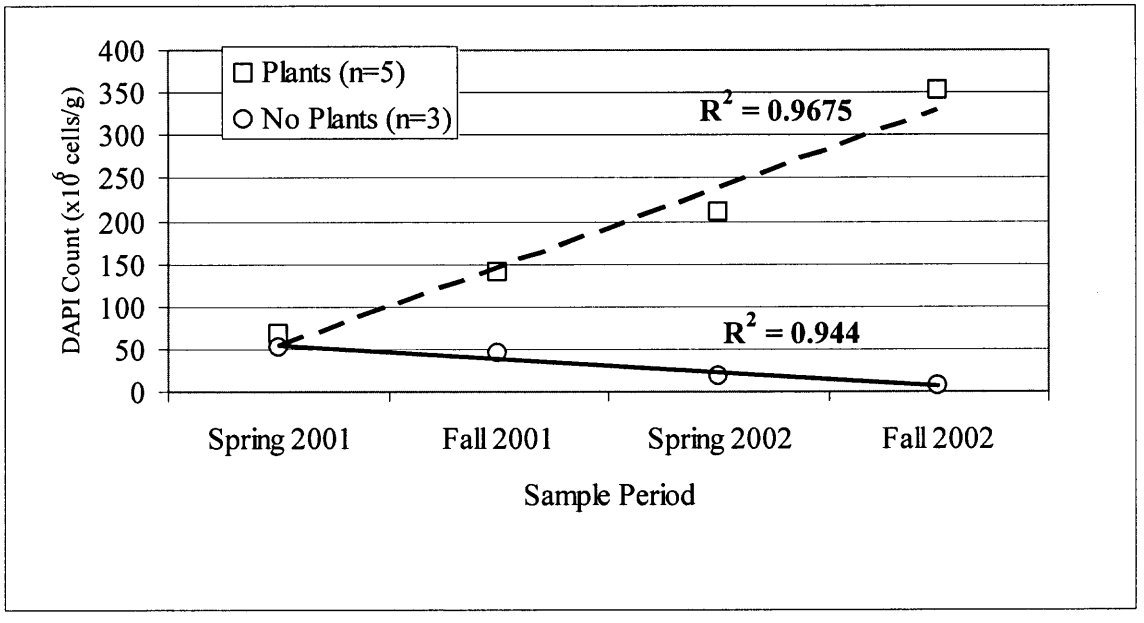


Figure 3.5b Microbial density trends in planted and bulk sediment at 2.5 cm.

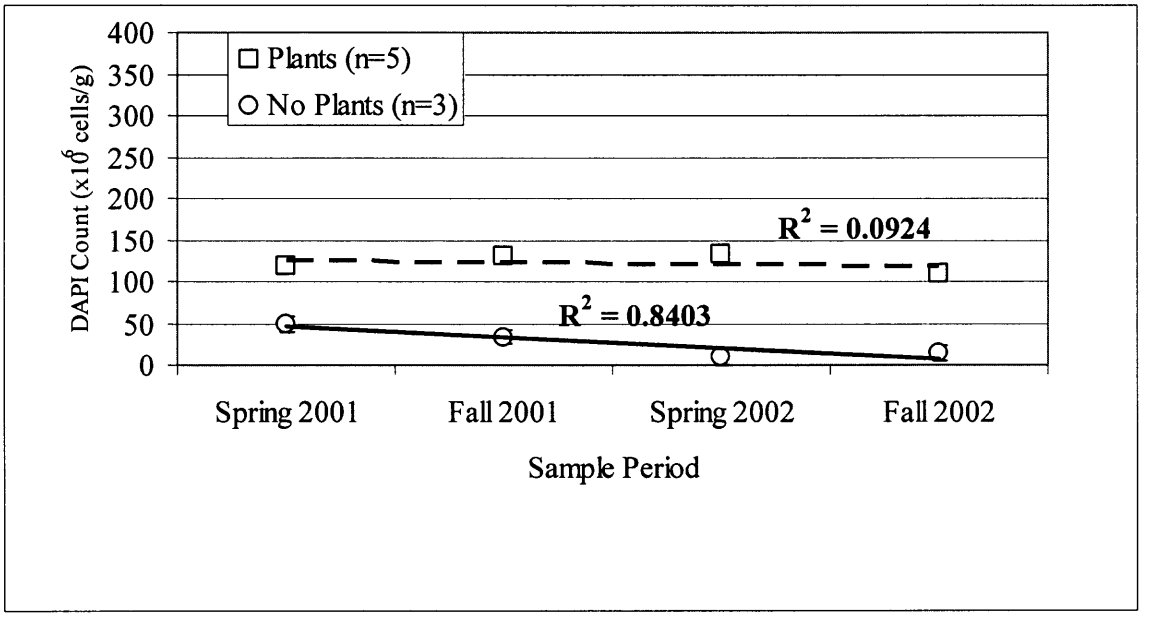


Figure 3.5c Microbial density trends in planted and bulk sediment at 7.5 cm.

The higher microbial densities found in the planted sediment were anticipated based on the pH data, ORP measurements, addition of organic carbon from root biomass. However, the decreasing trend in bulk sediment microbial density was not expected; the cause is unknown, and may be related to the change from fresh water Field Study conditions to salt water Greenhouse Study conditions. Regardless, the trend appears indicative of increased differences in microbial density between planted and bulk sediments beyond the study duration.

3.4.6 Petroleum Hydrocarbons

Test sediments for both treatments contained very high concentrations of PHC (Table 3.6 and Figure 3.6a). The mean PHC concentration in bulk sediment ranged from 53,590±32,046 ppm to 95,670±21,099 ppm at 2.5 cm, and from 45,100±14,915 ppm to 86,810±18,504 ppm at 7.5 cm (ANOVA $P<0.0001$). In the planted treatments, the PHC

averaged from 64,652±49,643 ppm to 89,014±59,383 ppm at 2.5 cm, and from 51,160±29,279 ppm to 70,605±51,452 ppm at 7.5 cm (ANOVA P=0.2379). The average PHC in the planted sediment at both depths for the last sample event (Fall 2002) is significantly lower than those in the bulk sediments ($P<0.05$). The statistical significance noted for mean PHC concentrations in the bulk sediments is due to lower PHC concentrations reported for the Fall 2002 and Spring 2002 sampling rounds at both depths.

Table 3.6 Total petroleum hydrocarbons in Vegetated and Bulk Sediments

PARAMETER	Treatment	Depth	Spring 2001		Fall 2001		Spring 2002		Fall 2002		ANOVA	
			Mean	+/- SD	Mean	+/- SD	Mean	+/- SD	Mean	+/- SD	p-Value	F-ratio
TPHC	No Plants	2.5 cm	76780	14699	**53590**	32046	**57420**	27427	95670	21099		
		7.5 cm	79410	30541	**46550**	23496	**45100**	14915	86810	18504	0.0000	6.7
	Plants	2.5 cm	89014	59383	66575	45585	83082	59608	64652 *	49643		
		7.5 cm	69535	43348	51160	29279	70605	51452	54608 *	52091	0.2379	1.3

Notes: (1) Sample mean and standard deviation (SD) data are in mg/Kg, dry weight basis.
(2) For "No Plants" treatments, n=10; F-critical=2.08
(3) For "Plants" treatments, n=20; F-critical=2.14
(4) **Bold** mean values are significant at $p<0.05$ based on one-way ANOVA and Tukey confidence interval analysis.
(5) Shaded mean values indicate significance at $p<0.05$ between depths within treatments.
(6) Single asterisk (*) indicates significant difference of means at $p<0.05$ between treatments at similar depths (t-test).

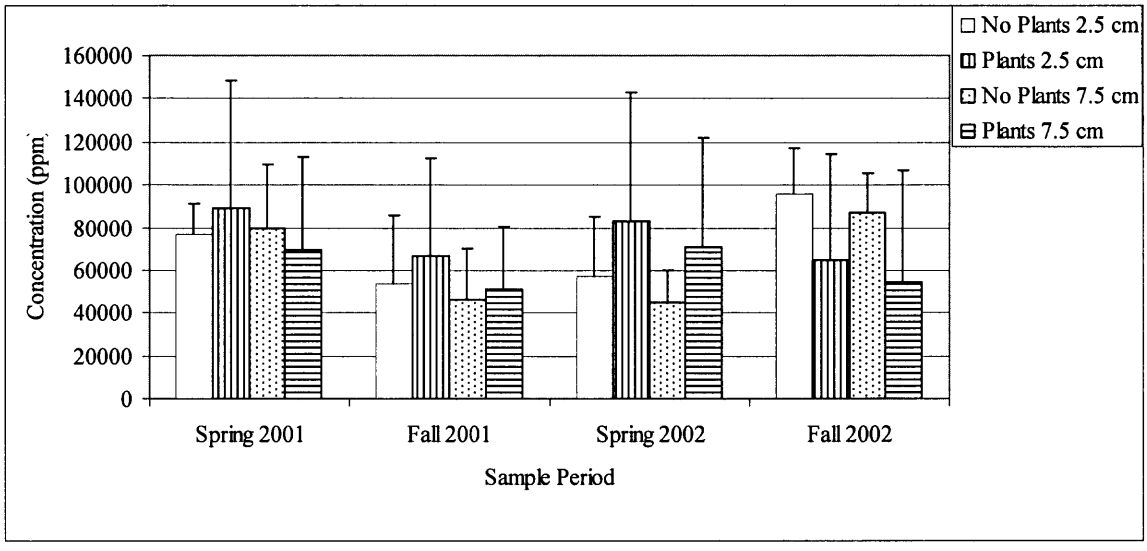


Figure 3.6a Summary statistics of total petroleum hydrocarbons in vegetated and bulk sediments at two depths.

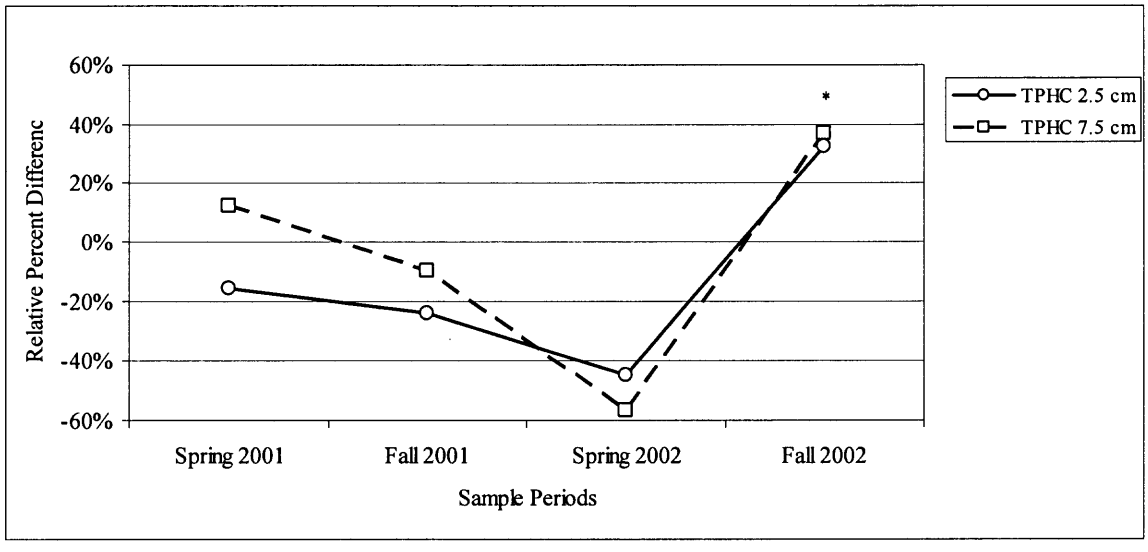


Figure 3.6b Relative percent difference of petroleum hydrocarbons in vegetated and bulk sediment.

Since the mean PHC concentrations for both depths in the last round of bulk sediment samples (Fall 2002) were not different from the beginning concentrations (Spring 2001), and no PHC was added to the test sediments, the data from the two intermediary sample rounds do not represent true reduction of PHC, but are likely an artifact of the sample analysis. This includes (1) potential laboratory bias (low), indicated by the lower PHC concentrations reported for the entire Fall 2001 set of samples at both depths and in both treatments; and (2) suspected positive interference from plant root material in the planted sediments that may have resulted in reporting artificially elevated PHC concentrations. The analytical method used for PHC measurement (USEPA Method 418.1) is subject to positive interference from naturally occurring organic compounds (e.g., plant biomass). For example, elevated PHC concentrations using Method 418.1 have been measured in plant tissue, including grass (14,000 mg/kg PHC), pine needles (16,000 mg/kg PHC), and oak leaves (18,000 mg/kg PHC) (API, 2001b).

To overcome these apparent analytical limitations the PHC data were normalized by calculating relative percent difference (RDP) between the planted and bulk mean sediment PHC concentrations for each sample period using:

$$RPD = [1 - (\text{mean planted PHC Conc.} \div \text{mean bulk sediment PHC Conc.})] \times 100 \quad (3.1)$$

Based on Equation (3.1), when the mean planted sediment PHC equals the mean bulk sediment PHC the RPD is zero; the RPD is positive when the mean planted sediment PHC is greater than the mean bulk sediment PHC; and the RPD is negative

when the mean planted sediment PHC is less than the mean bulk sediment PHC. The first three sample rounds (i.e., Spring 2001, Fall 2001 and Spring 2002) exhibit low or negative RPD (Figure 3.6b); the negative RPD is due to mean planted sediment PHC concentrations being higher than those in the bulk sediment in the Fall 2001 and Spring 2002. This is likely a result of positive interference during sample analysis from the plant roots and associated organic exudates, as described above. However, in this case the negative RPD is considered inconsequential since, based on the statistical analysis, the PHC data between the treatments for the first three of four sample events (Spring 2001, Fall 2001 and Spring 2002) are not significantly different ($P>0.05$). In contrast, the RPD's calculated for the Fall 2002 sample event were 32% and 37% for the 2.5 cm and 7.5 cm depths, respectively (Figure 3.6b), which are significant ($P<0.05$). While this does not confirm reduction of PHC in the planted sediment due to the variability in the data over time and the analytical method limitations, the modest increase in RPD in the Fall 2002 is statistically significant and may indicate early stages of PHC degradation in the planted sediment. As described above, as part of the study objectives, all of the sediment cores were watered with salt water to account for anticipated future conditions in the basin (i.e., restoring the study area to a tidally flowed salt marsh). Although *S. patens* are adapted to such conditions, the microbial consortia inhabiting the rhizosphere may have been adversely affected by the change, which is suspected of causing an extended microbial growth acclimation phase. This is a possible explanation for the modest decrease in PHC concentrations in the root zone for the last sampling event.

Another concern that is unique to PHC contamination in unconsolidated material such as sediment and soil is its potential physical effect on matrix porosity. The porosity

of unconsolidated material (e.g., sediment) may be altered by elevated PHC concentrations (Andrade, et al, 2004). It is assumed that for PHC to exist in soil or sediment at higher concentrations it must displace air and water, and occupy some of the available pore space. Since PHCs are hydrophobic and more viscous then water, they can clog pore space and decrease effective permeability when present at higher concentrations. Based on the grain size analysis of the composite samples described above, the total porosity of the test sediments is estimated to be approximately 25% to 30%. Although a few samples contained PHC over of 200,000 ppm (i.e., > 20%), the mean PHC concentrations were in the range of 45,000 ppm (4.5%) to 95,000 ppm (9.5%). For fresh oil spills, a comparison of the amount of PHC to the available porosity of the matrix may be appropriate by conversion of the PHC data to volume percent of oil using the density of the spilled material, and estimating the matrix porosity with in-situ bulk density and grain size analysis:

$$\text{Percent Oil Saturation} = \frac{[\text{PHC Conc.} \times \text{Oil Density}] \div [\text{Matrix Bulk Density}]}{\text{Matrix Porosity } (\eta)} \qquad (3.2)$$

However, this type of simple calculation is not necessarily appropriate for weathered PHC contamination, and will likely lead to erroneous conclusions (e.g., PHC volumes that far exceed matrix porosity). As an example, using the above calculation, the oil saturation for the greenhouse study sediment would be over 300% (which is clearly not possible) and the effective porosity (and permeability) of the test sediment in this study would be zero. To the contrary, the sediments used in this study were permeable to water based on numerous observations of water migration through the

matrix during watering of the test pots and sample collection, and no oily liquid phase was observed. These characteristics observed in the greenhouse study test sediment are suspected to be due to the weathered PHC being sorbed to the surfaces of fine particles (silt/clay/organic carbon) in the matrix. The weathered PHC may behave more as naturally occurring organic carbon than as non-aqueous phase liquid (Roy and McGill, 1998; Jonker et al., 2003). Thus, weathered PHC may also become part of the sediment matrix over time, in the same way TOC is considered a component of the sediment matrix (not as a substance that occupies pore space). This would explain three important characteristics of PHC-contaminated soils and sediments:

(1) positive interference caused by TOC in PHC analysis by Method 418.1 suggests that TOC and weathered PHC have similar chemical properties;

(2) soils and sediments can contain PHC concentrations above their theoretical volume capacity; and

(3) PHC concentrations that represent PHC volume well above the sample matrix porosity still exhibit water permeability.

The depositional processes and the timing of contamination are two important aspects of evaluating effects of PHC on porosity of unconsolidated material that appear to be largely overlooked in the literature (Testa et al., 1989; Dragun, 1995; API, 1996). However, these appear to be important aspects of evaluating PHC data for the presence of non-aqueous phase PHC and associated effect on pore space and permeability for two fundamental reasons. First, pore space in unconsolidated geologic material is a function of four related characteristics of unconsolidated geologic material; grain size distribution; bulk density; degree of compaction; and depositional processes. Differences in bulk density for subsurface soils (> 2 feet) and compaction in undisturbed areas may not vary

enough to significantly effect porosity. However, the dynamics of sediment deposition in aquatic environments (e.g., wetlands) provides a potential to allow a much larger variation in porosity of sediment matrices. Second, PHC can become tightly sorbed to particles that are redistributed in depositional areas with a porosity based on post-contamination sediment deposition. Thus, while a typical PHC-to-porosity volumetric comparison may be appropriate for a recent subsurface discharge of oil from an underground storage tank (UST) discharge, it may be inappropriate for application to sediment containing weathered PHC from historical discharges that occur simultaneous with sediment deposition. In the former example of the UST, the conceptual model holds that the liquid oil displaces water and occupies pore space in undisturbed strata with a fixed porosity. But in the latter example of sediment containing weathered PHC, assuming that the PHC (1) exists as a separate liquid phase, and/or (2) that porosity was constant during the addition of PHC are likely to result in erroneous conclusions regarding effects of PHC concentration on the presence of a liquid phase, and porosity of the sediment matrix.

In the case of the greenhouse study, the mechanism of the PHC contamination of the test sediment was through mixing, entrainment and deposition together via storm water migration over a long period of about 60 years, rather than oil seeping into pre-existing geologic strata.

3.4.7 Polycyclic Aromatic Hydrocarbons

3.4.7.1 Total PAH. Total polycyclic aromatic hydrocarbons (TPAH) represents the sum of the individual PAH concentrations determined using the analytical method identified above; summary statistics are provided on Table 3.7 and Figure 3.7.

Table 3.7 Total Polycyclic Aromatic Hydrocarbons in Bulk and Vegetated Sediments

PARAMETER	Treatment	Depth	Spring 2001		Fall 2001		Spring 2002		Fall 2002		ANOVA	
			Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	p-value	F-ratio
TPAH	No Plants	2.5 cm	108.5	23.3	117.7	35.3	109.7	26.4	124.3	21.0		
		7.5 cm	99.5	32.3	130.7	33.6	102.7	27.5	124.1	32.3	0.1938	1.46
	Plants	2.5 cm	96.2	102.8	57.0 **	50.7	71.6 *	60.9	91.3	76.2		
		7.5 cm	59.6 *	44.7	56.2 **	47.4	60.5 *	47.2	68.4 **	53.3	0.2975	1.22

Notes: (1) Sample mean and standard deviation (SD) data are in mg/Kg, dry weight basis.
(2) For "No Plants" treatments, n=10; F-critical=2.08
(3) For "Plants" treatments, n=20; F-critical=2.14
(4) **Bold** mean values are significant at $p<0.05$ based on one-way ANOVA and Tukey confidence interval analysis.
(5) **Shaded** mean values indicate significance at $p<0.05$ between depths within treatments.
(6) Single asterisk (*) indicates significant difference of means at $p<0.05$ between treatments at similar depths (t-test).
(7) Double asterisk (**) indicates significant difference of means at $p<0.01$ between treatments at similar depths (t-test).

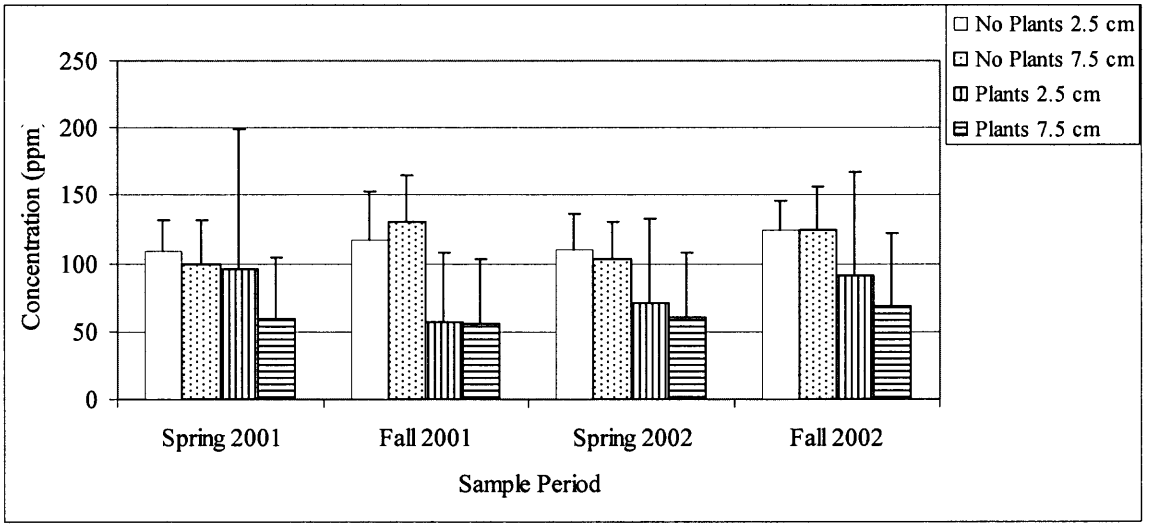


Figure 3.7 Summary statistics of total polycyclic aromatic hydrocarbons in vegetated and bulk sediments at two depths (surrogate corrected).

The individual PAHs included in the analysis are listed in Figure 3.8, along with molecular weight and chemical structures. As described above, the TPAH data were corrected [benzo(a)anthracene, chrysene and pyrene data only] based on percent surrogate recovery (Appendix C). The mean TPAH concentration in bulk sediment ranged from 108.5±23 ppm to 124.3±21 ppm at 2.5 cm, and from 99.5±32 ppm to 130.7±34 ppm at 7.5 cm (ANOVA P=0.1938). In the planted treatments, the TPAH averaged from 57.0±51 ppm to 96.2±103 ppm at 2.5 cm, and from 56.2±47 ppm to 68.4±53 ppm at 7.5 cm (ANOVA P=0.2975). Based on these data and statistical analyses, the average TPAH concentration did not change for the duration of the 18-month study period within either the planted sediment or bulk sediment treatments, and the mean TPAH concentration between the sample depths of 2.5 and 7.5 cm within treatments were not significantly different. While no temporal trends are exhibited by the TPAH data, the average TPAH in the planted sediment at both depths for all four

sampling events is are lower than the average TPAH in the bulk sediments. The differences in mean TPAH concentration between the bulk and planted sediments are statistically significant ($P<0.05$ to $P<0.01$) in two of the four sampling events at 2.5 cm and in all four sampling events at 7.5 cm. One explanation is that TPAH concentrations decreased in the root zone during the field study portion of the project under freshwater conditions. As described above, as part of the study objectives, all of the sediment cores were watered with salt water to account for anticipated future conditions in the basin (i.e., restoring the study area to a tidally flowed salt marsh). Although *S. patens* are adapted to such conditions, the microbial consortia inhabiting the rhizosphere may have been adversely affected, with some groups either killed or shocked into a long acclimation growth phase in response to the salt water.

3.4.7.2 Individual Polycyclic Aromatic Hydrocarbons. The evaluation of TPAH concentrations provides a good overall sense of the data, but the individual PAH compounds vary markedly in size, structure and degradability. The molecular weight (MW) and chemical structure for all 16 PAHs are presented in Figure 3.8. The individual PAHs are evaluated separately in the following sections, grouped in order of increasing structural complexity (i.e., number of aromatic rings) and MW. The summary statistics for PAH analytical data are presented in Tables 3.8 through 3.12.

Figure 3.8 Chemical structure of PAHs included in greenhouse study analysis.

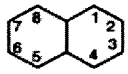
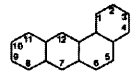
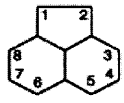
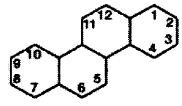
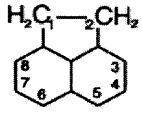
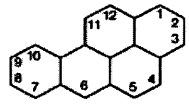
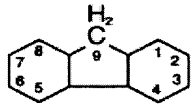
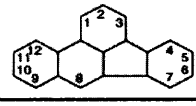
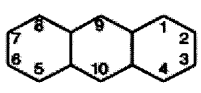
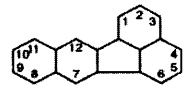
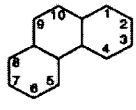
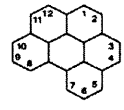
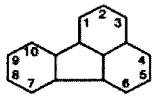
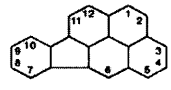
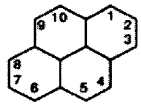
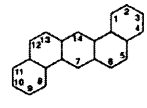
PAH	Structure	MW	Rings	PAH	Structure	MW	Rings
Naphthalene		128	2	Benzo(a)anthracene		228	4
Acenaphthylene		152	2	Chrysene		228	4
Acenaphthene		154	2	Benzo(a)pyrene		252	5
Fluorene		166	3	Benzo(b)fluoranthene		252	5
Anthracene		178	3	Benzo(k)fluoranthene		252	5
Phenanthrene		178	3	Benzo(g,h,i)perylene		276	6
Fluoranthene		190	4	Indeno(1,2,3-cd)pyrene		276	6
Pyrene		216	4	Dibenzo(a,h)anthracene		278	5

Table 3.8 Two-Ring PAHs in Vegetated and Bulk Sediments.

COMPOUND	Treatment	Depth	Spring 2001		Fall 2001		Spring 2002		Fall 2002		ANOVA	
			Mean	+/- SD	Mean	+/- SD	Mean	+/- SD	Mean	+/- SD	p-Value	F-ratio
Naphthalene	No Plants	2.5 cm	0.09	0.19	0.00	0.00	0.00	0.00	0.00	0.00		
MW = 128		7.5 cm	**0.15**	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.0160	2.7
Rings = 2	Plants	2.5 cm	0.05	0.21	0.00	0.00	0.00	0.00	0.00	0.00		
		7.5 cm	0.12	0.34	0.00	0.00	0.00	0.00	0.00	0.00	0.0634	2.0
Acenaphthylene	No Plants	2.5 cm	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
MW = 152		7.5 cm	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	NA	NA
Rings = 2	Plants	2.5 cm	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
		7.5 cm	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	NA	NA
Acenaphthene	No Plants	2.5 cm	**0.48**	0.65	0.00	0.00	0.00	0.00	0.00	0.00		
MW = 154		7.5 cm	**0.55**	0.72	0.00	0.00	0.00	0.00	0.00	0.00	0.0001	4.9
Rings = 2	Plants	2.5 cm	**0.54**	1.17	0.00	0.00	0.00	0.00	0.00	0.00		
		7.5 cm	0.33	0.61	0.14	0.63	0.00	0.00	0.28	0.86	0.0284	2.3

Notes: (1) Sample mean and standard deviation (SD) data are in mg/Kg, dry weight basis.

(2) For "No Plants" treatments, n=10; F-critical=2.08

(3) For "Plants" treatments, n=20; F-critical=2.14

(4) **Bold** mean values are significant at $p<0.05$ within treatments based on one-way ANOVA and Tukey confidence interval analysis.

(5) **Shaded** mean values indicate significance at $p<0.05$ between depths within treatments.

(6) Single asterisk (*) indicates significant difference of means at $p<0.05$ between treatments at similar depths (t-test).

(7) Double asterisk (**) indicates significant difference of means at $p<0.01$ between treatments at similar depths (t-test).

Two-Ring PAHs (MW 128 – 154)

Summary statistics for naphthalene, acenaphthylene and acenaphthene are presented in Table 3.8. Naphthalene was only detected in planted and bulk sediment samples collected from both depths in the initial sampling round (Spring 2001), with very low average concentrations from 0.05 to 0.15 ppm. Acenaphthylene was not detected in any of the 240 sediment samples analyzed for this study. Acenaphthene was detected in planted and bulk sediment samples at very low average concentrations (0.38 to 0.55 ppm) in the sediment samples collected from both depths in the initial sampling round (Spring 2001); it was also detected at average concentrations of 0.14 and 0.28 ppm in the Fall 2001 and Fall 2002 sampling rounds, respectively.

The naphthalene and acenaphthene concentrations are very low, at or near the corresponding analytical method detection limits, and they exhibit very low detection frequencies.

Table 3.9 Three-Ring PAHs in vegetated and bulk sediments.

COMPOUND	Treatment	Depth	Spring 2001		Fall 2001		Spring 2002		Fall 2002		ANOVA	
			Mean	+/- SD	Mean	+/- SD	Mean	+/- SD	Mean	+/- SD	p-Value	F-ratio
Fluorene	No Plants	2.5 cm	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
MW = 166		7.5 cm	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	NA	NA
Rings = 3	Plants	2.5 cm	**0.58**	1.68	0.00	0.00	0.00	0.00	0.00	0.00		
		7.5 cm	0.06	0.29	0.00	0.00	0.00	0.00	0.10	0.42	0.0472	2.1
Anthracene	No Plants	2.5 cm	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
MW = 178		7.5 cm	0.00	0.00	0.00	0.00	0.00	0.00	0.42	1.32	0.4385	1.0
Rings = 3	Plants	2.5 cm	**0.41**	1.26	0.00	0.00	0.00	0.00	0.00	0.00		
		7.5 cm	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0470	2.1
Phenanthrene	No Plants	2.5 cm	3.40	1.61	**1.97**	1.14	3.32	1.99	2.87	1.82		
MW = 178		7.5 cm	4.75	1.91	3.71	0.75	4.35	0.88	3.99	1.87	0.0065	3.1
Rings = 3	Plants	2.5 cm	**1.82**	3.17	0.20 *	0.91	0.00 *	0.00	0.13 *	0.58		
		7.5 cm	0.86 *	1.58	0.04 *	0.18	0.90 *	1.96	0.40 *	0.98	0.0025	3.3

Notes: (1) Sample mean and standard deviation (SD) data are in mg/Kg, dry weight basis.

(2) For "No Plants" treatments, n=10; F-critical=2.08

(3) For "Plants" treatments, n=20; F-critical=2.14

(4) **Bold** mean values are significant at $p<0.05$ within treatments based on one-way ANOVA and Tukey confidence interval analysis.

(5) **Shaded** mean values indicate significance at $p<0.05$ between depths within treatments.

(6) Single asterisk (*) indicates significant difference of means at $p<0.05$ between treatments at similar depths (t-test).

(7) Double asterisk (**) indicates significant difference of means at $p<0.01$ between treatments at similar depths (t-test).

Three-Ring PAHs (MW 166 – 178)

Summary statistics for fluorene, anthracene and phenanthrene are presented in Table 3.9. Like the 2-ring PAHs, fluorene and anthracene were largely undetected in both planted and bulk sediments, with average concentrations ranging from 0.06 to 0.58 ppm of fluorene and 0.41 to 0.42 ppm of anthracene. These concentrations are very low, at or near the corresponding analytical method detection limits, they exhibit very low detection frequencies, and no pattern or trend.

The mean phenanthrene concentration in bulk sediment (Figure 3.9a) ranged from 1.97±1.1 ppm to 3.4±1.6 ppm at 2.5 cm, and from 3.71±0.75 ppm to 4.75±1.9 ppm at 7.5 cm (ANOVA P=0.0065). In the planted treatments, the phenanthrene averaged from 0.13±0.58 ppm to 1.82±3.2 ppm at 2.5 cm, and from 0.04±0.18 ppm to 0.9±1.9 ppm at 7.5 cm (ANOVA P=0.0025). The statistical significance noted for mean phenanthrene concentrations in the bulk sediments is due to the slightly low phenanthrene concentration reported for the Fall 2001 sampling round at 2.5 cm. However, the mean phenanthrene concentrations at 2.5 cm in the last two rounds of bulk sediment samples (Spring 2002 and Fall 2002) were not different from the beginning concentrations (Spring 2001). The slightly low phenanthrene concentrations in the one intermediary sample round do not represent an actual reduction of phenanthrene, but are likely an artifact of the sample analysis. In contrast, the mean phenanthrene concentration at 2.5 cm in planted sediment samples at the start of the greenhouse study (Spring 2001) was higher than the last three sampling events (ANOVA P=0.0025). However, no significant differences in phenanthrene concentrations were noted for the four sampling rounds at 7.5 cm in planted sediment samples.

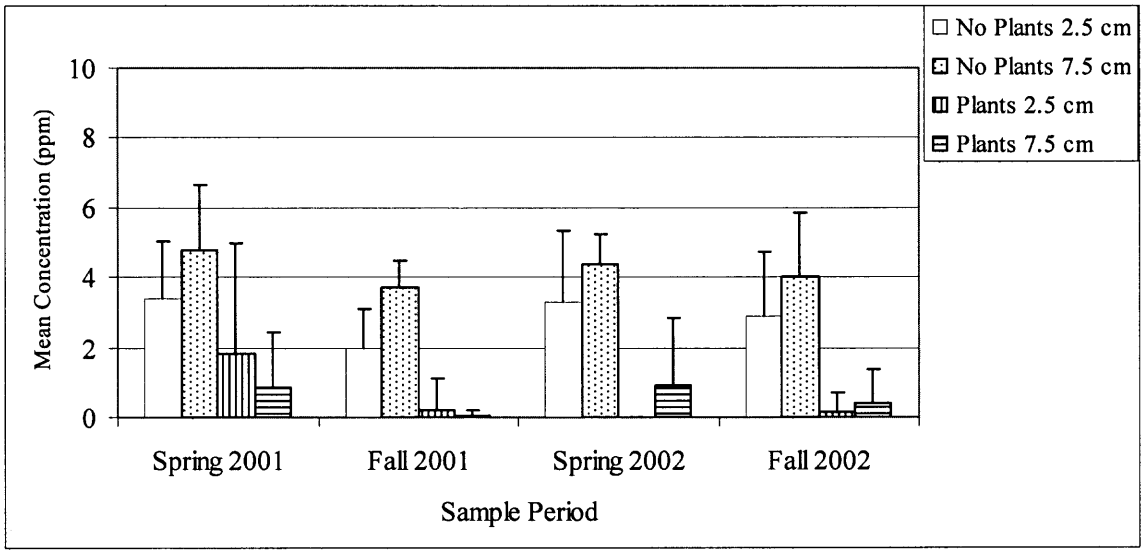


Figure 3.9a Summary statistics of phenanthrene in vegetated and bulk sediments at two depths.

Comparing treatments, the average phenanthrene concentrations in the planted sediment at 2.5 cm for the last three sampling events (Fall 2001, Spring 2002 and Fall 2002) are significantly lower than those in the bulk sediments ($P<0.01$). And the average phenanthrene concentrations in the planted sediment at 7.5 cm for all four sampling events are significantly lower than those in the bulk sediments ($P<0.01$). Regression analysis was performed for the mean phenanthrene concentrations in planted sediments at 2.5 cm plotted vs. time ($days^{-1}$) for the duration of the greenhouse study period (517 days). Since phenanthrene data are available from the earlier field study (Chapter 2) of the same sediments used in the greenhouse experiment, regression analysis was also performed for phenanthrene for the combined field and greenhouse study period (846 days). The half-life for phenanthrene was calculated assuming first-order reaction rate kinetics (Venosa et al., 1996; Hinga et al., 2003) using the following equations:

$$C_t = C_o e^{-kt} \quad (3.3)$$

$$t_{1/2} = \frac{-0.693}{k} \quad (3.4)$$

Where

C_t = Contaminant concentration at time t
C_o = Contaminant concentration at time t = 0
k = first-order rate constant
$t_{1/2}$ = time (days) required for C_o to decrease by 50%

The term "k" is the rate constant derived from the natural log function of the curve obtained from the data time-series plots (Figure 3.9b and 3.9c):

$$y = be^{-kx} \quad (3.5)$$

Where

y = phenanthrene concentration (ppm)
x = time (days)
b = y-intercept
k = first-order rate constant

From Figure 3.9b, the regression coefficient (R^2) = 0.7569 and k = -0.0049. Using Equation (3.4), a half-life of 141 days was calculated for phenanthrene in the planted sediments.

The average phenanthrene concentration from the three Field Study bulk sediment samples (Appendix B) is 2.86 ppm (n=3). This is consistent with the average of the phenanthrene data from bulk sediments at 2.5 cm used in the greenhouse study (2.88 ppm; n=40). Although samples were not collected at the beginning of the Field Study, the data from both studies provide a sound basis for assuming that the average

phenanthrene concentrations in the bulk sediment at the start of the field study remained consistent through both study periods. Therefore, the average phenanthrene concentration in bulk sediments form the greenhouse study (2.88 ppm) was used for the initial concentration in the second regression analysis (Figure 3.9c), which yielded R^2 =0.8627 and k = -0.0042, resulting in a half-life of 165 days for phenanthrene in the planted sediments.

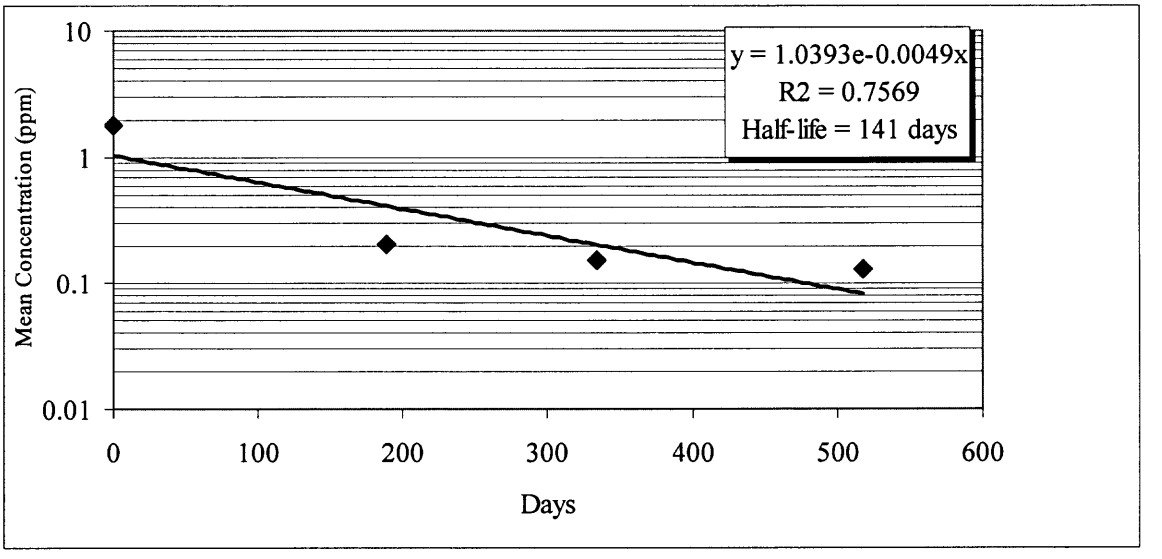


Figure 3.9b Phenanthrene decay in vegetated sediment at 2.5 cm during greenhouse study from April 5, 2001(t=0) through September 4, 2002 (t=517).

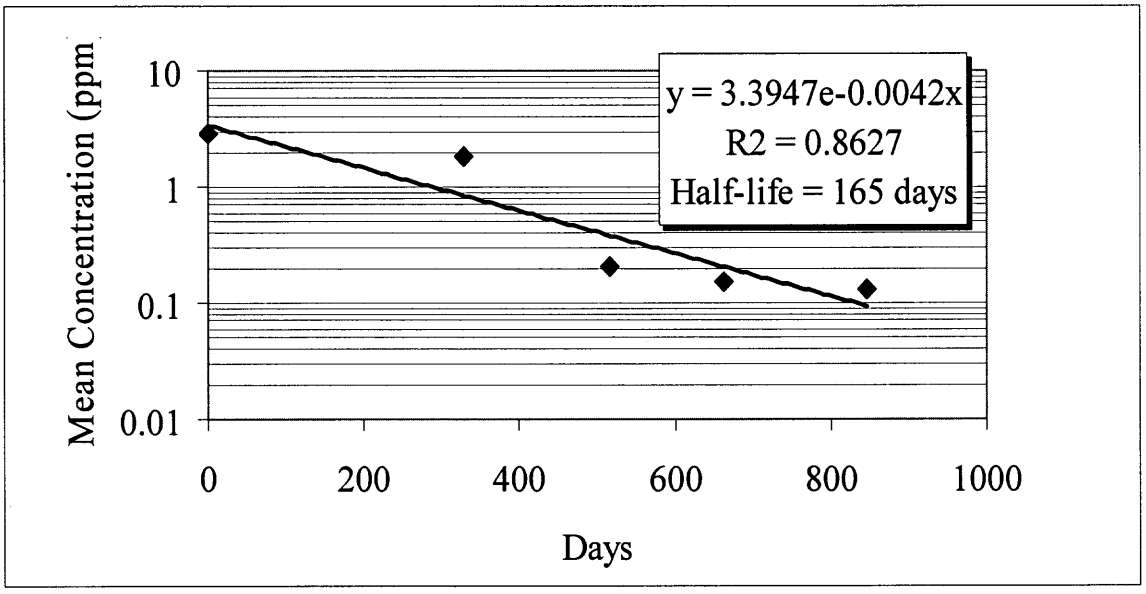


Figure 3.9c Phenanthrene decay in vegetated sediment at 2.5 cm from start of field study on May 11, 2001(t=0) through end of greenhouse study on September 4, 2002 (t=846).

Four-Ring PAHs (MW 190 – 228)

Summary statistics for fluoranthene, pyrene, benzo(a)anthracene and chrysene data are included in Table 3.10 and presented on Figures 3.10a through 3.10d. The pyrene, benzo(a)anthracene and chrysene data were surrogate-corrected as described in Appendix C. Average fluoranthene concentrations in bulk sediment (Figure 3.10a) ranged from 4.1±1.7 ppm to 7.6±2.8 ppm at the 2.5 cm depth, and from 4.6±1.4 ppm to 7.5±2.3 ppm at the 7.5 cm depth (ANOVA P=0.0031). In the planted treatments, the average fluoranthene concentrations in planted sediment ranged from 1.5±1.7 ppm to 2.6±3.9 ppm at the 2.5 cm depth, and from 1.3±1.4 ppm to 3.5±3.7 ppm at the 7.5 cm depth (ANOVA P=0.2498).

The mean pyrene concentration in bulk sediment (Figure 3.10b) ranged from 34.4±8.1 ppm to 47.1±9.5 ppm at 2.5 cm, and from 30.5±12.4 ppm to 48.8±11.8 ppm at 7.5 cm (ANOVA P<0.0001). In the planted treatments, the average pyrene ranged from

22.7±22 ppm to 35.5±41 ppm at 2.5 cm, and from 19.7±15.8 ppm to 23.1±21.4 ppm at 7.5 cm (ANOVA P=0.5046).

Average concentrations of benzo(a)anthracene in bulk sediment (Figure 3.10c) ranged from 8.7±2.1 ppm to 10.9±2.8 ppm at the 2.5 cm depth, and from 8.8±3.4 ppm to 10.8±6.2 ppm at the 7.5 cm depth (ANOVA P=0.3070). In the planted treatments, the average benzo(a)anthracene concentrations in planted sediment ranged from 4.2±3.8 ppm to 10.7±13.8 ppm at the 2.5 cm depth, and from 4.2±3.9 ppm to 5.9±5.5 ppm at the 7.5 cm depth (ANOVA P=0.0935).

Mean chrysene concentrations in bulk sediment (Figure 3.10d) ranged from 17.6±4.5 ppm to 23.9±5.5 ppm at 2.5 cm, and from 15.6±5.5 ppm to 29.8±9.3 ppm at 7.5 cm (ANOVA P<0.0001). In the planted treatments, the average chrysene ranged from 11.6±8.8 ppm to 16.9±12 ppm at 2.5 cm, and from 9.6±7.4 ppm to 12.3±9.6 ppm at 7.5 cm (ANOVA P=0.6313).

The four 4-ring PAHs combined account for roughly 80% of the TPAH detected. Of these, pyrene and chrysene are the two most abundant PAHs present in the sediment samples. The statistical significance in the bulk sediment treatments are due to a few low average concentrations that occur either at the beginning of the study (e.g., fluoranthene, pyrene and chrysene, Spring 2001) or in the third sampling round (e.g., pyrene and chrysene, Spring 2002). These are all slightly lower than the overall average for their respective treatments and do not represent degradation, due to the presence of higher concentrations in later replicate samples at both depths. Based on these data and statistical analyses, the average concentration of the 4-ring PAHs did not change for the duration of the 18-month study period within either the planted sediment or bulk

sediment treatments, and the mean concentrations of these compounds were not significantly different between the sample depths of 2.5 and 7.5 cm within treatments. However, the average concentrations of the 4-ring PAHs in the planted sediment at both depths for all four sampling events are lower than their average in the bulk sediments, with one exception (pyrene at 2.5 cm, Spring 2001). The differences in the mean concentrations of all four 4-ring PAHs between the bulk and planted sediments at both sample depths are statistically significant in the last three sampling events ($P<0.05$).

Table 3.10 Four-Ring PAHs in Vegetated and Bulk Sediments

COMPOUND	Treatment	Depth	Spring 2001		Fall 2001		Spring 2002		Fall 2002		ANOVA	
			Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	p-Value	F-ratio
Fluoranthene	No Plants	2.5 cm	**4.09**	1.68	6.34	2.85	6.48	1.85	7.55	2.78		
MW = 190		7.5 cm	4.63	1.44	7.48	2.30	6.30	2.05	7.36	2.33	0.0031	3.4
Rings = 4	Plants	2.5 cm	2.03	3.10	1.51 **	1.86	2.27 **	3.01	2.62 **	3.95		
		7.5 cm	1.31 **	1.45	1.78 **	1.85	2.88 **	2.93	3.48 **	3.71	0.2498	1.3
Pyrene +	No Plants	2.5 cm	34.68	6.37	45.64	15.04	34.44	8.14	47.11	9.49		
MW = 216		7.5 cm	**30.52**	12.45	48.76	11.78	**30.76**	5.61	43.37	12.85	<0.0001	5.0
Rings = 4	Plants	2.5 cm	35.54	40.84	22.70 **	21.99	22.75 *	18.15	26.36 **	22.66		
		7.5 cm	22.57	20.39	23.13 **	21.42	19.66 **	15.79	20.67 **	17.09	0.5046	0.9
Benzo(a)anthracene +	No Plants	2.5 cm	10.92	2.75	9.91	2.17	8.74	2.07	10.86	2.38		
MW = 228		7.5 cm	9.49	2.39	10.29	2.22	8.79	3.41	10.76	6.18	0.3070	1.2
Rings = 4	Plants	2.5 cm	10.72	13.81	5.34 **	5.92	4.24 **	3.83	6.86 **	6.01		
		7.5 cm	5.98 *	5.52	5.54 **	5.77	4.23 **	3.86	5.57 **	5.36	0.0935	1.8
Chrysene +	No Plants	2.5 cm	23.23	6.10	27.02	6.06	**17.62**	4.52	23.88	5.47		
MW = 228		7.5 cm	**15.64**	5.46	29.78	9.31	**16.03**	2.92	23.28	7.30	<0.0001	6.6
Rings = 4	Plants	2.5 cm	15.91	16.95	12.03 **	10.51	11.59 *	8.76	15.78 *	13.77		
		7.5 cm	11.10	8.84	13.23 **	12.33	9.59 **	7.44	12.12 **	9.88	0.6313	0.8

Notes: (1) Sample mean and standard deviation (SD) data are in mg/Kg, dry weight basis.
(2) For "No Plants" treatments, n=10; F-critical=2.08
(3) For "Plants" treatments, n=20; F-critical=2.14
(4) **Bold** mean values are significant at $p<0.05$ within treatments based on one-way ANOVA and Tukey confidence interval analysis.
(5) **Shaded** mean values indicate significance at $p<0.05$ between depths within treatments.
(6) Single asterisk (*) indicates significant difference of means at $p<0.05$ between treatments at similar depths (t-test).
(7) Double asterisk (**) indicates significant difference of means at $p<0.01$ between treatments at similar depths (t-test).
(8) Plus (+) indicates data for PAH compound is corrected for internal surrogate percent recovery (terphenyl-d14).

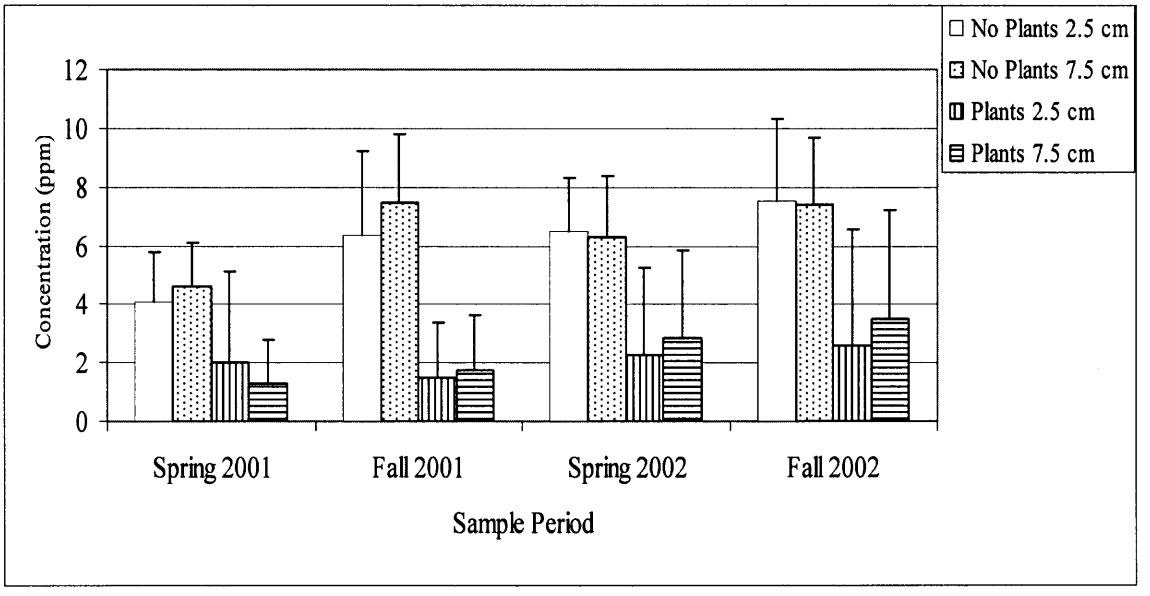


Figure 3.10a Summary statistics of fluoranthene in vegetated and bulk sediments at two depths.

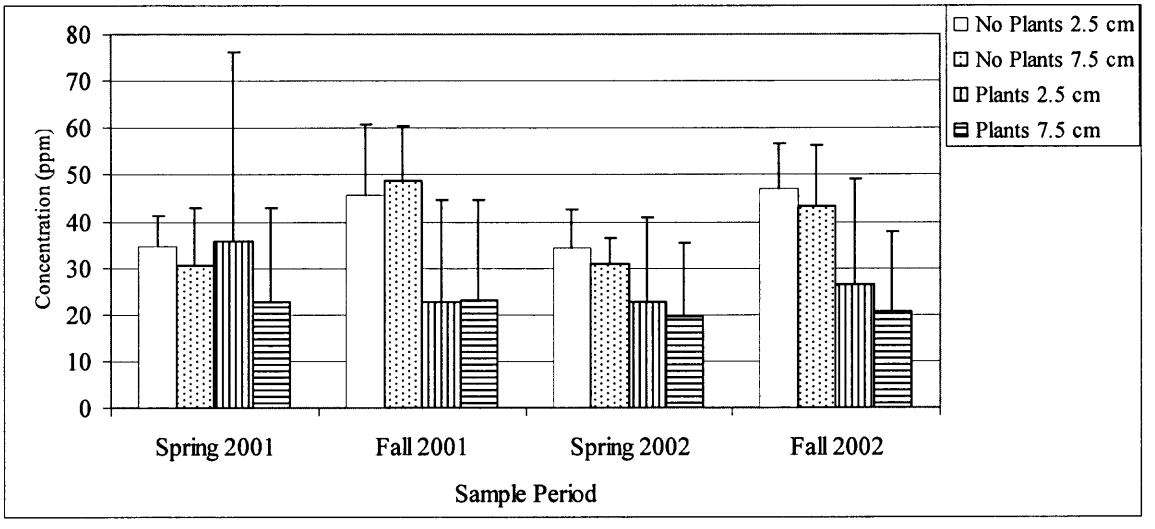


Figure 3.10b Summary statistics of pyrene in vegetated and bulk sediments at two depths (surrogate corrected).

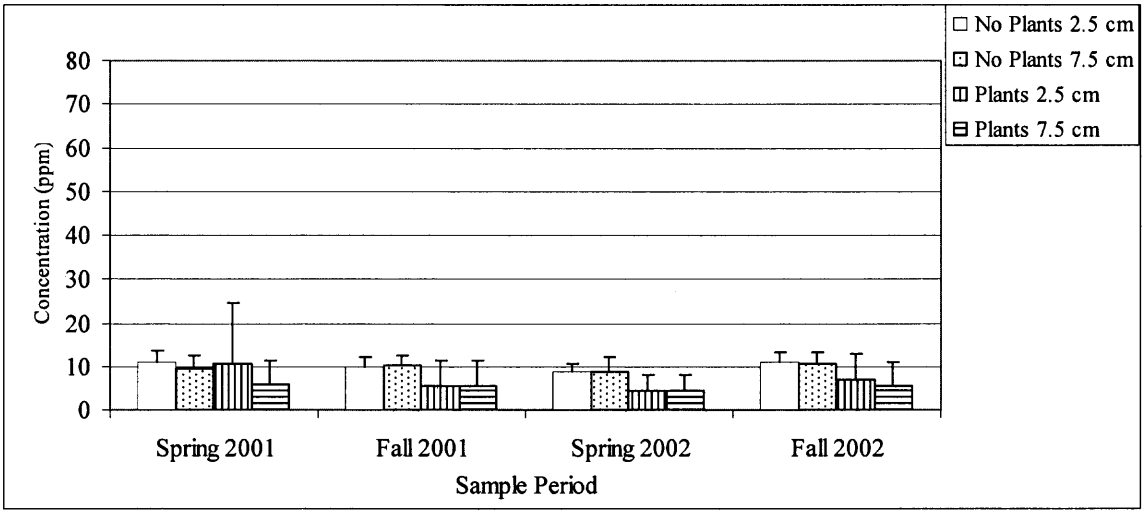


Figure 3.10c Summary statistics of benzo(a)anthracene in vegetated and bulk sediments at two depths (surrogate corrected).

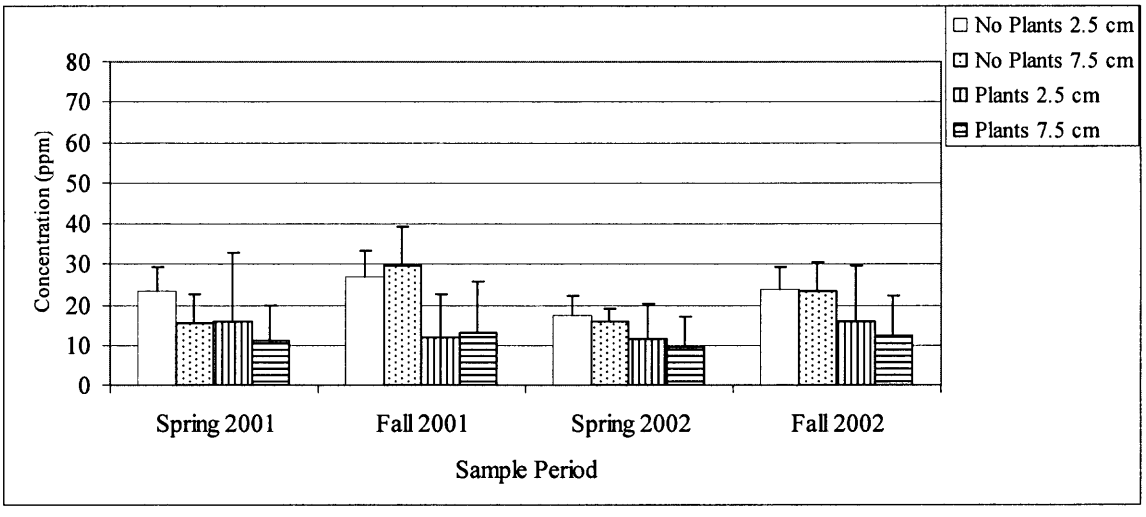


Figure 3.10d Summary statistics of chrysene in vegetated and bulk sediments at two depths (surrogate corrected).

Five-Ring PAHs (MW 252 – 278)

Summary statistics for benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene and dibenzo(a,h)anthracene data are included in Table 3.11 and Figure 3.11a through 3.11d. The mean benzo(a)pyrene concentrations in bulk sediment

(Figure 3.11a) ranged from 6.7±1.8 ppm to 8.8±2.4 ppm at 2.5 cm, and from 6.8±2.4 ppm to 9.6±2.9 ppm at 7.5 cm (ANOVA P=0.1494). In the planted treatments, the average benzo(a)pyrene ranged from 3.3±3 ppm to 8.7±8.3 ppm at 2.5 cm, and from 2.6±1.9 ppm to 6.4±5.8 ppm at 7.5 cm (ANOVA P=0.0024).

The average benzo(b)fluoranthene concentrations in bulk sediment (Figure 3.11b) ranged from 7.74±2.5 ppm to 11.8±4.1 ppm at the 2.5 cm depth, and from 7.8±3.2 ppm to 12.5±3.5 ppm at the 7.5 cm depth (ANOVA P=0.0527). In the planted treatments, average benzo(b)fluoranthene concentrations in planted sediment ranged from 4.3±3.7 ppm to 11.7±8.6 ppm at the 2.5 cm depth, and from 3.7±2.8 ppm to 7.9±6.2 ppm at the 7.5 cm depth (ANOVA P<0.0000).

Mean benzo(k)fluoranthene concentration in bulk sediment (Figure 3.11c) ranged from 0.57±0.5 ppm to 1.6±0.6 ppm at 2.5 cm, and from 0.54±0.6 ppm to 2.2±1.5 ppm at 7.5 cm (ANOVA P=0.0003). In the planted treatments, the average benzo(k)fluoranthene ranged from 0.34±0.43 ppm to 1.6±1.9 ppm at 2.5 cm, and from 0.32±0.4 ppm to 1.1±1.2 ppm at 7.5 cm (ANOVA P=0.0001).

Average concentrations of dibenzo(a,h)anthracene in bulk sediment (Figure 3.11d) ranged from 1.3±0.8 ppm to 3.8±1.2 ppm at the 2.5 cm depth, and from 1.3±0.6 ppm to 3.4±0.9 ppm at the 7.5 cm depth (ANOVA P<0.0001). In the planted treatments, the average dibenzo(a,h)anthracene concentrations in planted sediment ranged from 1.3±1.2 ppm to 3.3±2.7 ppm at the 2.5 cm depth, and from 0.97±0.9 ppm to 2.13±1.8 ppm at the 7.5 cm depth (ANOVA P=0.0043).

Statistical differences were detected by the ANOVA for most of the data; the exception being benzo(a)pyrene in bulk sediments (ANOVA P=0.1494). However, the mean concentrations are similar over time and no trends are observed in the data. The mean concentrations are the same or higher in the last sampling round (Fall 2002) for all four 5-ring PAHs. The differences appear related to analytical instrument response as indicated by the variable surrogate recoveries. These data were not corrected for surrogate recovery because there is a greater potential that the correction itself may add error due to unknown differences that may exist between the surrogate used (terphenyl-d14) and the compounds associated with various phases of the chemical analysis, e.g., extraction efficiency and detector response (see Appendix C). Other than a few exceptions, the mean concentrations of benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene and dibenzo(a,h)anthracene in planted sediments are lower than those found in the bulk sediments at both sample depths. These differences are generally not significant at the 2.5 cm sample depth, but are significant for much of the data at the 7.5 cm depth ($P<0.05$).

Table 3.11 Five-Ring PAHs in Vegetated and Bulk Sediments

COMPOUND	Treatment	Depth	Spring 2001		Fall 2001		Spring 2002		Fall 2002		ANOVA	
			Mean	+/- SD	Mean	+/- SD	Mean	+/- SD	Mean	+/- SD	p-Value	F-ratio
Benzo(a)pyrene	No Plants	2.5 cm	6.73	1.78	7.65	2.90	8.82	2.37	8.66	1.93		
MW = 252		7.5 cm	6.75	2.44	9.21	3.24	8.53	3.46	9.55	2.94	0.1494	1.6
Rings = 5	Plants	2.5 cm	5.26	5.41	3.27 *	3.04	6.09	5.25	**8.73**	8.25		
		7.5 cm	3.34 *	2.19	2.65 *	1.90	5.05 *	4.14	6.44	5.81	0.0024	3.4
Benzo(b)fluoranthene	No Plants	2.5 cm	7.74	2.48	10.38	4.29	11.82	4.05	11.39	3.06		
MW = 252		7.5 cm	7.84	3.24	12.49	3.48	11.22	6.10	11.79	3.98	0.0527	2.1
Rings = 5	Plants	2.5 cm	5.61	5.02	4.28 *	3.70	8.99	6.37	11.69	8.63		
		7.5 cm	3.85 *	2.64	3.69 *	2.85	7.30	5.78	7.88	6.23	0.0000	5.3
Benzo(k)fluoranthene	No Plants	2.5 cm	**0.57**	0.50	1.60	0.62	1.64	0.59	1.34	0.70		
MW = 252		7.5 cm	**0.54**	0.64	1.95	1.02	1.64	1.15	2.19	1.46	0.0003	4.5
Rings = 5	Plants	2.5 cm	0.34	0.43	0.38 *	0.35	**1.62**	1.92	**1.59**	2.05		
		7.5 cm	0.32	0.45	0.44 *	0.38	0.90 *	0.77	1.14 *	1.17	0.0001	4.7
Dibenzo(a,h)anthracene	No Plants	2.5 cm	3.18	1.03	**1.34**	0.84	3.75	1.25	2.27	0.72		
MW = 278		7.5 cm	3.18	1.21	**1.33**	0.63	3.36	0.95	2.43	0.87	0.0000	9.2
Rings = 5	Plants	2.5 cm	2.59	2.49	**1.31**	1.19	2.93	3.18	3.26	2.69		
		7.5 cm	1.63 *	1.03	**0.97**	0.90	2.13 *	1.56	2.13	1.78	0.0043	3.1

Notes: (1) Sample mean and standard deviation (SD) data are in mg/Kg, dry weight basis.
(2) For "No Plants" treatments, n=10; F-critical=2.08
(3) For "Plants" treatments, n=20; F-critical=2.14
(4) **Bold** mean values are significant at $p<0.05$ within treatments based on one-way ANOVA and Tukey confidence interval analysis.
(5) **Shaded** mean values indicate significance at $p<0.05$ between depths within treatments.
(6) Single asterisk (*) indicates significant difference of means at $p<0.05$ between treatments at similar depths (t-test).
(7) Double asterisk (**) indicates significant difference of means at $p<0.01$ between treatments at similar depths (t-test).

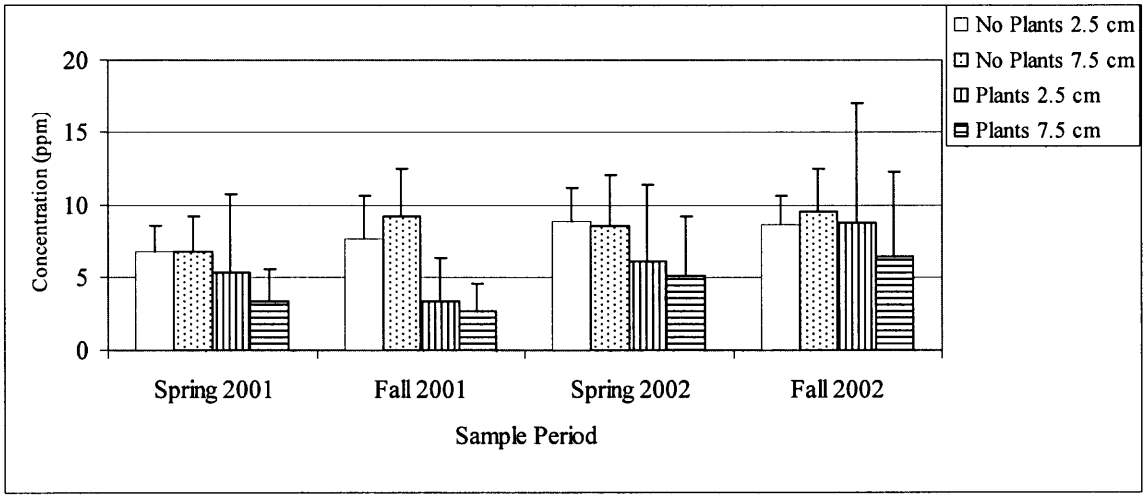


Figure 3.11a Summary statistics of benzo(a)pyrene in vegetated and bulk sediments at two depths.

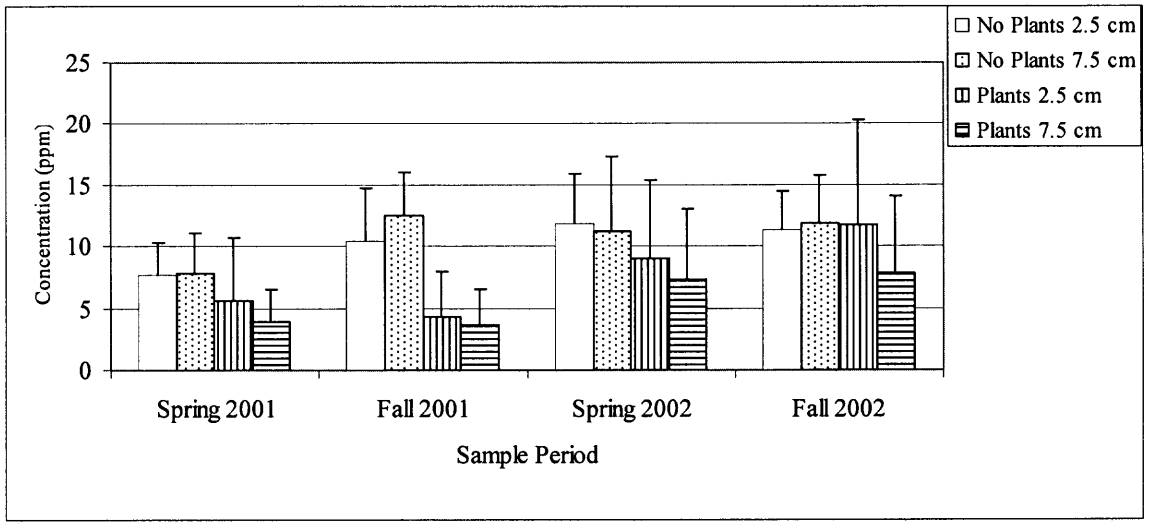


Figure 3.11b Summary statistics of benzo(b)fluoranthene in vegetated and bulk sediments at two depths.

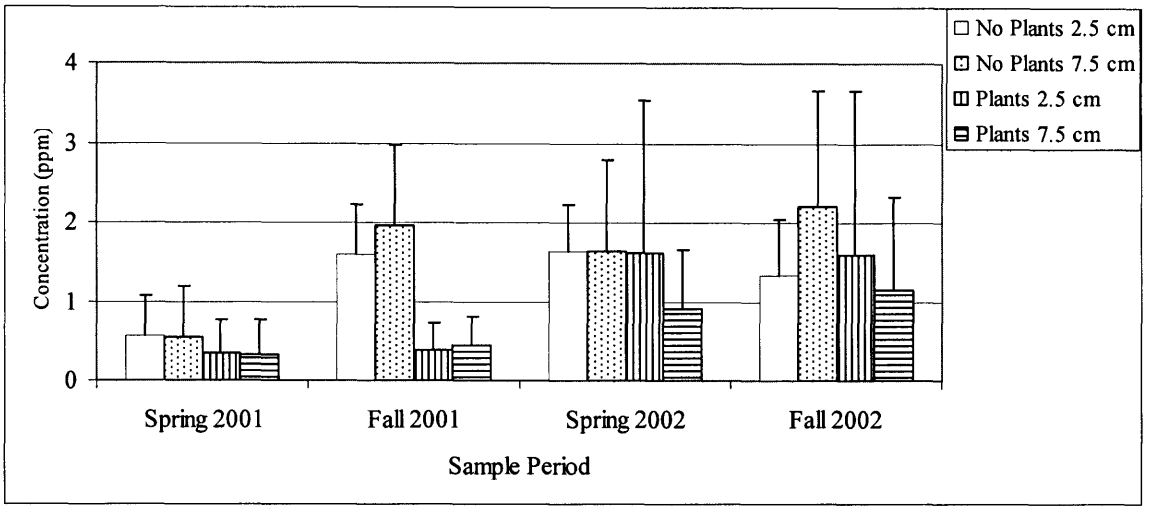


Figure 3.11c Summary statistics of benzo(k)fluoranthene in vegetated and bulk sediments at two depths.

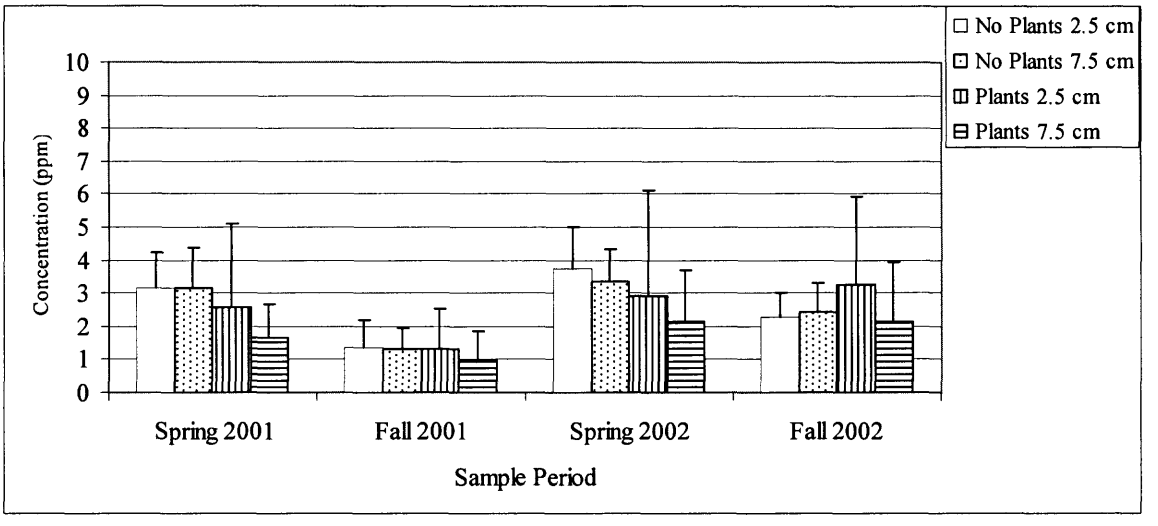


Figure 3.11d Summary statistics of dibenzo(a,h)anthracene in vegetated and bulk sediments at two depths.

Six-Ring PAHs (MW 276)

Summary statistics for benzo(g,h,i)perylene and indeno(1,2,3.cd)pyrene data are included in Table 3.12 and presented on Figure 3.12a and 3.12b. Average benzo(g,h,i)perylene concentrations in bulk sediment (Figure 3.12a) ranged from 4.3±2.9 ppm to 10.4±3.4 ppm at the 2.5 cm depth, and from 4.2±2.2 ppm to 11.9±9.7 ppm at the

7.5 cm depth (ANOVA P=0.0004). In the planted treatments, the average benzo(g,h,i)perylene concentrations in planted sediment ranged from 4.6±4.3 ppm to 11.4±11.6 ppm at the 2.5 cm depth, and from 3.5±3.4 ppm to 6.2±3.7 ppm at the 7.5 cm depth (ANOVA P=0.0117).

The mean indeno(1,2,3-cd)pyrene concentrations in bulk sediment (Figure 3.12b) ranged from 1.5±0.9 ppm to 3.9±2.0 ppm at 2.5 cm, and from 1.4±0.7 ppm to 3.6±1.9 ppm at 7.5 cm (ANOVA P=0.0001). In the planted treatments, the average indeno(1,2,3.cd)pyrene ranged from 1.4±1.2 ppm to 3.3±3.5 ppm at 2.5 cm, and from 1.1±0.9 ppm to 2.1±1.6 ppm at 7.5 cm (ANOVA P=0.0028).

Statistical differences detected by the ANOVA are attributable solely to low mean concentrations for both 6-ring PAHs in Fall 2001. The mean concentrations are similar over time and no trends are observed in the data. As with the 5-ring PAHs described above, the differences appear related to analytical instrument response as indicated by the variable surrogate recoveries. These data were also not corrected for surrogate recovery for the reasons provided above for 5-ring PAHs. The mean concentrations of for both 6-ring PAHs in planted sediments are similar to those found in the bulk sediments at the 2.5 cm sample depth, but are lower than in the bulk sediments at the 7.5 cm depth. These differences are generally not significant (P>0.05).

Table 3.12 Six-Ring PAHs in Vegetated and Bulk Sediments

COMPOUND	Treatment	Depth	Spring 2001		Fall 2001		Spring 2002		Fall 2002		ANOVA	
			Mean	+/- SD	Mean	+/- SD	Mean	+/- SD	Mean	+/- SD	p-Value	F-ratio
Benzo(g,h,i)perylene	No Plants	2.5 cm	10.42	3.35	**4.34**	2.98	9.84	4.29	5.98	1.84		
MW = 276		7.5 cm	11.87	9.69	**4.24**	2.22	8.51	2.12	6.39	2.23	0.0004	4.4
Rings = 6	Plants	2.5 cm	11.44	11.62	4.59	4.31	8.56	10.94	11.03	12.78		
		7.5 cm	6.16	3.70	**3.50**	3.41	5.86	3.94	6.06	4.98	0.0117	2.7
Indeno(1,2,3-cd)pyrene	No Plants	2.5 cm	3.86	2.01	**1.49**	0.89	3.24	1.19	2.37	0.63		
MW = 276		7.5 cm	3.57	1.86	**1.45**	0.70	3.18	1.39	2.53	0.81	0.0001	5.0
Rings = 6	Plants	2.5 cm	3.33	3.50	**1.39**	1.22	2.60	2.41	3.20	2.17		
		7.5 cm	1.97 *	1.18	**1.07**	0.92	2.01 *	1.39	2.10	1.62	0.0028	3.3

Notes: (1) Sample mean and standard deviation (SD) data are in mg/Kg, dry weight basis.
(2) For "No Plants" treatments, n=10; F-critical=2.08
(3) For "Plants" treatments, n=20; F-critical=2.14
(4) **Bold** mean values are significant at $p<0.05$ within treatments based on one-way ANOVA and Tukey confidence interval analysis.
(5) Shaded mean values indicate significance at $p<0.05$ between depths within treatments.
(6) Single asterisk (*) indicates significant difference of means at $p<0.05$ between treatments at similar depths (t-test).
(7) Double asterisk (**) indicates significant difference of means at $p<0.01$ between treatments at similar depths (t-test).

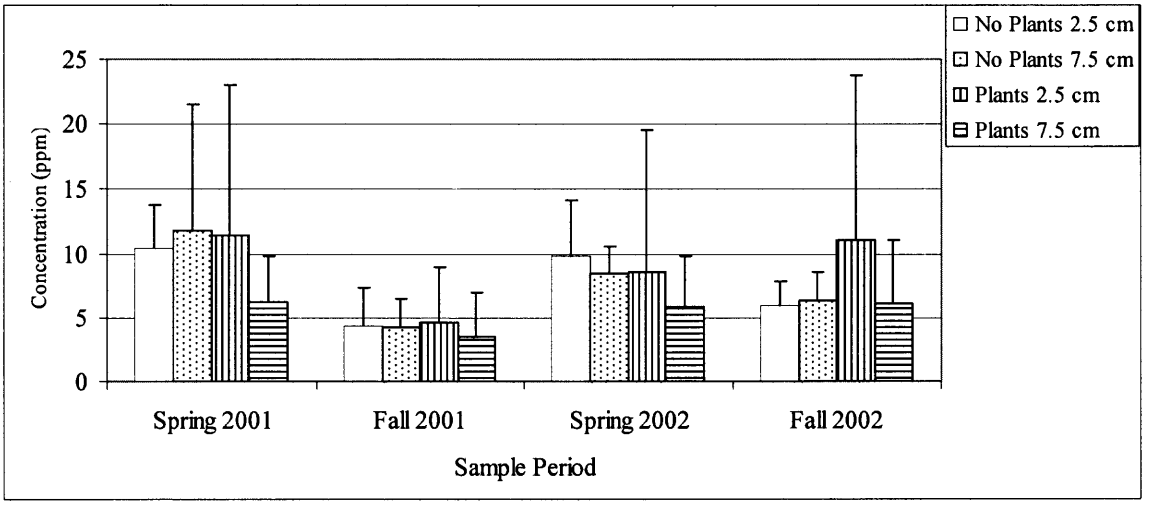


Figure 3.12a Summary statistics of benzo(g,h,I)perylene in vegetated and bulk sediments at two depths.

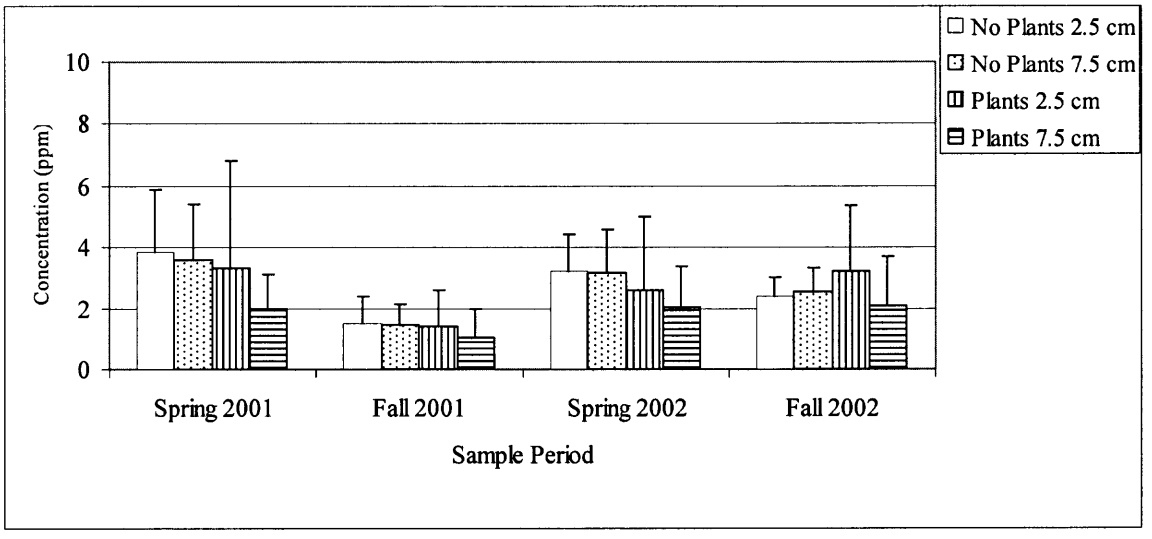


Figure 3.12b Summary statistics of indeno(1,2,3-cd)pyrene in vegetated and bulk sediments at two depths.

Evaluation of Normalized PAH Data

The only PAH that clearly exhibited a change in concentrations over time was phenanthrene. However, the significant differences among the higher MW PAHs (MW 190 to 278) in the planted sediment and bulk sediment data indicate that these PAHs

decreased to lower concentrations in the root zone. To compare the differences in planted vs. bulk sediment PAH concentrations for PAH compounds with MW 178 to 278, the mean concentrations were normalized using the RPD calculation described above for PHC data evaluation. This includes calculating a ratio of the mean bulk sediment data divided by the mean planted sediment data for individual PAHs per sample event. In this way the differences in the planted vs. the bulk sediment concentrations can be compared without the effect of any time-related laboratory bias. The assumption made here is that the analytical bias (including variable surrogate recovery) is reduced when comparison is made using the data generated in the same time period, i.e., sampling event. This assumption is supported by the consistent pattern of surrogate recovery data that is apparent over time described in Appendix C.

The calculation of the RPD for the individual PAHs was made separately for each of the two sample depths (2.5 cm and 7.5 cm) for all four sampling events (Spring 2001, Fall 2001, Spring 2002, Fall 2002). RPDs calculated from PAH concentrations in planted sediments that were significantly lower ($P<0.05$) than those in bulk sediments based on the statistical analysis of the data described above were included without adjustment. RPDs that were not determined to be significantly lower than those in bulk sediments based on the statistical analysis ($P>0.05$) were set at zero percent (i.e., no difference in planted vs. bulk sediment PAH concentration). The PAH concentrations did not significantly change with time for the duration of the study period (the exception being phenanthrene). Therefore, a mean RPD (RPD_M) was calculated as an average of the RPDs from the four sampling dates, grouped together by molecular weight (MW) for each sample depth (2.5 cm and 7.5 cm).

The RPD_M exhibit decreasing linear trends with increasing MW for both sample depths (Figure 3.13). The RPD_M at 2.5 cm decrease from 82% for the lower MW PAH to 0% for the highest MW PAHs (R2 = 0.9308; Slope = -0.0071). The RPD_M at 7.5 cm exhibit a decreasing linear trend (R2 = 0.8299) from 87% for the lower MW PAH down to 21% for the highest MW PAHs (Slope = -0.0054). Based on the ORP data from the two sampling depths, redox conditions in the test sediments were oxidizing at the 2.5 cm sample depth, but alternated between hypoxic and reducing seasonally at the 7.5 cm depth in the root zone.

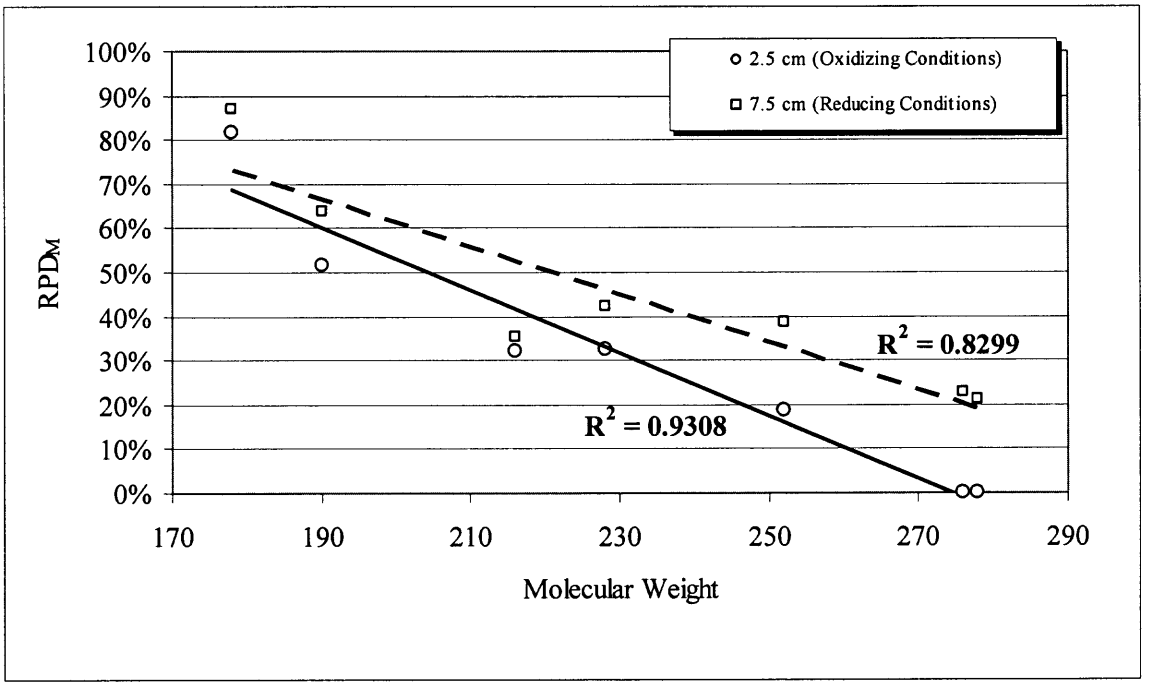


Figure 3.13 Molecular weight vs. mean relative percent difference (RPDM) of pooled PAH concentrations in bulk and vegetated sediment under oxidizing conditions (2.5 cm) and reducing conditions (7.5 cm).

Three general observations are made from the RPD_M linear correlation plot and ORP data discussed above: (1) the differences in planted and bulk sediment PAH concentrations decreased linearly with increasing MW; (2) RPD's for lower MW PAHs

(MW 178 to 216) were similar for both sample depths; and (3) greater significant differences in planted and bulk sediment for the higher MW PAHs (MW 228 to 278) occur at 7.5 cm, where reducing conditions had developed in the planted treatments. Although the observed trend may be a result of PAH degradation, such degradation would have had to occur prior to the greenhouse study (i.e., during the field study). This could be explained by the potential effect of changing from a fresh water system in the field to a saltwater regime in the greenhouse, as was described earlier in this chapter. However, the study data do not confirm microbial degradation of PAH.

3.5 Discussion

Ninety-five percent of the plants survived past two growing seasons. This is an important finding since plant survival through changing seasonal temperatures, flowering and senescence in grossly contaminated sediment could not be assumed prior to this study.

Biogeochemical data indicate observed rhizosphere effects, such as increased plant-derived organic carbon and neutralizing shifts in pH, that are important to plant health and microbial growth. However, the increasing pH measured in both bulk and planted sediments is likely a result of the natural pH buffering capacity of the salt water used during the experiment. Microbial densities increased in the root zone and decreased marginally in the bulk sediments; increases in microbial activity in the planted sediment are assumed from significant decreases in redox potential indicated by ORP measurement data.

The microbial carbon mass was calculated using the microbial densities from the planted sediments at the 2.5 cm depth, an estimated mean cell volume of 0.063 μm^3 (Schönholzer et al. 1999) and a biovolume-carbon transformation factor of 310 femtogram C per μm^3 (Fry, 1990). Based on this calculation, the total estimated microbial carbon mass added to the planted sediments at the 2.5 cm depth was 1.4, 2.8 and 5.5 mg C/kg soil (dry weight basis) for the Fall 2001, Spring 2002 and Fall 2002 sampling dates, respectively. The microbial carbon mass amounts estimated are not nearly large enough to explain the difference of approximately 35% in PHC concentrations between bulk and planted sediments (i.e., approx. 30000 mg/kg carbon). The difference in bulk and planted sediment PHC concentrations in the Fall 2002 samples is likely due to matrix heterogeneity or sequestration of PHC to root biomass (i.e., lowered analytical extraction efficiency). However, the change in phenanthrene concentrations in the planted sediments at the 2.5 cm depth represents a decrease of approximately 1.6 mg C/kg soil (dry weight basis). Microbial degradation of phenanthrene has been reported in various soils, intertidal sediments, and in rhizosphere soils and sediments (Aitken et al., 1998; Berardesco et al., 1998; Daane et al., 2002; Corgie et al., 2004). Daane et al. (2002) isolated bacteria (Paenibacillus validus) that use phenanthrene as a sole carbon source from the rhizosphere of *Spartina alterniflora* that were growing in a PHC-contaminated salt marsh.

Based on an average of the pooled data, pyrene and chrysene represent approximately 65% of the TPAH in the sediment samples (pyrene $\cong$ 40% and chrysene $\cong$ 25%), and benzo(a)anthracene and benzo(g,h,i)perylene represent about 20% of the TPAH (approx. 10% each), with the other 10 PAHs detected each accounting for less

than 10% of the TPAH. Also, a comparison of PAH concentrations in the planted and bulk sediments links depth and redox conditions to decreases in PAHs as a function of MW. Data from investigations of PAH degradation by Chang et al. (2002) suggest a similar (albeit imperfect) trend of increasing degradability with increasing MW in anaerobic sediments.

Experiments conducted by Kludze and DeLaune (1995) demonstrate reducing conditions (below –100 mV) in the planted sediment of *S. patens* caused changes in plant function, including decrease in plant growth, CO_2 fixation and emissions of CH_4. In related laboratory studies, *S. patens* root and shoot dry weight decreased by 37% and 25%, respectively, in response to rhizosphere redox intensities between –200 and –300 mV (Kludze, 1994). Based on the biomass and ORP data from the greenhouse study, reductions in root and shoot biomass were not observed and the ORP was above –100 mV. Although, the mean ORP measured in the greenhouse study sediments was not this low, potential effects on plant physiology due to reducing conditions should be monitored for studies where redox is expected to be below –100 mV.

The organic carbon content of soil and sediment can effect partitioning and bioavailability of organic and inorganic nutrients and contaminants. Wetlands are among the most biologically productive ecosystems and wetland soils may contain up to 40% organic matter (Zedler, 2001). According to Tedrow (1986), two types of soils are predominant in the vicinity of the study site (i.e., Perth Amboy): Wethersfield, an upland soil containing from 0.6 to 7.4% carbon in the upper 13 inches; and Tidal Marsh, that contains about 15% organic matter on average, but can vary from less than 1% to near 50%. Based on results from analysis of 45 sediment samples collected from Woodbridge

Creek (adjacent to the study site) in 2003, the nearby estuary sediments contain an average of 6.4% total organic carbon (TOC), ranging from 0.2 to 32.8% TOC (Chevron, 2003). The TOC analysis (Lloyd Kahn Method; USEPA, 1988) does not differentiate organic carbon and petroleum hydrocarbons, and based on other associated sampling data, PHC may account for a significant portion of the reported TOC concentrations in those samples. Based on a cursory review of the data, pooled mean PHC concentration in the study samples ($\cong$ 6.8%) is surprisingly similar to the average percent TOC in the from Woodbridge Creek (6.4%), and is within the range of the carbon content reported for the local soil types.

Since the basis for decay of the organic contaminants is in essence microbially-mediated enzymatic transformation, increasing microbial densities are generally desirable. According to Bouwer and Wright (1988), increasing the microbial density by adding substrate and nutrients has been linked to significant decreases in the half-life of organic contaminants. However, a major limitation to this type of biostimulation technique is the inability to effectively deliver the nutrients evenly to remote subsurface pore space. According to Norris (1994), typical bioremediation (i.e., without plants) is not practical for grossly contaminated media that is nearly saturated with heavy-end oily PHC (i.e., above 20,000 ppm). Although the PHC concentrations in the sediments used in this study ranged from 45,000 to 95,000 ppm, the plant roots apparently provide a mechanism to overcome this limitation for the sediment used in this study, based on the observed microbial densities.

Data obtained from different depths indicate that significantly different redox conditions developed in the rhizosphere that may have affected degradation of individual PAH depending on MW. In natural, unpolluted bulk sediment, anerobic conditions dominate within millimeters below the sediment surface due in part to microbial degradation of naturally-occuring organic carbon (Atlas and Bartha, 1998). In the rhizosphere of wetland plants like *S. patens*, the depth of the oxic zone is expected to increase via plant root aeration (Jaffe et al, 2002). However, the results of the greenhouse study suggest that the predicted increase in oxidation of the sediments in the root zone appears to reverse in saturated sediments that are grossly contaminated with PHC. This is consistent with other literature that reported hypoxic and anoxic conditions in submerged sediments can extend to the sediment-water interface when under eutrophic conditions caused by excess organic carbon from either natural or anthropogenic sources (Jorgensen and Richardson, 1996). The consistent seasonal changes in redox conditions were not expected; such conditions could not be assumed to occur in sediments grossly contaminated with PHC and PAH just on the basis of the oil potentially hindering diffusion of DO through the sediment matrix. In addition, the presence of the wetland roots would be expected to enhance the oxic zone in the shallow sediments. Reversal of the expected redox conditions is apparently a biotic response to the additional organic carbon loading in the form of PHC. For bulk sediments, the response is in the form of decreased microbial activity, either through poor substrate bioavailability (i.e., hydrophobic nature of PHC) or toxicity due to high contaminant concentration, allowing oxic conditions to prevail. For the planted sediments, the additions of natural (plant) organic carbon in the form of root biomass (and related root turnover) and exudates

enhances microbial activity, creating reducing conditions that are most pronounced at the end of the growing season. The seasonal variation of redox conditions in the sediment at different depths represents a potentially significant mechanism for organic contaminant degradation by fostering conditions that encourage development of dynamic microbial niches. Based on the ORP and microbial density data, the seasonal variation was more pronounced in the planted sediment. Alternating redox conditions to enhance degradation of organic matter is not new; publicly operated treatment works facilities have been using aerobic and anaerobic reactors for years to reduce organic pollutants in wastewater.

Evaluation of biodegradation using growth-dependant models, such as the Monod parameters (Knightes and Peters, 2000) assumes microbial biomass increases with degradation of the organic substances. But this may not be a correct assumption in all cases, since microbial degradation of organic substances can be growth-linked or non-growth-linked (Alexander, 1999). Growth-linked degradation is associated with increasing microbial densities; non-growth-linked degradation occurs without increases in microbial density. The later may result from microbial use of C during a stationary growth phase or cellular respiration is consuming C for non-catabolic activity. Based on the microbial density data, decreases in concentrations of PHC and PAH in the planted sediment appear to be growth-linked at the 2.5 cm depth and non-growth-linked at the 7.5 cm depth. The ORP data indicate redox conditions were oxidizing at 2.5 cm and microaerobic to reducing at 7.5 cm.

Phenanthrene decreased in the planted sediment under oxidizing conditions, with half-life estimates of 141 to 165 days. The longer half-life (165 days) is considered to be more reliable than the shorter (141 days) for phenanthrene, since it is based on a longer time period (846 days), more data points, and the data exhibited a better linear correlation. The half-life estimates calculated for phenanthrene from these data are generally consistent with other research that reported phenanthrene half-life in soil and sediment up to 126 days (Kanaly and Harayama, 2000).

Analytical Data Uncertainties

Most of the samples for PHC required significant dilution of the solid-liquid extract to perform the analysis. As a result a dilution factor of 25 and 100 was used to calculate the reported PHC concentrations for many samples; however, a dilution factor of 50 was used for the majority of samples. Dilution of the samples to this extent is indicative of the extremely high PHC concentrations encountered in most of the samples. At this high of concentration, the general precision and accuracy of the PHC data are expected to be lower than for samples containing PHC at concentrations more in the range of the method standards (i.e., that require little or no dilution). Also, as described above, PHC analysis by Method 418.1 is subject to positive interference by naturally occurring organic substances. Thus, the PHC data are considered semi-quantitative, and useful more as an indicator of general conditions, rather than as absolute concentrations for comparison to remedial standards.

The high concentrations of PHC also represent matrix interference for PAH analysis that was evident in the data. The sample analysis was performed by GCMS operating in selective ion mode (SIM) to overcome the potential interference at the point of the instrument detector and achieve low detection limits. However, the high PHC concentrations also potentially affect sample extraction efficiencies, GC column performance and matrix spike recovery. This is a relatively common analytical problem for environmental samples – especially those that contain high concentrations of PHC.

While the PAH surrogate recoveries for the PAH data were within the acceptability range for Method 8270 (18% to 137% for terphenyl-d14), they represent a laboratory bias that influenced the data evaluation in this study. While the data are considered usable for this study with appropriate correction for variable surrogate recovery (see Appendix C), a tighter range of surrogate recovery acceptance limits and surrogates selected based on target compounds in future analyses of similar samples should be employed to improve end-user data evaluation.

CHAPTER 4

CONCLUSIONS

The field study demonstrated that *Spartina patens* could not survive in the NFB sediments containing 12% or more TPHC; however, the plants grown in sediments containing from 6.3% to 11.9% TPHC exhibited better than 80% survival, and growth was significant. Sediment samples within the plots where plants survived exhibited decreases of 24% TPHC and up to 51% PAH compared to bulk sediment from around the plots. Sediment metals concentrations were below remedial action levels and phytoextraction coefficients for metals were very low (less than 1, with the exception of 1.37 for manganese).

Based on the greenhouse study, planted sediments contained 1% to 2% root biomass (not present in bulk sediments). The pH in planted sediments exhibited an increasing linear trend toward neutral (i.e., pH 7) with time; the pH was higher and changed at a faster rate in the planted sediment as compared to bulk sediment. Microbial density (DAPI cell count) in planted sediment at a depth of 2.5 cm exhibited a linear increasing trend with time, and was generally found at higher numbers than those in bulk sediment at both the 2.5 and 7.5 cm depths. Oxidizing redox conditions prevailed in bulk sediments and in planted sediments at the 2.5 cm depth. Reducing redox conditions developed in the planted sediments at the 7.5 cm depth by the end of each growing season (Fall 2001 and Fall 2002); decreased redox in the rhizosphere is assumed to be associated with increases in microbial activity.

PHC concentrations were unchanged in first three sample rounds, but was 35% to 37% lower in the planted sediments vs. bulk sediment at the end of the study at both the

2.5 and 7.5 cm sample depths after 2 years. Planted sediment PAH concentrations were generally lower than in bulk sediments. Low molecular weight (MW) PAH's (2 and 3-rings) were either absent or at relatively low concentrations in all treatments. Phenanthrene concentrations in bulk sediments did not change, but were reduced by 95% in rhizosphere with half-life estimates of 141 to 165 days in the rhizosphere at the 2.5 cm depth. Temporal changes in high MW PAHs (4 to 6-rings) were not observed over the greenhouse study period. Differences in PAH concentrations between rhizosphere and bulk sediments exhibit a decreasing trend with increasing PAH molecular weight. Consistent, significantly lower concentrations of PAH in the rhizosphere vs. bulk sediment suggests degradation of PAHs occurred during the field study under freshwater conditions. Evaluation of the relative percent difference (RPD) in individual PAH concentrations from the rhizosphere vs. bulk sediments indicates that lower MW PAHs may degrade better under oxidizing conditions, and higher MW PAHs under reducing conditions, which developed in the rhizosphere. Large differences in microbial consortia and chemodynamics of PAH degradation exist between oxidizing and reducing environments in sediment, which appear to be enhanced in the rhizosphere.

Future Direction

Root depth is one limitation noted for rhizodegradation. However, based on the foregoing, it cannot be assumed that the same microbial processes are occurring at all depths in the root zone. Thus, spatio-temporal variations and cycling of redox conditions should be considered in future study design to properly evaluate biodegradation of organic contaminants at different depths in the rhizosphere. In addition, assuming that

microbial degradation of organic contaminants in the rhizosphere is growth-linked may be incorrect based on Alexander (1999), especially under reducing (anaerobic) conditions; and natural microbial diversity may decline due to exposure to petroleum (Nyman, 1999). Therefore, future research should be aimed at identifying implied connections between growth-linked and non-growth-linked decay of organic contaminants by rhizosphere microorganisms under oxidizing and reducing conditions.

Limitations identified as a result of this study include changing from essentially a fresh water regime in the field study to salt water for the greenhouse study and use of laboratory prepared salt water using de-ionized water and aquarium sea salt. These aspects had a purpose in the original study design, and were unavoidable without significantly increasing the scope and cost of the project. This was necessary to control the variables in the study, and introduction of another treatment (i.e., 10 replicates maintained with natural estuary water) was beyond the scope of the original project. Natural estuarine water contains microbial consortia that would be expected to colonize the sediments. Surface waters with a history of exposure to petroleum hydrocarbons have been shown to exhibit microbial communities with enhanced capacity for degradation of PHC and PAH (Carman et al., 1996; Hayes et al., 1999). Woodbridge Creek appears to be an ideal candidate for supplying such microbiota given the industrial history of the area and proximity to several oil refineries. Woodbridge Creek sediments contain total PAH concentrations of up to 140 ppm (Chevron, 2003). Thus, restoring the natural hydrology, including the influx of tidal flow from Woodbridge Creek appears to be a potentially important mechanism for inoculating the basin sediments with microorganisms naturally selected to degrade TPHC and PAH. To test this, subsequent

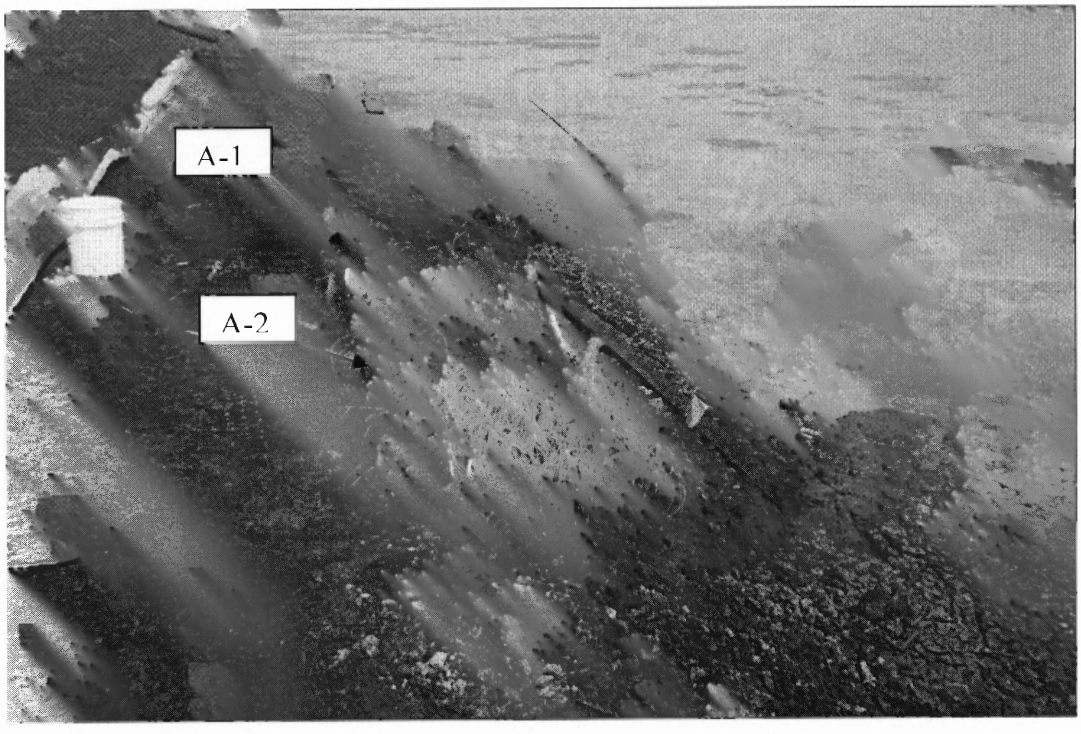
research would incorporate use of Woodbridge Creek water into the existing experimental design. In addition, molecular methods, such as PCR could be used to identify the microbial community structure and PHC degraders both in creek water and in the sediments over time. Additional analysis for TPHC and PAH would also be required to evaluate any effects due to the anticipated changes to the microbial population in the rhizosphere and bulk sediments.

Other directions for research include isolation and identification of the specific phenanthrene degrading microorganism(s) present in the rhizosphere of the test sediments, and investigations of associated enzyme systems and activities. Community structure under different redox conditions in the rhizosphere should also be investigated. One aspect of future research that is of practical importance involves evaluating potential for leaching and transport of PAH from contaminated rhizosphere sediments to the surface water (solubility in tide water).

APPENDIX A

PHOTOGRAPHS OF FIELD STUDY PLOTS

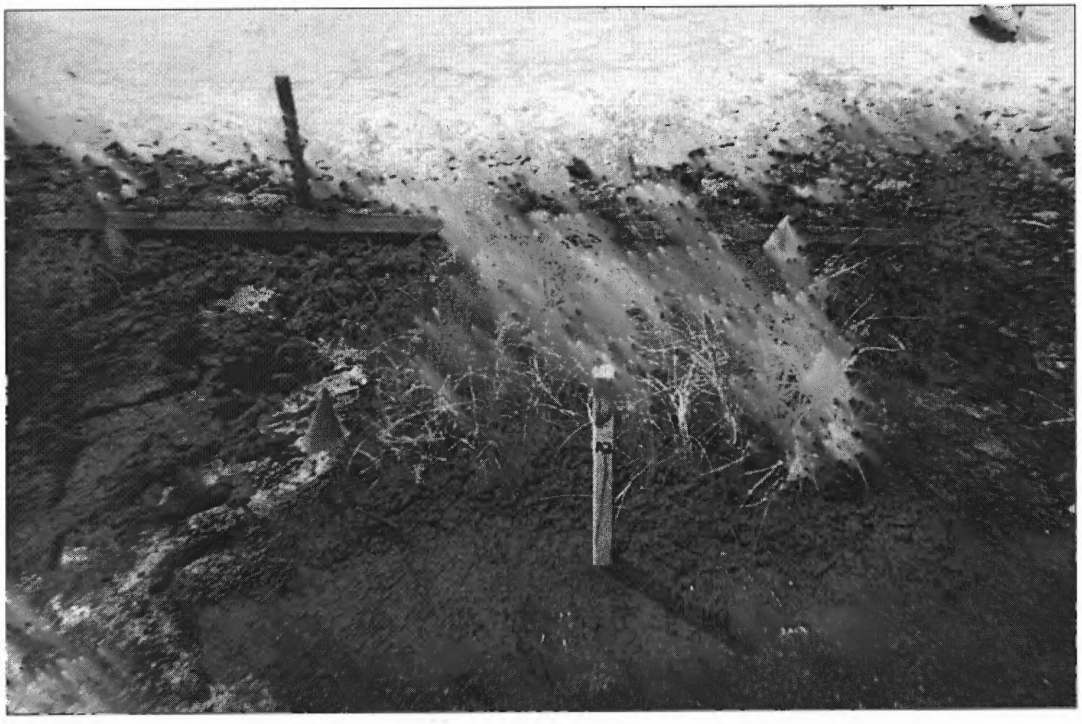
Slides A.1 through A.11 are photographs of the field study plots containing *Spartina patens* shot on various dates from May 2000 through November 2000.



Slide A.1 Plots A-1 (upper) and A-2 (lower) after planting on 5/17/00.

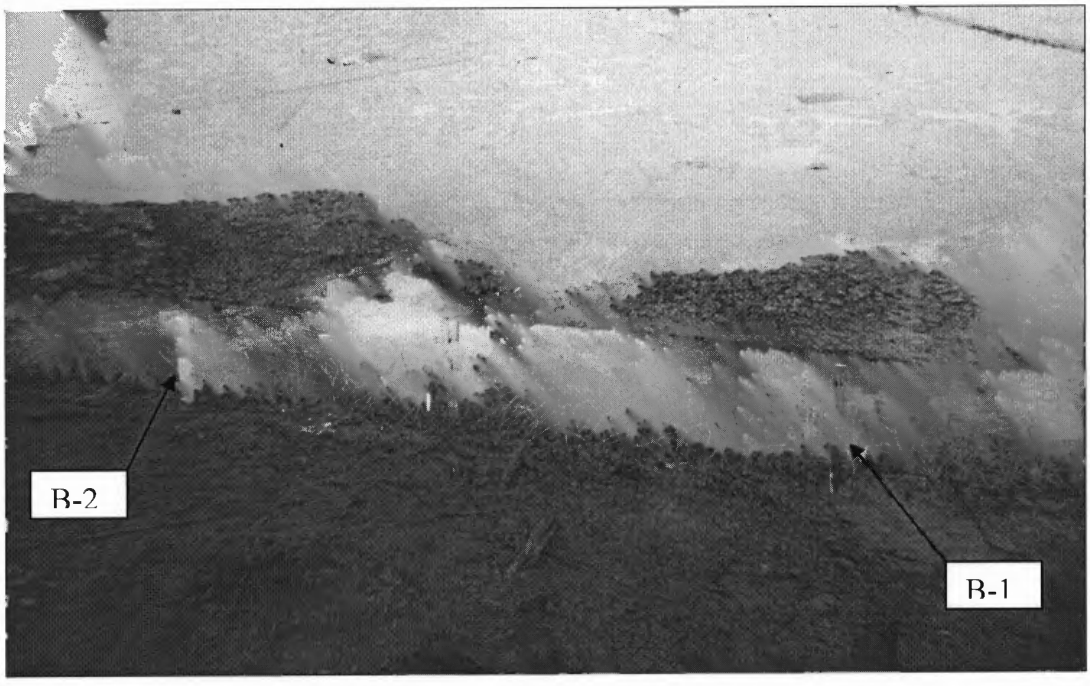
Slide A.2 Plot A1 on 6/2/00.

Slide A.3 Plot A1 on 8/17/00.

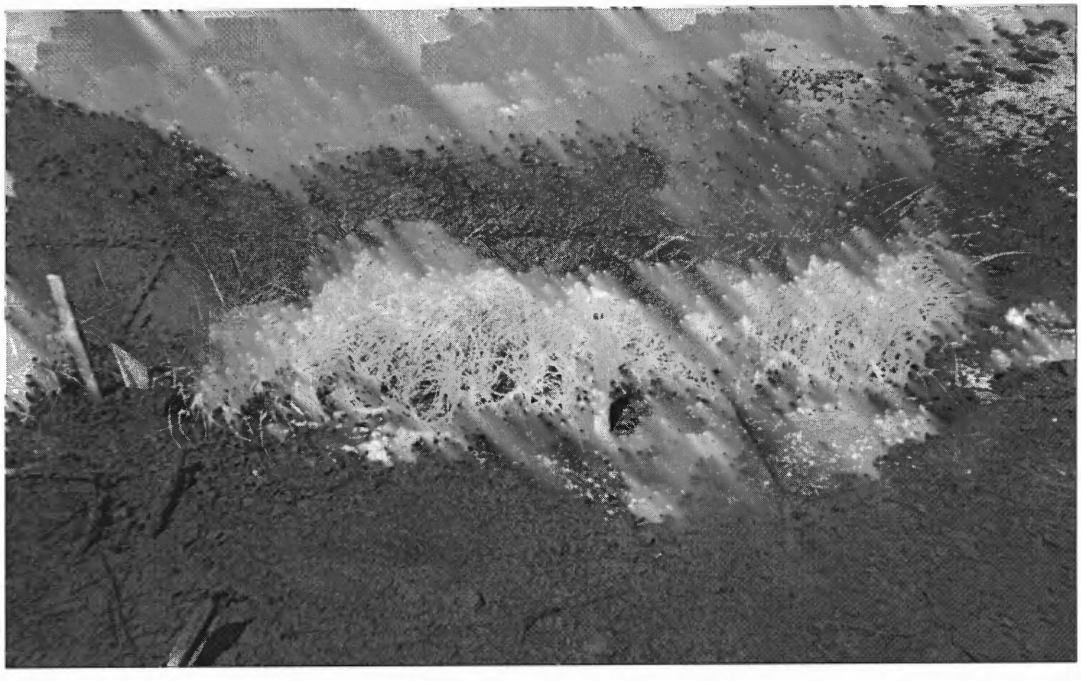


Slide A.4 Plot A2 on 6/8/00.

Slide A.5 Plot A2 on 8/17/00.

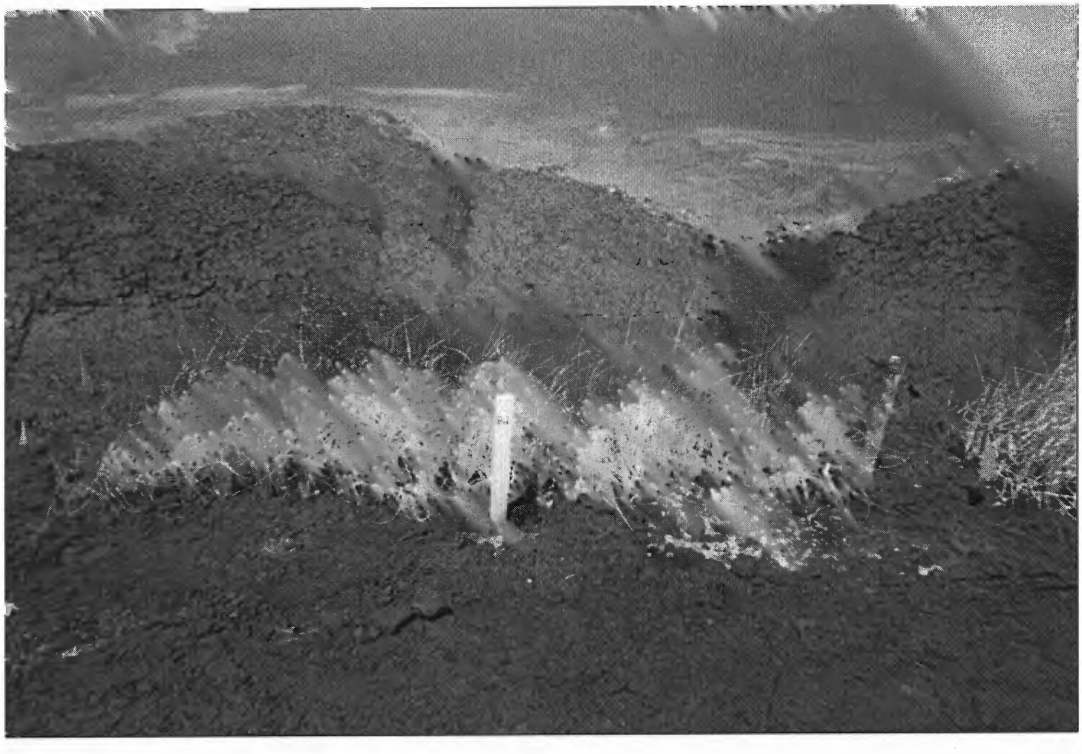


Slide A.6 Plots B-1 (left) and B-2 (right) on 6/14/00.



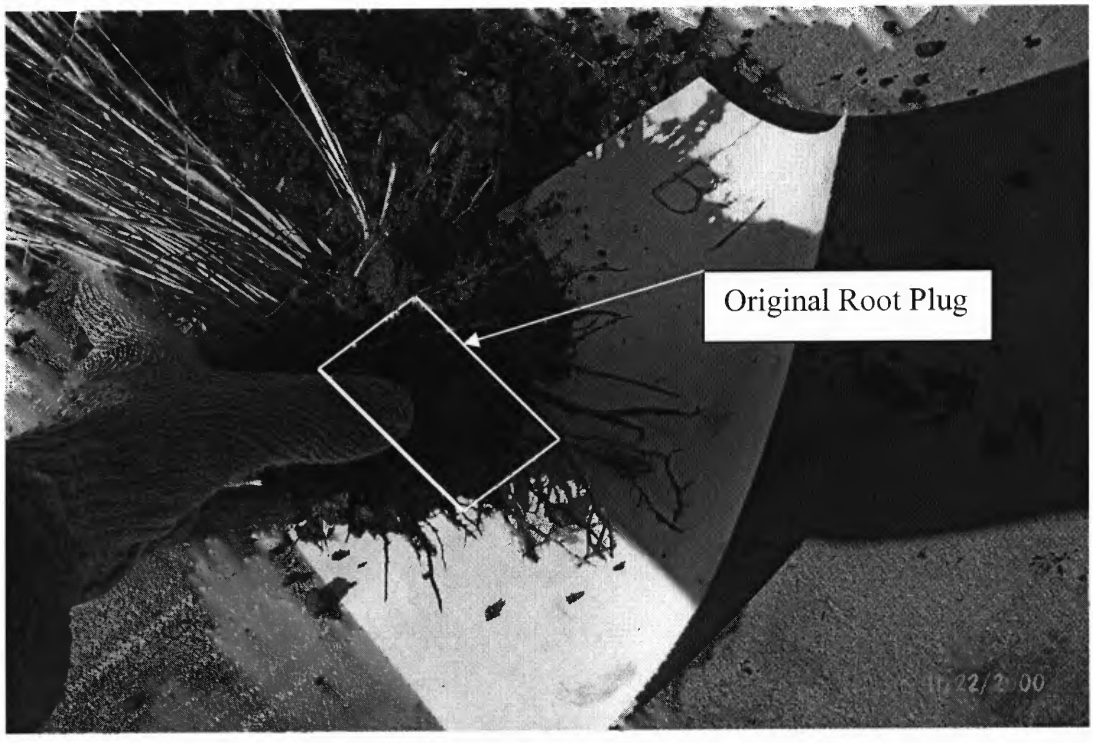
Slide A.7 Plot B1 on 7/11/00.

Slide A.8 Plot B1 on 11/21/00.



Slide A.9 Plot B2 on 7/11/00.

Slide A.10 Plot B2 on 11/21/00.



Slide A.11 Healthy root system of *S.patens* plant removed from Plot B-1 on 11/22/00 and approximate size of root plug planted in May 2000.

APPENDIX B

FIELD STUDY ANALYTICAL RESULTS

The results from chemical analysis of sediment samples collected at the conclusion of the Field Study in November 2000 are provided in the following Tables B.1 through Table B.6.

Table B.1 Total Petroleum Hydrocarbons in sediment samples collected during the Field Study

Sample No./Depth (feet)	Sample Date	TPHC (mg/kg)
A-1 (1)/0.0-0.5	11/21/2000	**238000**
A-2 (1)/0.0-0.5	11/21/2000	**120000**
B-1 (1)/0.0-0.5	11/21/2000	**62500**
B-1 (2)/0.0-0.5	11/21/2000	**88700**
B-2 (1)/0.0-0.5	11/21/2000	**108000**
B-2 (2)/0.0-0.5	11/21/2000	**105000**
Control-1/0.0-0.5	11/21/2000	**119000**
Control-2/0.0-0.5	11/21/2000	**66900**
Control-3/0.0-0.5	11/21/2000	**113000**

Total Petroleum Hydrocarbons (TPHC)
Concentratrion in parts per million (mg/kg).

Table B.2 Volatile Organic Compounds in Sediment Samples collected during Field Study

Sample No.: / Date Sampled:	A-1(1) 11/21/2000	A-2(1) 11/21/2000	B-1(1) 11/21/2000	B-1(2) 11/21/2000	B-2(1) 11/21/2000	B-2(2) 11/21/2000	Control-1 11/21/2000	Control-2 11/21/2000	Control-3 11/21/2000
VOCs (ppm)									
Acetone	ND	ND	ND	ND	ND	ND	ND	ND	ND
Benzene	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bromodichloromethane	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bromoform	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bromomethane	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-Butanone (MEK)	ND	ND	ND	ND	ND	ND	ND	ND	ND
Carbon disulfide	ND	ND	ND	ND	ND	ND	ND	ND	ND
Carbon tetrachloride	ND	ND	ND	ND	ND	ND	ND	ND	ND
Chlorobenzene	ND	ND	ND	ND	ND	ND	ND	ND	ND
Chloroethane	ND	ND	ND	ND	ND	ND	ND	ND	ND
Chloroform	ND	ND	ND	ND	ND	ND	ND	ND	ND
Chloromethane	ND	ND	ND	ND	ND	ND	ND	ND	ND
Dibromochloromethane	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,1-Dichloroethane	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2-Dichloroethane	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,1-Dichloroethene	ND	ND	ND	ND	ND	ND	ND	ND	ND
cis-1,2-Dichloroethene	ND	ND	ND	ND	ND	ND	ND	ND	ND
trans-1,2-Dichloroethene	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2-Dichloropropane	ND	ND	ND	ND	ND	ND	ND	ND	ND
cis-1,3-Dichloropropene	ND	ND	ND	ND	ND	ND	ND	ND	ND
trans-1,3-Dichloropropene	ND	ND	ND	ND	ND	ND	ND	ND	ND
Ethylbenzene	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-Hexanone	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-Methyl-2-pentanone(MIBK)	ND	ND	ND	ND	ND	ND	ND	ND	ND
Methylene chloride	ND	ND	ND	ND	ND	ND	ND	ND	ND
Styrene	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,1,2,2-Tetrachloroethane	ND	ND	ND	ND	ND	ND	ND	ND	ND
Tetrachloroethene	ND	ND	ND	ND	ND	ND	ND	ND	ND
Toluene	ND	ND	ND	ND	ND	ND	0.173	ND	ND
1,1,1-Trichloroethane	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,1,2-Trichloroethane	ND	ND	ND	ND	ND	ND	ND	ND	ND
Trichloroethene	ND	ND	ND	ND	ND	ND	ND	ND	ND
Vinyl chloride	ND	ND	ND	ND	ND	ND	ND	ND	ND
Xylene (total)	ND	ND	ND	ND	ND	ND	ND	ND	ND
Total Targeted VOCs (ppm)	ND	ND	ND	ND	ND	ND	0.173	ND	ND
Total Non-Targeted VOCs (ppm)	77	4.6	37.8	15.7	77.1	15.3	36.1	0	39
Total VOCs (ppm)	77	4.6	37.8	15.7	77.1	15.3	36.273	0	39

ND = not detected.
J = compound detected below reporting limit.
ppm = patrs per million.
All samples obtained from 0.0-0.5 ft-bg.

Table B.3 Semi-volatile Organic Compounds in Sediment Samples collected during Field Study

Sample No./Depth: / Date Sampled:	A-1(1) 11/21/2000	A-2(1) 11/21/2000	B-1(1) 11/21/2000	B-1(2) 11/21/2000	B-2(1) 11/21/2000	B-2(2) 11/21/2000	Control-1 11/21/2000	Control-2 11/21/2000	Control-3 11/21/2000
SVOCs (ppm)									
2-Chlorophenol	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-Chloro-3-methyl phenol	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4-Dichlorophenol	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4-Dimethylphenol	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4-Dinitrophenol	ND	ND	ND	ND	ND	ND	ND	ND	ND
4,6-Dinitro-o-cresol	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-Methylphenol	ND	ND	ND	ND	ND	ND	ND	ND	ND
3&4-Methylphenol	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-Nitrophenol	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-Nitrophenol	ND	ND	ND	ND	ND	ND	ND	ND	ND
Pentachlorophenol	ND	ND	ND	ND	ND	ND	ND	ND	ND
Phenol	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4,5-Trichlorophenol	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4,6-Trichlorophenol	ND	ND	ND	ND	ND	ND	ND	ND	ND
Acenaphthene	ND	ND	ND	ND	ND	ND	ND	ND	1.74 J
Acenaphthylene	ND	ND	ND	ND	ND	ND	ND	ND	ND
Anthracene	ND	ND	ND	ND	ND	ND	ND	ND	ND
Benzo(a)anthracene	ND	6.43	ND	3.4	1.85 J	3.84	6.16	1.39 J	4.1
Benzo(a)pyrene	ND	12.8	1.72 J	2.86	2.4	3.62	5.8	1.38 J	8.27
Benzo(b)fluoranthene	ND	ND	4.2	ND	4.12	5	11.9	4.06	8.63
Benzo(g,h,i)perylene	ND	7.02	4.02	4.23	5.07	5.35	12	3.68	11.9
Benzo(k)fluoranthene	ND	ND	0.81 J	ND	ND	ND	ND	ND	1.75 J
4-Bromophenyl phenyl ether	ND	ND	ND	ND	ND	ND	ND	ND	ND
Butyl benzyl phthalate	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-Chloronaphthalene	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-Chloroaniline	ND	ND	ND	ND	ND	ND	ND	ND	ND
Carbazole	ND	ND	ND	ND	ND	ND	ND	ND	ND
Chrysene	31.3	18.7	5.31	7.77	4.59	9.38	17.7	5.99	7.72
bis(2-Chloroethoxy)methane	ND	ND	ND	ND	ND	ND	ND	ND	ND
bis(2-Chloroethyl)ether	ND	ND	ND	ND	ND	ND	ND	ND	ND
bis(2-Chloroisopropyl)ether	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-Chlorophenyl phenyl ether	ND	ND	ND	ND	ND	ND	ND	ND	ND

SVOC = semi-volatile organic compound
ND = not detected.
J = compound detected below reporting limit.
ppm = parts per million.

Table B.3 Semi-volatile Organic Compounds in Sediment Samples collected during Field Study (continued)

Sample No./Depth: / Date Sampled:	A-1(1) 11/21/2000	A-2(1) 11/21/2000	B-1(1) 11/21/2000	B-1(2) 11/21/2000	B-2(1) 11/21/2000	B-2(2) 11/21/2000	Control-1 11/21/2000	Control-2 11/21/2000	Control-3 11/21/2000
1,2-Dichlorobenzene	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3-Dichlorobenzene	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,4-Dichlorobenzene	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4-Dinitrotoluene	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,6-Dinitrotoluene	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,3'-Dichlorobenzidine	ND	ND	ND	ND	ND	ND	ND	ND	ND
Dibenzo(a,h)anthracene	ND	ND	ND	ND	ND	ND	ND	ND	ND
Dibenzofuran	ND	ND	ND	ND	ND	ND	ND	ND	ND
Di-n-butyl phthalate	ND	ND	ND	ND	ND	ND	ND	ND	ND
Di-n-octyl phthalate	ND	ND	ND	ND	ND	ND	ND	ND	ND
Diethyl phthalate	ND	ND	ND	ND	ND	ND	ND	ND	ND
Dimethyl phthalate	ND	ND	ND	ND	ND	ND	ND	ND	ND
bis(2-Ethylhexyl)phthalate	8.16 J	5	2.52	3.73	2.98	3.34	6.54	3.09	2.5
Fluoranthene	ND	ND	ND	ND	ND	ND	ND	ND	ND
Fluorene	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hexachlorobenzene	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hexachlorobutadiene	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hexachlorocyclopentadiene	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hexachloroethane	ND	ND	ND	ND	ND	ND	ND	ND	ND
Indeno(1,2,3-cd)pyrene	ND	ND	ND	ND	ND	ND	ND	ND	3.19
Isophorone	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-Methylnaphthalene	ND	ND	ND	ND	ND	ND	ND	ND	2.1 J
2-Nitroaniline	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-Nitroaniline	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-Nitroaniline	ND	ND	ND	ND	ND	ND	ND	ND	ND
Naphthalene	ND	ND	ND	ND	ND	ND	ND	ND	ND
Nitrobenzene	ND	ND	ND	ND	ND	ND	ND	ND	ND
N-Nitroso-di-n-propylamine	ND	ND	ND	ND	ND	ND	ND	ND	ND
N-Nitrosodiphenylamine	ND	ND	ND	ND	ND	ND	ND	ND	ND
Phenanthrene	ND	ND	ND	3.54	ND	2.53 J	4.14 J	ND	2.94
Pyrene	56.3	37.8	11.5	17.2	8.5	19.2	40.6	10.5	22.3
1,2,4-Trichlorobenzene	ND	ND	ND	ND	ND	ND	ND	ND	ND
Total Targeted SVOCs (ppm)	**95.76**	**87.75**	**30.08**	**42.73**	**29.51**	**52.26**	**104.84**	**30.09**	**77.14**
Total Non-Targeted SVOCs (ppm)	**4728**	**1754**	**645**	**914**	**704**	**1022**	**1873**	**803**	**2157**
Total SVOCs (ppm)	**4823.76**	**1841.75**	**675.08**	**956.73**	**733.51**	**1074.26**	**1977.84**	**833.09**	**2234.14**

SVOC = semi-volatile organic compound

ND = not detected.

J = compound detected below reporting limit.

ppm = parts per million.

Table B.4 Pesticides in Sediment Samples collected during Field Study

Sample No./Depth: / Date Sampled:	A-1(1) 11/21/2000	A-2(1) 11/21/2000	B-1(1) 11/21/2000	B-1(2) 11/21/2000	B-2(1) 11/21/2000	B-2(2) 11/21/2000	Control-1 11/21/2000	Control-2 11/21/2000	Control-3 11/21/2000
Pesticides (ppm)									
Aldrin	ND	ND	ND	ND	ND	ND	ND	ND	ND
alpha-BHC	ND	ND	ND	ND	ND	ND	ND	ND	ND
beta-BHC	ND	ND	ND	ND	ND	ND	ND	ND	ND
delta-BHC	ND	ND	ND	ND	ND	ND	ND	ND	ND
gamma-BHC (Lindane)	ND	ND	ND	ND	ND	ND	ND	ND	ND
alpha-Chlordane	ND	ND	ND	ND	ND	ND	ND	ND	ND
gamma-Chlordane	ND	ND	ND	ND	ND	ND	ND	ND	ND
Dieldrin	ND	ND	ND	ND	ND	ND	ND	ND	ND
4,4'-DDD	ND	ND	ND	ND	ND	ND	ND	ND	ND
4,4'-DDE	ND	ND	ND	ND	ND	ND	ND	ND	ND
4,4'-DDT	0.208	ND	0.0827	ND	ND	0.0621	0.12	0.0746	0.11
Endrin	ND	ND	ND	ND	ND	ND	ND	ND	ND
Endosulfan sulfate	ND	ND	ND	ND	ND	ND	ND	ND	ND
Endrin aldehyde	ND	ND	ND	ND	ND	ND	ND	ND	ND
Endosulfan-I	ND	ND	ND	ND	ND	ND	ND	ND	ND
Endosulfan-II	ND	ND	ND	ND	ND	ND	ND	ND	ND
Heptachlor	ND	ND	ND	ND	ND	ND	ND	ND	ND
Heptachlor epoxide	ND	ND	ND	ND	ND	ND	ND	ND	ND
Methoxychlor	ND	ND	ND	ND	ND	ND	ND	ND	ND
Endrin ketone	ND	ND	ND	ND	ND	ND	ND	ND	ND
Toxaphene	ND	ND	ND	ND	ND	ND	ND	ND	ND

ND = not detected.

J = compound detected below reporting limit.

ppm = patrs per million.

Table B.5 Metals in Sediment Samples collected during Field Study

Sample No./Depth:	A-1(1)		A-2(1)		B-1(1)		B-1(2)		B-2(1)		B-2(2)		Control-1		Control-2		Control-3	
Date Sampled:	11/21/2000		11/21/2000		11/21/2000		11/21/2000		11/21/2000		11/21/2000		11/21/2000		11/21/2000		11/21/2000	
Metals (ppm)																		
Aluminum	6360		9860		5720		8770		5640		6100		7330		7020		7660	
Antimony	1.5		1.5	U	1.7		2.4		4		2		1.9		1.8		2	
Arsenic	13.1		11.8		6.3		9.7		12.3		9.1		9		9.2		10.9	
Barium	107		76.7		42.7		59.2		43.5		51		56.9		43		40.9	
Beryllium	0.77	U	0.74	U	0.67	U	0.77	U	0.68	U	0.71	U	0.73	U	0.62	U	0.78	U
Cadmium	2.4		1.3		0.67	U	0.77	U	0.68	U	0.71	U	0.73	U	0.62	U	0.78	U
Calcium	1290		740	U	1810		1110		926		1770		1800		1390		1340	
Chromium	251		101		70.1		105		74.4		89.2		83		50.5		51.4	
Cobalt	11.9		13.8		6.7	U	7.7	U	6.8	U	7.1	U	7.23	U	6.2	U	7.8	U
Copper	360		202		96.7		95.5		112		159		122		127		111	
Iron	13200		20900		13200		23100		19500		25200		24900		24600		24400	
Lead	114		83.4		126		277		297		222		159		239		337	
Magnesium	1390		1980		1780		1960		1420		1790		2180		1450		1940	
Manganese	62.5		101		61.4		90.5		85.4		103		130		96		118	
Mercury	0.96		0.36		0.15		0.38		0.23		0.65		0.29		0.077		0.19	
Nickel	69.7		63.8		19.6		21.5		18.8		27		27.3		21.1		25.1	
Potassium	770	U	2240		670	U	1220		731		757		1020		818		936	
Selenium	10.6		3.9		1.8		1.5		1.5		1.9		2		1.2	U	1.6	U
Silver	1.5	U	1.5	U	1.3	U	1.5	U	2.2		1.5		1.5	U	1.2	U	1.6	U
Sodium	770	U	740	U	670	U	770	U	680	U	710	U	730	U	620	U	780	U
Thallium	1.5	U	1.5	U	1.3	U	1.5	U	1.4	U	1.4	U	1.5	U	1.2	U	1.6	U
Vanadium	127		69.8		56.3		61.7		61.7		58.9		63.8		53.3		59.8	
Zinc	460		221		99.9		84.9		94.8		134		125		97.4		146	

ppm = patrs per million.

U = under detection limit; not detected.

Table B.6 TAL Metals in Plant Tissue Samples collected during Field Study

Sample No./Depth:	B-1(1)/LC	B-1(1)/RC	B-1(2)/LC	B-1(2)/RC	B-2(1)/LC	B-2(1)/RC	B-2(2)/LC	B-2(2)/RC
Date Sampled:	11/21/2000	11/21/2000	11/21/2000	11/21/2000	11/21/2000	11/21/2000	11/21/2000	11/21/2000
Metals (ppm)								
Aluminum	164	339	83.8	160	375	195	145	801
Antimony	<0.98	<1.0	<0.94	<0.97	<0.93	<0.98	<0.91	<1.1
Arsenic	<0.98	6.5	<0.94	2.6	<0.93	<0.98	<0.91	2.9
Barium	<20.0	<20.0	<19.0	<19.0	<19.0	<20.0	<18.0	<22.0
Beryllium	<0.49	<0.5	<0.47	<0.48	<0.47	<0.49	<0.45	<0.54
Cadmium	<0.49	<0.5	<0.47	<0.48	<0.47	<0.49	<0.45	<0.54
Calcium	1440	<500.0	524	<480.0	757	<490.0	<450.0	<540.0
Chromium	1.3	7	0.97	2.8	7.1	4.8	2.2	9.8
Cobalt	<4.9	<5.0	<4.7	<4.8	<4.7	<4.9	<4.5	<5.4
Copper	5.5	15.7	4.8	8.6	21.2	15	9.5	21.5
Iron	447	6840	227	2910	1350	3040	560	7990
Lead	8	31	3.1	11.5	13.2	19.5	5.1	92.3
Magnesium	566	<500.0	<470.0	<480.0	<470.0	<490.0	<450.0	<540.0
Manganese	120	20.9	87.6	11.6	40.8	10.2	27.5	28.5
Mercury	<0.033	<0.032	<0.032	<0.03	<0.03	<0.03	<0.03	<0.03
Nickel	<3.9	<4.0	<3.8	<3.9	3.8	<3.9	<3.6	4.5
Potassium	2570	<500.0	2280	<480.0	1370	<490.0	1850	<540.0
Selenium	<0.98	<1.0	<0.94	<0.97	1.1	<0.98	<0.91	<1.1
Silver	<0.98	<1.0	<0.94	<0.97	<0.93	<0.98	<0.91	<1.1
Sodium	<490.0	<500.0	481	<480.0	<470.0	<490.0	<450.0	<540.0
Thallium	<0.98	<1.0	<0.94	<0.97	<0.93	<0.98	<0.91	<1.1
Vanadium	<4.9	6.2	<4.7	<4.8	<4.7	<4.9	<4.5	11.4
Zinc	24.2	28.3	21.7	16.5	33.6	6.9	22.6	26

Plant Tissue = Leaves and Roots
LC = Leaf composite (3 - into - 1)
RC = Root Composite (3 - into - 1)
ppm = parts per million; concentration is in mg metal/kg plant tissue (dry weight basis)

APPENDIX C

SURROGATE CORRECTION OF PAH DATA

C.1 Abstract

As part of the Greenhouse Study (Chapter 3), four groups of 60 sediment samples were collected roughly 180-day apart (total 240 samples) from 10 sediment cores without plants (i.e., bulk or unplanted sediments) and 20 sediment cores containing *Spartina patens* (i.e., planted sediments). The sediment cores were obtained from the Field Study described in Chapter 2. All 240 sediment samples from the Greenhouse Study were analyzed for polycyclic aromatic hydrocarbons (PAH) using USEPA SW-846 Methods, along with several other parameters, including total petroleum hydrocarbons (TPHC) by USEPA Method 418.1. Most of the samples for PHC required significant dilution of the solid-liquid extract (25x to 100x) to perform the analysis. Dilution of the samples to this extent is indicative of the high PHC concentrations encountered in most of the samples, which averaged from 45,000 to 95,000 mg/kg (or parts per million [ppm]) TPHC. Based on prior knowledge of the site conditions from the Field Study (Chapter 2), the concentrations of TPHC and some individual PAHs were expected to be elevated. In addition, some concentrations of some of the individual PAHs were expected to decrease to below the method detection limits by the end of the study. Therefore, the sample analysis was performed by gas chromatography/mass spectrometry (GCMS) operating in selective ion mode (SIM) to overcome the potential interference at the point of the instrument detector and to achieve low detection limits. The analytical method did

achieve the lower detection limits; however, the analytical results indicated an overall increase in the concentrations of some individual PAHs by the end of the study period in treatments with and without plants. Since no PAHs were added during the test, the apparent increase in PAH concentrations was suspected of being an artifact of the laboratory analysis. A review of the data revealed a temporal laboratory bias associated with instrument response sensitivity, as indicated by variable surrogate recovery and blank spike recovery data. Thus, a method adapted from Swartz et al. (2003) is described for correcting the analytical results for three of the prominent PAHs detected in the samples based on surrogate recovery data.

C.2 Introduction

Although the biodegradation literature reviewed for this study generally do not indicate correction of PAH data based on surrogate recovery (Coates et al., 1997; Smith et al., 1999; Liste and Alexander, 2000; Kanaly et al., 2000; Chang et al., 2002), there are several notable published studies that do describe this type of data correction (Carman et al., 1996; McDonald et al., 2000; Swartz et al., 2003). In fact, in the context of using deuterated surrogates for correcting PAH data, Swartz et al. indicated no other reports in the literature were found that included non-normalized recovery data for PAHs. Thus, it appears that PAH data may be corrected in air particulates investigations (McDonald et al., 2000; Swartz et al., 2003) more so than in sediment and soil investigations (Carman et al., 1996; Coates et al., 1997; Smith et al., 1999; Liste and Alexander, 2000; Kanaly et al., 2000; Chang et al., 2002).

Swartz et al. (2003) is a publication from the USEPA's Office of Research and Development, Research Triangle Park, North Carolina (USA), and provides the most practical guidance regarding use of surrogate standard recovery for normalizing (i.e., correcting) PAH data of the references cited. Several concerns are noted by Swartz et al. (2003) including (1) potential for error in using recovery of one surrogate for interpretation of data for many compounds; and (2) normalizing (i.e., correcting) data based on surrogate recovery without providing algorithm details. According to the USEPA (Swartz et al., 2003), a good example of addressing these concerns by correcting individual PAH data based on recovery of deuterated PAHs that exhibit comparable chromatographic retention time (RT) and stability is found in McDonald et al. (2000). Regarding correction of their PAH data for varying instrumentation response factors, McDonald et al (2000) states: "The compounds that were analyzed without authentic standards were quantified using response factors of standards with similar structures."

C.3 Materials and Methods

The sediment samples were all analyzed by USEPA SW-846 Methods 8270C and 3550B for the following PAHs:

	PAH	CAS No.	MW	Rings
1	Naphthalene	91-20-3	128	2
2	Acenaphthylene	208-96-8	152	2
3	Acenaphthene	83-32-9	154	2
4	Fluorene	86-73-7	166	3
5	Anthracene	120-12-7	178	3
6	Phenanthrene	85-01-8	178	3
7	Fluoranthene	206-44-0	190	4
8	Pyrene	129-00-0	216	4
9	Benzo(a)anthracene	56-55-3	228	4
10	Chrysene	218-01-9	228	4
11	Benzo(a)pyrene	50-32-8	252	5
12	Benzo(b)fluoranthene	205-99-2	252	5
13	Benzo(k)fluoranthene	207-08-9	252	5
14	Benzo(g,h,i)perylene	191-24-2	276	6
15	Indeno(1,2,3-cd)pyrene	193-39-5	276	6
16	Dibenzo(a,h)anthracene	53-70-3	278	5

The PAH analysis was performed using a Hewlett Packard 5890/5972 gas chromatograph/mass spectrometer (GCMS) in selective-ion mode (SIM) to accommodate low sample volumes (~1 g) while maintaining low detection levels (<0.3 mg/kg) and minimizing matrix interference. The GC was operated with a 30-meter DB-5 column (0.25mm I.D., 0.25mm stationary phase) and a step-temperature program that had an initial temperature of 45 ^{o}C, increased to 130 ^{o}C at 20 ^{o}C per minute, increased from 130 ^{o}C to 250 ^{o}C at 10 ^{o}C per minute, and increased to a final temperature of 310 ^{o}C at 15 ^{o}C per minute, which was held for 10 minutes.

All samples were spiked with internal standards and surrogates for PAH analysis. Internal standards added to sample extracts after extraction and prior to injection into the GC included acenaphthene-d10, phenanthrene-d10, chrysene-d12 and perylene-d12. Nitrobenzene-d5, 2-fluorobiphenyl and *p*-terphenyl-d14 ("terphenyl") were added to the samples prior to extraction as internal surrogate standards. Of these, terphenyl is the surrogate representative of PAHs for data quality evaluation. Reagent-grade laboratory pure water was used for method and spiked blank analysis at a frequency of one blank and blank spike per 20 samples. All 16 of the PAHs listed above were added to the blank spikes at 1,000 µg/l, and blank spike recovery was determined for all blank spike samples.

Terphenyl data are reported as percent recovery (%R):

$$\text{Terphenyl (\%R)} = \frac{C_{ptp}}{C^{o}_{ptp}} \times 100 \qquad \text{(C.1)}$$

Where

C^{o}_{ptp} = initial (spiked) terphenyl concentration
C_{ptp} = final (recovered) terphenyl concentration

The PAH data are reported in mg PAH per kg sediment (mg/kg) on a dry-weight basis using percent solids data determined by ASTM Method 4643-93. TPAH data represent the sum of the individual PAH concentrations determined using the analytical method identified above.

Statistical analyses of the study data were performed using StatTools software (Professional Edition, version 1.0.1; Palisade Corporation © 2003). Data are reported as summary statistics, including the arithmetic mean plus/minus (±) the standard deviation of the mean. One-way analysis of variance (ANOVA) and Tukey confidence interval tests were performed for comparison of sample means at both depth intervals within treatments, with P-values and F-ratios calculated per treatment using a statistical significance level of 95%. Statistical significance was evaluated between treatments at corresponding sample depths using two-tailed t-tests; the resulting summary statistics are reported at either the 95% or 99% significance level. For time-series trends and linear regression analysis of the data in this study, a regression coefficient (R^2) of around 0.8 or greater is considered significant.

C.4 Results

According to USEPA Method 8270B (USEPA, 1996), PAH analytical results can vary over a fairly wide range and still be viewed acceptable. As an example the following ranges for PAH recovery from are Multi-Laboratory Performance Data listed in USEPA Method 8270B (USEPA, 1996):

	PAH	Percent Recovery (%R)
1	Naphthalene	21 – 133
2	Acenaphthylene	33 – 145
3	Acenaphthene	47 – 145
4	Fluorene	59 – 121
5	Anthracene	27 – 133
6	Phenanthrene	54 –120
7	Fluoranthene	26 –137
8	Pyrene	52 – 115
9	Benzo(a)anthracene	33 –143
10	Chrysene	17 – 168
11	Benzo(a)pyrene	17 –163
12	Benzo(b)fluoranthene	24 – 159
13	Benzo(k)fluoranthene	11 – 162
14	Benzo(g,h,i)perylene	D – 219
15	Indeno(1,2,3-cd)pyrene	D – 171
16	Dibenzo(a,h)anthracene	D – 227

D = detected

Surrogate recovery data can also vary over a range of percent recovery. Lee and Yi (1999) reported a "recovery ratio" ranging from 60% to 140% for recovery of the surrogate decachlorobiphenyl for analysis of PAHs in sediments. For the Greenhouse Study PAH data, the surrogate control (acceptance) limits for terphenyl-d14 were established at 18% to 137% (percent recovery). These control limits were developed according to Method 8270B, Section 8.5 (USEPA, 1996) and USEPA Method 8000, Section 8.6 (USEPA, 1996). This is consistent with the USEPA's precision and bias values for terphenyl-d14, which are given in Method 8270B (USEPA, 1996) as 78.6±32.4% (percent mean recovery ± SD). The terphenyl surrogate recoveries for the Greenhouse Study data set were 82.9±27.5% (percent mean recovery ± SD), ranging from 30% to 136%.

Although internal surrogate recoveries were within the established acceptability limits, it is apparent from the sample summary data plotted vs. time that the variability in the data for TPAH (Figure C.1) and some of the individual PAHs (Figure C.2, C.3, and C.4) follow a pattern similar to the surrogate recovery data plotted vs. time (Figure C.5, C.6 and C.7).

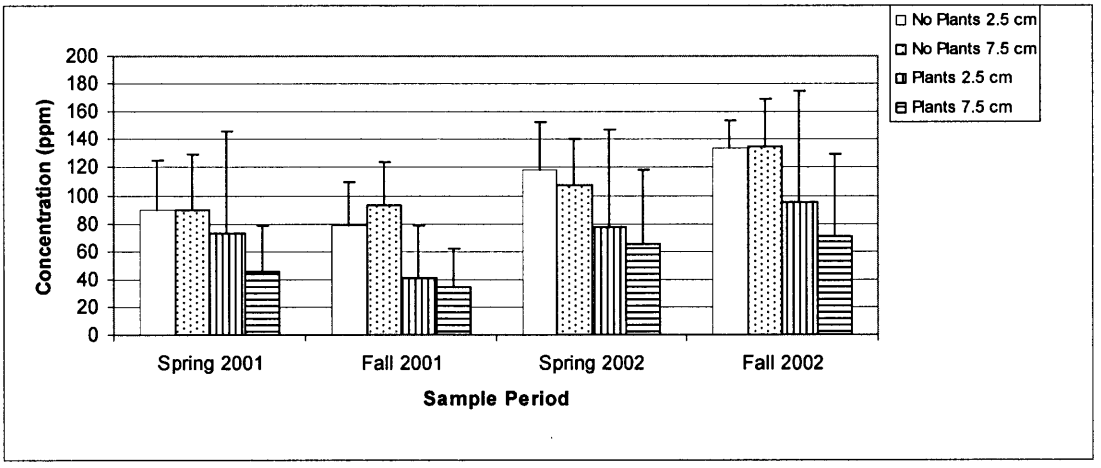


Figure C.1 Mean total polycyclic aromatic hydrocarbon concentrations in planted and bulk sediments at two depths (uncorrected).

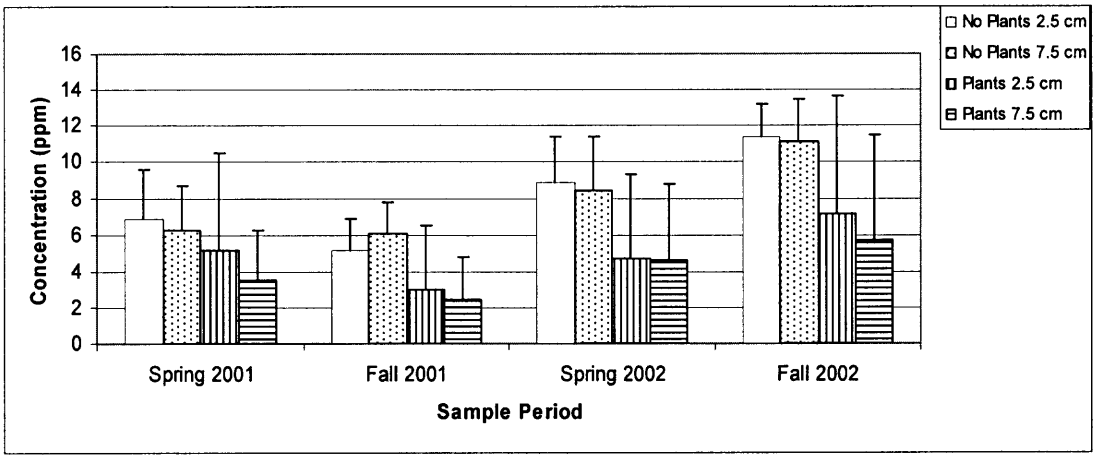


Figure C.2 Mean benzo(a)anthracene concentrations in planted and bulk sediments at two depths (uncorrected).

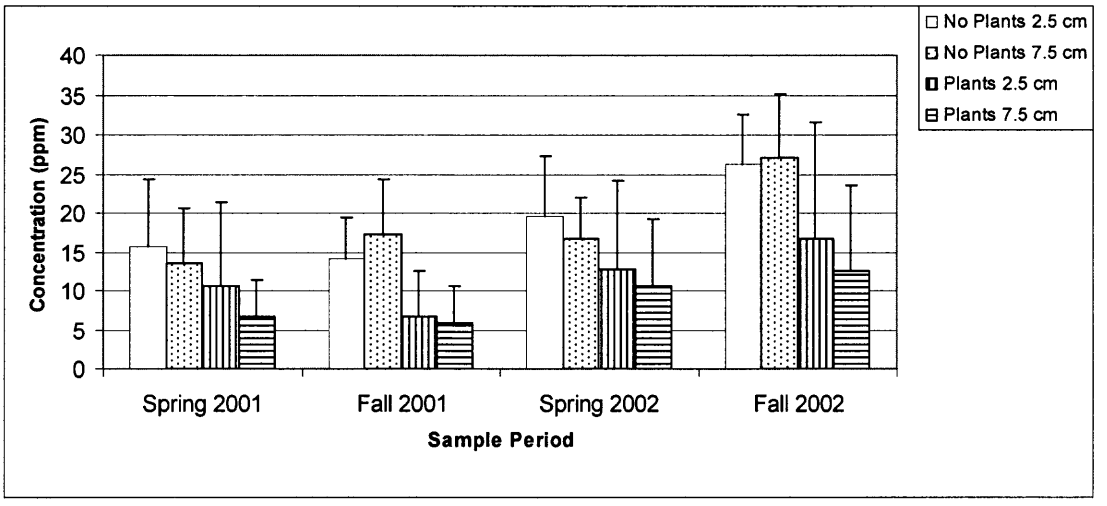


Figure C.3 Mean chrysene concentrations in planted and bulk sediments at two depths (uncorrected).

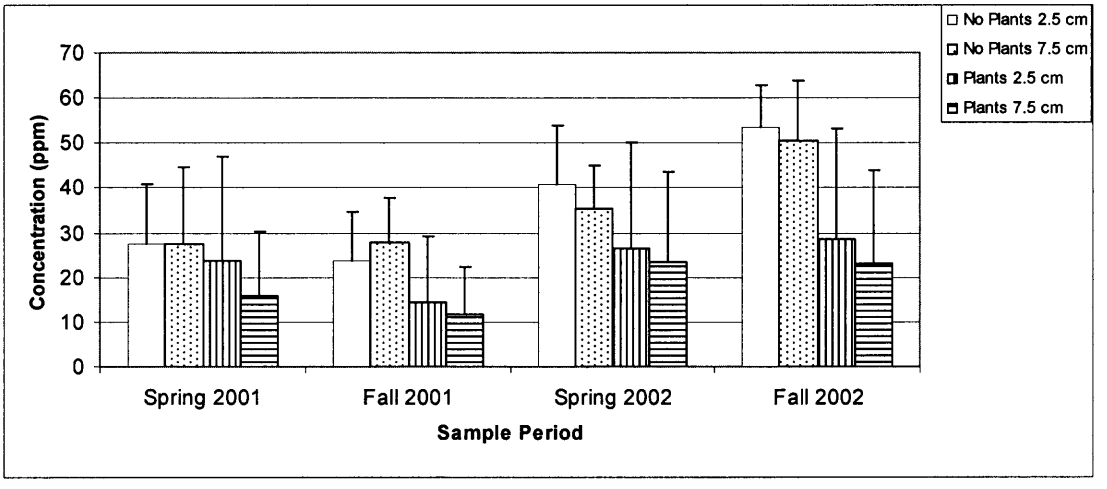


Figure C.4 Mean pyrene concentrations in planted and bulk sediments at two depths (uncorrected).

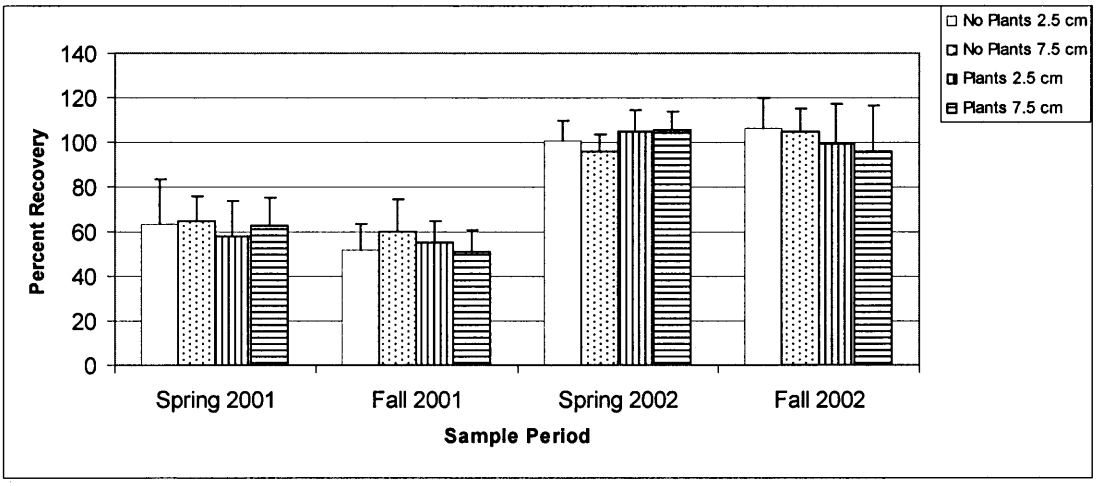


Figure C.5 Mean percent recovery (%R) of internal surrogate terphenyl-d14 in planted and bulk sediments at two depths applicable to benzo(a)anthracene.

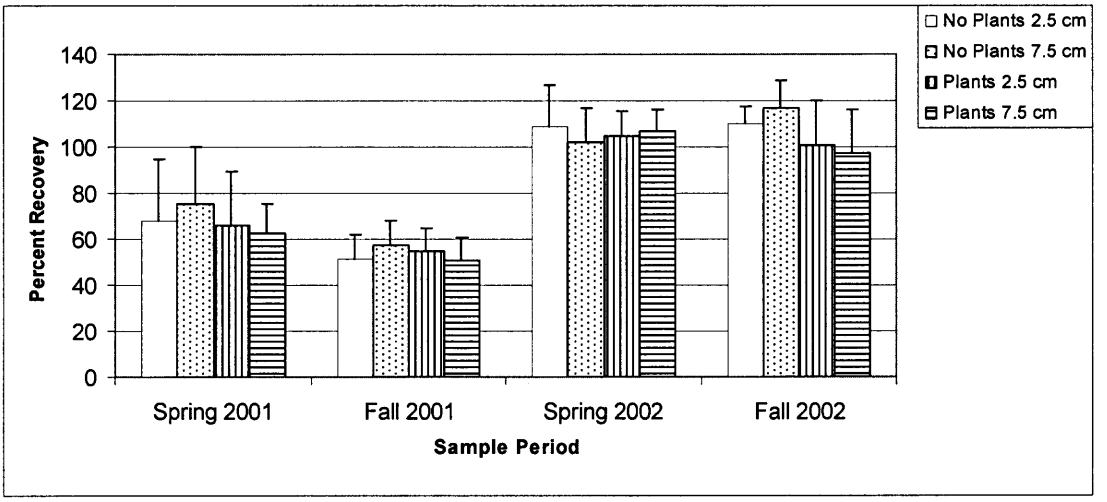


Figure C.6 Mean percent recovery (%R) of internal surrogate terphenyl-d14 in planted and bulk sediments at two depths applicable to chrysene.

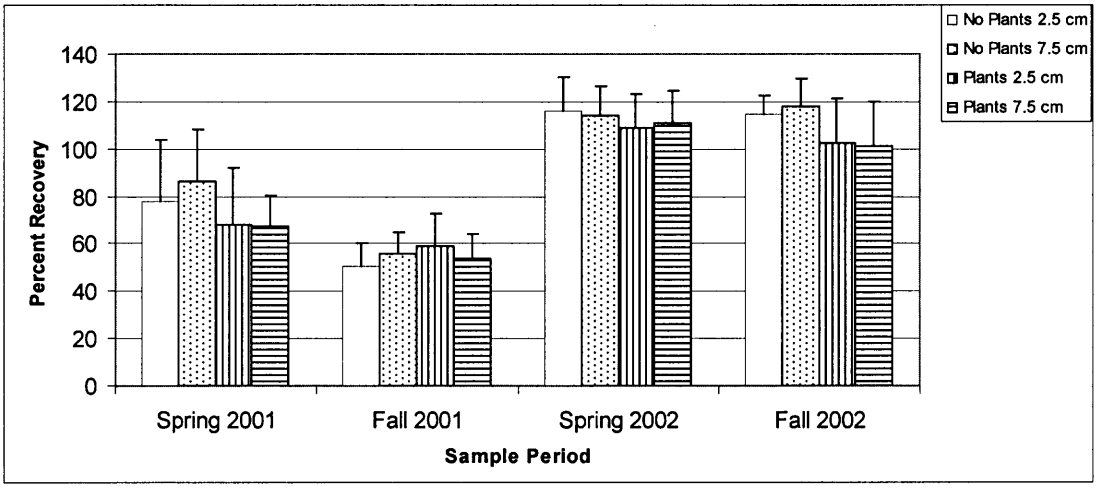


Figure C.7 Mean percent recovery (%R) of internal surrogate terphenyl-d14 in planted and bulk sediments at two depths applicable to pyrene.

Blank spike recovery data are virtually absent from the literature referenced in this paper. However, the blank spike recoveries for the Greenhouse Study PAH data are similar to terphenyl (Figure C.8) and demonstrate a reasonably good correlation ($R^2 = 0.7825$). Thus, the surrogate recovery appears to be controlled by instrument detector response, rather than any matrix interference.

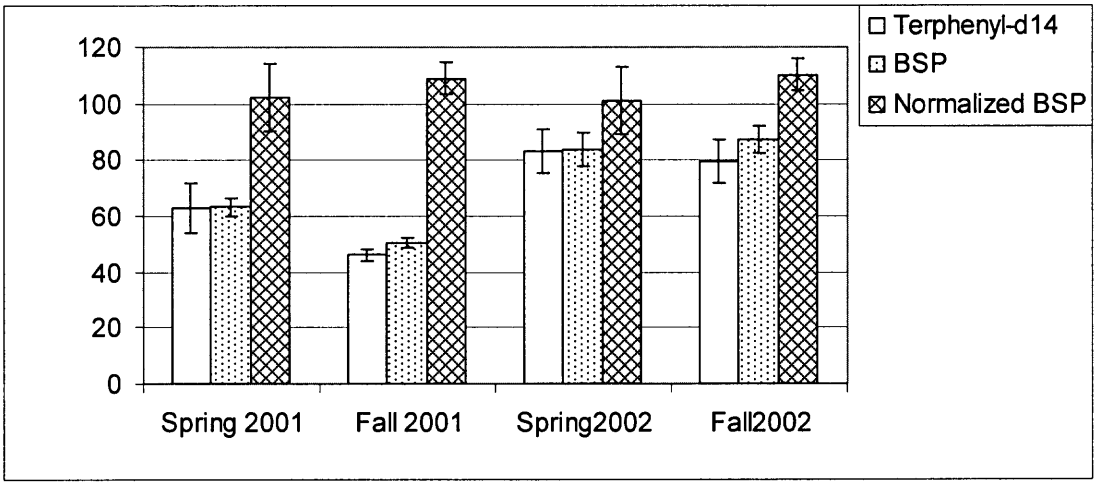


Figure C.8 Summary of blank spike internal standards (benzo(a)anthracene, chrysene and pyrene) and surrogate (terphenyl-d14) percent recovery.

The significant implication of the surrogate and blank spike recovery data is that variability in the PAH data vs. time appear to be due largely to artifacts of the laboratory analysis, of which the surrogate and blank spike recovery are indicators. Therefore, correction of the data is warranted to reduce the errors in the data and decrease uncertainty in data interpretation and use.

The following criteria were set for surrogate correction of the Greenhouse Study PAH data, which incorporate the concerns raised by Swartz et al. (2003):

(1) Similarity of surrogate and PAH
(2) Comparison of Data Profile
(3) Blank Spike Recovery
(4) Surrogate Correction Algorithm

Similarity of Surrogate and Selected PAH Compounds

The chemical formula of the PAH surrogate (*p*-terphenyl-d14) is $C_{18}D_{14}$ and the MW is 244 (g/mole). The RT for elution form the GC column was approximately 15.3 minutes. The PAHs that appear to be most similar to terphenyl in MW and RT are pyrene ($C_{16}H_{10}$; MW=216; RT=14.9), benzo(a)anthracene ($C_{18}H_{12}$; MW=228; RT=18.3), and chrysene ($C_{18}H_{12}$; MW=228; RT=18.4). The PAHs with MW outside this range of MWs and RTs were not included because their MW and RT were too far under or over those of the surrogate, and their data did not match the profile described below.

Comparison of Data Profile

A simple comparison of the PAH data, surrogate recovery data and blank spike recovery data plotted vs. time (Figures C.1 through C.7) indicate a similar pattern for the four sample events. The pattern can be described as the percentage of the maximum surrogate and BSP recovery, and the percentage of the maximum concentration for PAH data. The percentage of the maximum recovery for terphenyl-d14 and the BSP, and the average PAH percent of maximum concentrations (for pyrene, benzo(a)anthracene and chrysene only), are presented on Figure C.9. For terphenyl-d14, the percent of the maximum equals 76%, 56%, 100% and 96% for the Spring 2001, Fall 2001, Spring 2002 and Fall 2002 sample periods, respectively. A similar pattern is also present in the blank spike recoveries and in the PAH data for pyrene, benzo(a)anthracene and chrysene (treatments with and without plants combined; Figure C.9). In this report, the pattern being described is referred to as a "surrogate profile" for the laboratory artifact (or bias), which is related to the instrument response in this data set. Only the individual PAH data that exhibited the surrogate profile were corrected, i.e., only the data for pyrene, benzo(a)anthracene and chrysene.

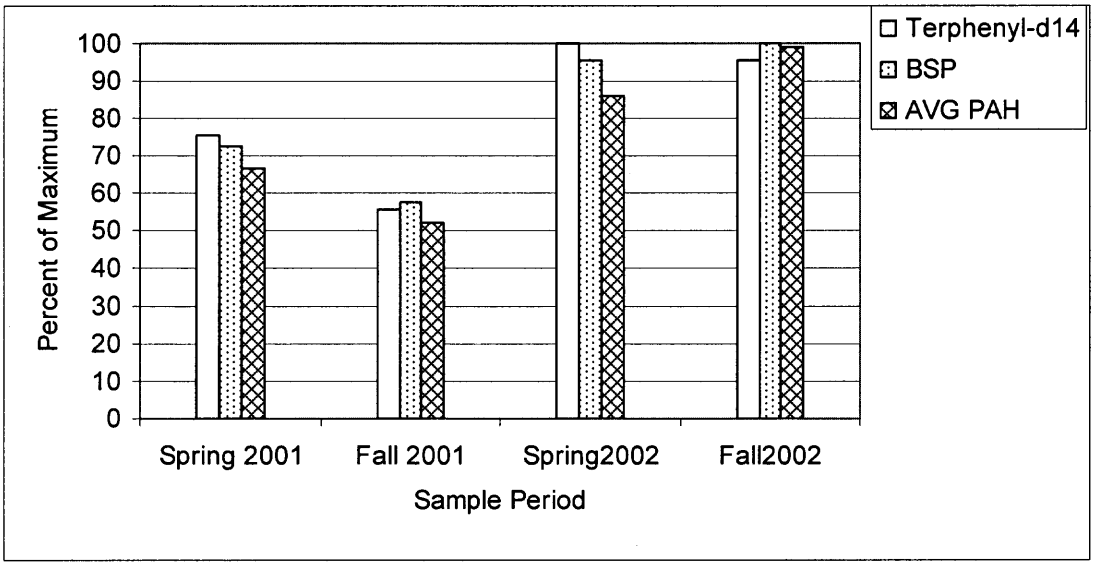


Figure C.9 Percent of maximum surrogate recovery, blank spike recovery and mean PAH data [average of benzo(a)anthracene, chrysene and pyrene] from four sample periods.

Blank Spike Recovery

The blank spike recovery data for the Greenhouse Study pyrene, benzo(a)anthracene and chrysene data are presented in Table C.1. These data demonstrate the similarity between the surrogate recovery data and the data from the selected PAHs. Correction of the blank spike recovery data using the surrogate correction algorithm described below indicates the data are corrected to 100±10 % R using this method (Figure C.8). The blank spike data also provides evidence to support the idea that the variability in surrogate recoveries is driven by instrument response, rather than matrix interference, which supports the need for correction of the data for the three PAHs identified above.

Table C.1 Blank Internal Standard Recovery for Benzo(A)Anthracene, Chrysene and Pyrene Sediment Data and Corrected Internal Standard Recovery using Percent Recovery of Internal Surrogate (Terphenyl-D14)

Accutest Job No.	Sample Period	Replicates	PAH Compound	BSP (%)	Corrected BSP (%)
E88702	Spring 2001	1 - 10	Benzo(a)anthracene	**115**	**183**
			Chrysene	**118**	**187**
			Pyrene	**120**	**190**
			p-terphenyl-d14	63	100
E88813	Spring 2001	11 - 20	Benzo(a)anthracene	66	92
			Chrysene	66	92
			Pyrene	66	92
			p-terphenyl-d14	72	100
E88881	Spring 2001	21 - 30	Benzo(a)anthracene	63	117
			Chrysene	57	106
			Pyrene	62	115
			p-terphenyl-d14	54	100
N457	Fall 2001	1 - 10	Benzo(a)anthracene	51	106
			Chrysene	54	113
			Pyrene	51	106
			p-terphenyl-d14	48	100
N553	Fall 2001	11 - 20	Benzo(a)anthracene	49	104
			Chrysene	50	106
			Pyrene	48	102
			p-terphenyl-d14	47	100
N645	Fall 2001	21 - 30	Benzo(a)anthracene	52	118
			Chrysene	48	109
			Pyrene	51	116
			p-terphenyl-d14	44	100
N9651	Spring 2002	1 - 10	Benzo(a)anthracene	82	109
			Chrysene	89	119
			Pyrene	88	117
			p-terphenyl-d14	75	100
N9736	Spring 2002	11 - 20	Benzo(a)anthracene	74	87
			Chrysene	82	96
			Pyrene	77	91
			p-terphenyl-d14	85	100
N9885	Spring 2002	21 - 30	Benzo(a)anthracene	94	104
			Chrysene	83	92
			Pyrene	84	93
			p-terphenyl-d14	90	100
N21418	Fall 2002	1 - 10	Benzo(a)anthracene	79	108
			Chrysene	86	118
			Pyrene	85	116
			p-terphenyl-d14	73	100
N21515	Fall 2002	11 - 20	Benzo(a)anthracene	89	114
			Chrysene	84	108
			Pyrene	88	113
			p-terphenyl-d14	78	100
N21650	Fall 2002	21 - 30	Benzo(a)anthracene	89	101
			Chrysene	92	105
			Pyrene	95	108
			p-terphenyl-d14	88	100

BSP = Blank spike percent recovery
Bold data are anomalies and are not included in totals.

Surrogate Correction Algorithm

PAH data were corrected based on terphenyl surrogate recovery data as a measure of instrument response using a method adapted (Swartz et al., 2003) as follows:

$$C = \frac{A_x \cdot RF_x}{V} \cdot \frac{C^o_{ptp}}{\frac{A_{ptp} \cdot RF_{ptp}}{V_{ptp}}} \quad \text{(C.2)}$$

Where

C = final corrected concentration (µg/ml)
C^o = initial concentration (µg/ml)
A = the area count from the GCMS
RF = response factor (µg/area counts)
V = volume of the solution (mL)
x = individual PAH
ptp = p-terphenyl-$_{14}$

Rearranging equation (C.2) yields:

$$C = \frac{C^o_x}{\frac{C_{ptp}}{C^o_{ptp}}} \quad \text{(C.3)}$$

The denominator in equation 2 is equal to percent recovery (as decimal percent) of the surrogate (terphenyl-d14) determined for all of the PAH data with each sediment sample analysis. Therefore, the PAH data for selected compounds (described below) were corrected for surrogate recovery using:

$$C = \frac{C^o_x}{CF_{ptp}} \quad \text{(C.4)}$$

Where

CF_{ptp} = correction factor in decimal percent recovery for p-terphenyl-d14

Table C.2 Correction Factors for selected PAH Data based on Percent Recovery* of Internal Surrogate (Terphenyl-D14)

	Correction Factors for benzo(a)anthracene				Correction Factors for chrysene				Correction Factors (Pyrene)			
Replicate	Spg 2001	Fall 2001	Spg 2002	Fall 2002	Spg 2001	Fall 2001	Spg 2002	Fall 2002	Spg 2001	Fall 2001	Spg 2002	Fall 2002
C-1(A)	0.64	0.56	1.21	1.04	1.04	0.56	1.30	1.10	1.04	0.55	1.30	1.10
C-2(A)	0.44	0.58	1.00	1.12	0.44	0.58	1.00	1.12	0.44	0.52	1.00	1.10
C-3(A)	0.50	0.75	1.08	0.93	0.50	0.70	1.35	1.05	0.90	0.70	1.35	1.05
C-4(A)	0.74	0.42	1.03	1.11	0.74	0.42	1.03	1.11	0.72	0.42	0.99	1.34
C-5(A)	0.83	0.61	0.97	1.27	1.10	0.61	0.97	1.15	1.10	0.52	1.25	1.15
C-6(A)	0.89	0.52	0.98	0.92	0.64	0.52	1.35	0.92	0.64	0.52	1.35	1.15
C-7(A)	0.49	0.54	0.95	0.92	0.49	0.54	0.95	1.15	0.96	0.56	1.15	1.15
C-8(A)	0.59	0.45	0.96	1.09	0.59	0.45	0.96	1.10	0.70	0.50	1.08	1.10
C-9(A)	0.90	0.36	1.00	0.96	0.95	0.36	1.11	1.10	0.95	0.36	1.11	1.10
C-10(A)	0.31	0.41	0.87	1.28	0.31	0.41	0.87	1.20	0.31	0.41	1.05	1.20
V-11(A)	0.62	0.66	1.13	0.79	0.62	0.66	1.13	0.79	0.82	0.88	1.20	1.05
V-12(A)	0.55	0.33	1.03	0.99	0.55	0.33	1.03	0.99	0.55	0.33	1.03	0.99
V-13(A)	0.87	0.46	0.88	0.96	0.87	0.46	0.88	0.96	0.87	0.46	0.88	0.96
V-14(A)	0.54	0.45	0.86	0.89	0.54	0.45	0.86	0.89	0.54	0.45	0.86	0.89
V-15(A)	0.65	0.57	0.98	0.59	0.65	0.57	0.98	0.59	0.65	0.57	0.98	0.59
V-16(A)	0.78	0.64	1.21	1.13	1.08	0.64	1.30	1.02	1.08	0.87	1.30	1.02
V-17(A)	0.70	0.46	1.12	0.99	1.16	0.46	1.12	1.30	1.18	0.46	1.26	1.30
V-18(A)	0.71	0.56	0.95	0.73	0.71	0.56	0.95	0.73	0.96	0.67	0.95	0.73
V-19(A)	0.51	0.39	1.03	1.18	0.51	0.39	1.03	1.18	0.51	0.49	1.22	1.25
V-20(A)	0.63	0.63	1.16	0.95	0.63	0.63	1.16	1.22	0.63	0.63	1.32	1.22
V-21(A)	0.53	0.65	1.05	1.36	0.53	0.65	1.05	0.96	0.53	0.65	1.26	0.96
V-22(A)	0.36	0.59	0.98	1.02	0.44	0.59	0.98	1.36	0.44	0.59	0.98	1.36
V-23(A)	0.38	0.57	1.18	1.16	0.55	0.57	1.11	1.00	0.55	0.69	1.11	1.00
V-24(A)	0.91	0.73	1.07	1.05	0.91	0.73	1.07	1.05	0.91	0.73	1.07	1.14
V-25(A)	0.47	0.50	0.94	0.89	0.47	0.50	0.94	0.89	0.47	0.50	0.94	0.89
V-26(A)	0.43	0.58	1.07	0.82	0.43	0.58	1.07	0.82	0.43	0.58	1.07	0.82
V-27(A)	0.36	0.58	1.09	1.22	1.05	0.58	1.09	1.22	1.05	0.57	1.12	1.13
V-28(A)	0.49	0.50	0.98	1.09	0.49	0.50	0.98	1.09	0.47	0.50	0.98	1.09
V-29(A)	0.66	0.55	1.14	1.01	0.66	0.55	1.14	1.01	0.60	0.55	1.08	1.01
V-30(A)	0.41	0.60	1.09	1.07	0.41	0.60	1.09	1.07	0.41	0.60	1.18	1.07
C-1(B)	0.71	0.78	1.06	1.09	0.71	0.72	1.25	1.00	0.80	0.72	1.25	1.00
C-2(B)	0.48	0.64	1.06	1.06	0.48	0.64	1.06	1.35	0.70	0.55	1.11	1.35
C-3(B)	0.51	0.66	0.93	1.15	0.51	0.66	0.93	1.15	0.51	0.57	1.20	1.22
C-4(B)	0.84	0.64	0.97	1.17	1.15	0.64	0.97	1.10	1.15	0.57	1.02	1.10
C-5(B)	0.71	0.88	0.98	1.10	0.71	0.68	0.98	1.10	1.05	0.68	1.20	1.15
C-6(B)	0.63	0.49	0.98	1.09	0.63	0.49	0.98	1.15	0.70	0.57	1.25	1.15
C-7(B)	0.75	0.55	0.90	1.08	0.78	0.55	1.30	1.25	0.78	0.57	1.30	1.25
C-8(B)	0.65	0.46	0.94	0.87	1.20	0.46	0.94	1.15	1.20	0.46	1.14	1.15
C-9(B)	0.61	0.45	0.82	0.96	0.61	0.45	0.82	1.35	0.94	0.45	0.99	1.35
C-10(B)	0.58	0.46	0.97	0.89	0.78	0.46	0.97	1.10	0.78	0.46	0.94	1.10
V-11(B)	0.78	0.45	0.96	1.11	0.78	0.45	0.96	1.11	0.78	0.71	0.96	1.20
V-12(B)	0.61	0.59	0.92	0.66	0.61	0.59	0.92	0.66	0.61	0.59	0.92	0.66
V-13(B)	0.60	0.51	1.20	0.93	0.80	0.51	1.20	0.93	0.80	0.51	1.26	0.93
V-14(B)	0.57	0.48	0.89	0.73	0.57	0.48	0.89	0.73	0.57	0.48	0.89	0.73
V-15(B)	0.66	0.61	0.94	0.63	0.66	0.61	0.94	0.63	0.66	0.61	0.94	0.63
V-16(B)	0.87	0.51	1.04	0.81	0.87	0.51	1.04	0.81	0.87	0.51	1.35	1.20
V-17(B)	0.80	0.30	1.21	1.18	0.80	0.30	1.21	1.18	0.75	0.30	1.34	1.25
V-18(B)	0.75	0.42	1.06	1.09	0.75	0.42	1.06	1.20	0.75	0.42	1.16	1.20
V-19(B)	0.65	0.34	1.13	0.75	0.65	0.34	1.13	0.75	0.65	0.38	1.29	1.19
V-20(B)	0.50	0.40	1.05	0.76	0.50	0.40	1.05	1.07	0.98	0.50	1.05	1.07
V-21(B)	0.59	0.65	1.05	1.17	0.59	0.65	1.05	1.17	0.59	0.65	1.17	1.17
V-22(B)	0.57	0.53	1.02	0.98	0.57	0.53	1.02	0.98	0.57	0.53	1.02	0.98
V-23(B)	0.46	0.49	1.16	1.34	0.46	0.49	1.14	1.15	0.48	0.49	1.14	1.15
V-24(B)	0.62	0.52	1.13	1.02	0.62	0.52	1.13	1.02	0.62	0.52	1.13	1.02
V-25(B)	0.50	0.57	1.05	1.13	0.50	0.57	1.05	1.13	0.50	0.57	1.05	1.05
V-26(B)	0.56	0.63	1.00	0.90	0.56	0.63	1.00	0.90	0.56	0.63	1.00	0.90
V-27(B)	0.42	0.49	1.12	1.26	0.42	0.49	1.12	1.26	0.63	0.67	1.14	1.10
V-28(B)	0.74	0.63	1.03	0.97	0.74	0.63	1.03	0.97	0.74	0.63	1.03	0.97
V-29(B)	0.55	0.48	1.01	0.81	0.55	0.48	1.26	0.81	0.79	0.48	1.26	0.81
V-30(B)	0.60	0.57	1.07	0.99	0.60	0.57	1.07	0.99	0.60	0.57	1.07	0.99

* Surrogate recovery shown as decimal percent

The CF's used for surrogate correction of the Greenhouse Study data are presented in Table C.2. Summary statistics of the uncorrected and corrected analytical results for TPAH, pyrene, benzo(a)anthracene and chrysene in study sediment samples are presented in Tables C.3 and C.4.

Table C.3 Summary Statistics of Uncorrected TPAHs, Pyrene, Benzo(a)anthracene and Chrysene Data in Planted and Bulk Sediments

COMPOUND	Treatment	Depth	Spring 2001		Fall 2001		Spring 2002		Fall 2002		ANOVA	
			Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	p-Value	F-ratio
TPAH	No Plants	2.5 cm	89.8	34.9	78.2	31.6	118.2	34.4	**133.5**	20.2		
		7.5 cm	89.8	40.0	92.9	31.0	107.5	33.2	**135.3**	34.1	0.0005	4.29
	Plants	2.5 cm	73.3	72.1	40.1 *	38.0	77.0 *	70.0	**94.5**	80.0		
		7.5 cm	45.8 **	32.6	34.3 **	27.1	65.7 *	52.8	71.2 **	58.3	0.0138	2.63
Pyrene	No Plants	2.5 cm	27.59	13.20	23.79	11.00	40.79	13.09	**53.43**	9.38		
MW = 216		7.5 cm	27.58	16.88	27.77	9.99	35.44	9.47	**50.55**	13.42	0.0000	8.5
Rings = 4	Plants	2.5 cm	23.53	23.58	14.38	14.89	26.44	23.72	28.46 **	24.80		
		7.5 cm	15.66	14.69	11.72 **	10.43	23.43 *	20.05	23.00 **	21.05	0.0731	1.9
Benzo(a)anthracene	No Plants	2.5 cm	6.85	2.75	5.18	1.71	8.86	2.57	**11.37**	1.83		
MW = 228		7.5 cm	6.25	2.39	6.08	1.65	8.40	3.03	11.14	2.34	0.0000	10.0
Rings = 4	Plants	2.5 cm	5.18	5.30	3.00 *	3.54	4.69 *	4.63	7.10 *	6.57		
		7.5 cm	3.52 *	2.71	2.44 **	2.35	4.59 *	4.18	5.65 **	5.82	0.0406	2.2
Chrysene	No Plants	2.5 cm	15.73	8.66	14.11	5.21	19.65	7.61	**26.30**	6.34		
MW = 228		7.5 cm	13.46	7.07	17.20	7.20	16.59	5.29	**26.99**	8.02	0.0000	5.5
Rings = 4	Plants	2.5 cm	10.57	10.78	6.63 **	5.85	12.84	11.27	**16.69** *	14.86		
		7.5 cm	6.66 **	4.70	5.84 **	4.66	10.63	8.50	12.49 **	11.12	0.0048	3.1

Notes: (1) Sample mean and standard deviation (SD) data are in mg/Kg, dry weight basis.
(2) For "No Plants" treatments, n=10; F-critical=2.08
(3) For "Plants" treatments, n=20; F-critical=2.14
(4) **Bold** mean values are significant at $p<0.05$ within treatments based on one-way ANOVA and Tukey confidence interval analysis.
(5) **Shaded** mean values indicate significance at $p<0.05$ between depths within treatments.
(6) Single asterisk (*) indicates significant difference of means at $p<0.05$ between treatments at similar depths (t-test).
(7) Double asterisk (**) indicates significant difference of means at $p<0.01$ between treatments at similar depths (t-test).

Table C.4 Summary Statistics of Uncorrected TPAHs, Pyrene, Benzo(a)anthracene and Chrysene Data in Planted and Bulk Sediments

COMPOUND	Treatment	Depth	Spring 2001		Fall 2001		Spring 2002		Fall 2002		ANOVA	
			Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	p-Value	F-ratio
TPAH +	No Plants	2.5 cm	108.5	23.3	117.7	35.3	109.7	26.4	124.3	21.0		
		7.5 cm	99.5	32.3	130.7	33.6	102.7	27.5	124.1	32.3	0.1938	1.46
	Plants	2.5 cm	96.2	102.8	57.0 **	50.7	71.6 *	60.9	91.3	76.2		
		7.5 cm	59.6 *	44.7	56.2 **	47.4	60.5 *	47.2	68.4 **	53.3	0.2975	1.22
Pyrene + MW = 216 Rings = 4	No Plants	2.5 cm	34.68	6.37	45.64	15.04	34.44	8.14	47.11	9.49		
		7.5 cm	**30.52**	12.45	48.76	11.78	**30.76**	5.61	43.37	12.85	<0.0001	5.0
	Plants	2.5 cm	35.54	40.84	22.70 **	21.99	22.75 *	18.15	26.36 **	22.66		
		7.5 cm	22.57	20.39	23.13 **	21.42	19.66 **	15.79	20.67 **	17.09	0.5046	0.9
Benzo(a)anthracene + MW = 228 Rings = 4	No Plants	2.5 cm	10.92	2.75	9.91	2.17	8.74	2.07	10.86	2.38		
		7.5 cm	9.49	2.39	10.29	2.22	8.79	3.41	10.76	6.18	0.3070	1.2
	Plants	2.5 cm	10.72	13.81	5.34 **	5.92	4.24 **	3.83	6.86 **	6.01		
		7.5 cm	5.98 *	5.52	5.54 **	5.77	4.23 **	3.86	5.57 **	5.36	0.0935	1.8
Chrysene + MW = 228 Rings = 4	No Plants	2.5 cm	23.23	6.10	27.02	6.06	**17.62**	4.52	23.88	5.47		
		7.5 cm	**15.64**	5.46	29.78	9.31	**16.03**	2.92	23.28	7.30	<0.0001	6.6
	Plants	2.5 cm	15.91	16.95	12.03 **	10.51	11.59 *	8.76	15.78 *	13.77		
		7.5 cm	11.10	8.84	13.23 **	12.33	9.59 **	7.44	12.12 **	9.88	0.6313	0.8

Notes: (1) Sample mean and standard deviation (SD) data are in mg/Kg, dry weight basis.
(2) For "No Plants" treatments, n=10; F-critical=2.08
(3) For "Plants" treatments, n=20; F-critical=2.14
(4) **Bold** mean values are significant at $p<0.05$ within treatments based on one-way ANOVA and Tukey confidence interval analysis.
(5) Shaded mean values indicate significance at $p<0.05$ between depths within treatments.
(6) Single asterisk (*) indicates significant difference of means at $p<0.05$ between treatments at similar depths (t-test).
(7) Double asterisk (**) indicates significant difference of means at $p<0.01$ between treatments at similar depths (t-test).
(8) Plus (+) indicates data for PAH compound is corrected for internal surrogate percent recovery (terphenyl-d14).

C.4 Discussion

The uncorrected results indicated that the PAH concentrations increased during the Greenhouse Study (Figures C.1 through C.4, and Table C.3), which is obviously incorrect, since no PAHs were added to any treatments during the study period. From the sediment sample PAH analytical results, pyrene and chrysene represent approximately 75% of the TPAH in the sediment samples (pyrene ≅ 40%; chrysene ≅ 25%; benzo(a)anthracene ≅ 10%), with the other 11 PAHs detected each accounting for less than 20% of the TPAH. Therefore, correction of pyrene, benzo(a)anthracene and chrysene data has significant influence on the TPAH data.

Statistical significance within treatments was identified in the last sampling period of the uncorrected data (i.e., Fall 2002; Table C.3) in all treatments with the exception of pyrene in the planted sediments (ANOVA P=0.0731). After surrogate correction (Table C.4), statistical significance within treatments was limited to only the bulk sediments (i.e., No Plants) for pyrene and chrysene. In addition, the significance based on Tukey's confidence interval test (ANOVA), was distributed to earlier sampling events, rather than indicating the highest concentrations at the end of the study period. The significance in the bulk sediment treatments are due to a few low average concentrations that occur either at the beginning of the study (e.g., pyrene and chrysene, Spring 2001) or in the third sampling round (e.g., pyrene and chrysene, Spring 2002). These are all slightly lower than the overall average for their respective treatments and do not represent degradation, due to the presence of higher concentrations in later replicate samples at both depths. Based on these data and statistical analyses, the average concentration of TPAH, pyrene, benzo(a)anthracene

and chrysene did not change for the duration of the 18-month study period within either the planted sediment or bulk sediment treatments, and the mean concentrations of these compounds were not significantly different between the sample depths of 2.5 and 7.5 cm within treatments.

The surrogate correction had little effect on comparisons of TPAH, pyrene, benzo(a)anthracene and chrysene concentrations between bulk sediment and planted sediments using two-sample t-tests. The average concentrations of these parameters in the planted sediment at both depths for all four sampling events are lower than their average in the bulk sediments, with one exception (pyrene at 2.5 cm, Spring 2001). These differences are between the bulk and planted sediments at both sample depths are statistically significant in all of the last three sampling events ($P<0.05$).

Based on the results of the statistical analysis (Tables C.3 and C.4), the surrogate correction of the data for these three PAHs do not change the overall study findings. But making the correction decreased the uncertainties of the evaluation by providing an explanation for the apparent increase in PAH concentrations during the study, and by allowing identification and reduction of laboratory error in the data set.

Typical sample handling and analyses performed for academic research is completed under controlled conditions, and may therefore be costly and infeasible for a large-scale field application. As shown in this study, state-of-art instrumentation and USEPA-approved analytical procedures are still subject to limitations from sources of error associated with natural heterogeneity of soil and sediment, matrix interference and varying surrogate recoveries, especially in samples complicated with elevated contaminant concentrations. Data from severely contaminated sites are

likely to suffer these analytical limitations. This is a relatively common analytical problem for environmental samples that contain high concentrations of PHC. The conventional approach to address heterogeneity and data quality concerns may be to increase the sample number and validate all data. However, the collection of a large number of samples and state-of-art analyses performed in compliance with current EPA methods can be costly, with no benefit to data usability for evaluating biodegradation in sediments and soil. Some of these costs and limitations may be avoided by developing a sampling plan that:

(1) Incorporates a statistical design to optimize the number of data points required;

(2) Establishes project-specific matrix spike and surrogate recovery acceptance limits; and

(3) Anticipates data evaluation for laboratory bias and surrogate correction as part of the data evaluation process.

C.5 Conclusions

The use of PAH surrogate correction allows the differences in the planted vs. the bulk sediment concentrations to be compared while limiting the effect of the laboratory bias long-term variability in instrument response. It is assumed here that the analytical bias (including variable surrogate recovery) is reduced when comparison is made using the data generated in the same time period, i.e., sampling event. This assumption is supported by the consistent pattern of surrogate recovery data that is apparent over time, described in this paper as the surrogate profile. However, surrogate correction should only be applied after careful review of the data to assure that the surrogate recovery is comparable to the specific compounds being corrected.

In addition, the surrogate correction criteria and algorithm should be documented.

While the PAH surrogate recoveries for the PAH data were within the acceptability range for Method 8270 (18% to 137% for terphenyl-d14), they represent a laboratory bias that influenced the data evaluation in this study. Although PAH data generated by routine laboratory analysis by USEPA methods may be considered usable, specification of a tighter range of surrogate recovery acceptance limits and including surrogates selected based on target compounds in future analyses, and careful application of appropriate correction for variable surrogate recovery can reduce data uncertainty and improve end-user data evaluation.

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