ABSTRACT

GREGORY III, SAMUEL THORNE. Differences in Sediment Organic Matter Composition and PAH Weathering Between Non-Vegetated and Recently Vegetated Fuel-Oiled Sediments. (Under the direction of Elizabeth Guthrie Nichols and Damian Shea).

Previous results have shown a strong correlation between vegetation and dissipation of polycyclic aromatic hydrocarbon (PAH) contamination over long time frames. Evidence of phytoremediation over shorter time frames is difficult to detect, particularly in sites where overall PAH concentrations have not begun to significantly decrease. This study assessed the impact of vegetation on a highly contaminated sediment over a short time frame in order to track the effects of plant derived organic carbon (PdOC) in a variety of sediment compartments. The Indiana Harbor Canal is an industrialized area with historic petroleum contamination of near-shore sediments. In 2002, near-shore sediments were planted with hybrid poplar and willow trees as part of a phytoremediation treatability study. Site disturbance allowed for colonization by cattails and *Phragmites*. In 2004, non-vegetated and vegetated oiled-shore sediments were collected and analyzed for 42 polycyclic aromatic hydrocarbons (PAHs), total organic carbon, and percent modern carbon ($\Delta^{14}\text{C}$ radiocarbon). Sediments were collected from several locations, field-composited, homogenized, sieved, and chemically fractionated. There is a significant input of modern carbon in both labile and recalcitrant fractions of vegetated sediments that is absent in the non-vegetated control. *Phragmites* sediments contained more modern carbon (plant carbon) and more weathered PAH ratios than willow, cattail, and non-vegetated sediments. Organic carbon-normalized PAH concentrations were greater in *Phragmites* humin than other sediments. Humin fractions of willow trees were more weathered than non-vegetated

humin, but modern carbon values were not always different. Cattail humin contained more modern carbon than non-vegetated humin, but only de-ashed humin was more weathered than non-vegetated de-ashed humin. Results show that sediment composition and PAH weathering indices are not indicative of reduced PAH concentrations. Instead, PAH weathering appears related to increased modern carbon (plant carbon) content. Substantial increases in modern carbon content were observed after two years of plant growth. These results suggest that PdOC is diffusing into a variety of sediment compartments where it may be enhancing carbon cycling and beginning to mitigate PAH concentrations.

Differences in Sediment Organic Matter Composition and PAH Weathering Between Non-Vegetated and Recently Vegetated Fuel-Oiled Sediments

By
Samuel Thorne Gregory III

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APPROVED BY:

Dr. Elizabeth Guthrie Nichols
Co-Chair of Advisory Committee

Dr. Damian Shea
Co-Chair of Advisory Committee

Dr. Chris S. Hofelt

BIOGRAPHY

Samuel Thorne Gregory III. (or just Thorne) was born on July 17, 1980 in Wilson N.C. Being born a little early, he ended up a Cancer instead of a Virgo as was expected. His first 12 years were spent in the town of Dunn, N.C., known more for its physical location (at the intersection of 421 and I-95) than any special characteristics about the place. After spending many hours in the Dunn public library, Thorne moved to New Jersey where he received all but one year of his mandatory education before moving back to Sanford N.C. for his final year of high school. Thorne then enrolled at the University of North Carolina, like his parent and grandparents before him. He graduated in 2002 with a B.S. in chemistry but decided that the synthetic inorganic research he had been pursuing was not what he wanted to do for the rest of his life. In fact, it was the warning labels on the reagents that caught his attention, so he enrolled in the graduate program in Toxicology at NCSU. Thorne is an avid biker, runner, and a voracious reader. His previous jobs include reptile breeder, disk jockey for both FM and AM radio stations, computer repair technician, and teaching assistant. When asked what it was like to wrangle cold blooded creatures with brains the size of a pea, or about the monotony of hearing the same thing day in and day out....his teaching assistant experience is what comes to mind.

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CHAPTER 1. LITERATURE REVIEW

1.1 Polycyclic Aromatic Hydrocarbons. Risks and Accessibility

Polycyclic Aromatic Hydrocarbons (PAHs) are persistent organic contaminants with a variety of acute, chronic, mutagenic, and carcinogenic effects (Durant *et al.*, 1996, 1999, Cavalieri *et al.*, 1988). PAHs are byproducts of both incomplete combustion processes, forming pyrogenic PAHs, and catagenesis reactions, creating petrogenic PAHs (Fernandes *et al.*, 2003; Tissot *et al.*, 1978). High concentrations of petrogenic PAHs are often found in residual soils and sediments at sites of historical petroleum transport and refining. The contaminant levels at many sites often exceed environmental regulations and can encompass large areas making them difficult to remediate with standard technologies. Alternative treatment options at PAH contaminated sites that are less invasive and expensive are of great interest to a variety of regulatory groups and private industries. One such treatment option is phytoremediation, or the use of vegetation to mitigate the environmental impacts and risks of soil and water contamination. A variety of studies have investigated plants and their associated microbial communities for the *in situ* treatment of organic contaminants, such as PAHs, in soils and sediments (Widdowson *et al.*, 2005; Davis *et al.*, 2002; Mueller *et al.*, 2006).

The overall risk associated with any level of sediment contamination is highly dependent on the bioavailability of the compound. The more bioavailable a contaminant, the more likely it will cause a deleterious effect on any given organism. It is the structure and lipophilicity of a given compound that determines its overall bioavailability and thereby plays a major role in the efficacy of phytoremediation of the contaminant (Binet *et al.*, 2000). More bioavailable and water soluble contaminants are generally more accessible to microorganisms and therefore more likely to be metabolized. Bioavailability also depends on the aging and

environmental weathering of the contaminant (Hatzinger *et al.*, 1995; Erickson *et al.*, 1993), and the physical properties, such as organic matter and particle size, of the sediment in question (Pignatello, 1989). As contaminants age, they preferentially diffuse into sediment pores and absorb into or are adsorbed onto sediment organic matter in matrix pores where they can then be incorporated into stable soil/sediment organic matter phases (Ehlers *et al.*, 2003).

The fraction of PAHs in sediments that are currently or potentially available to living organisms is known as the bioaccessible fraction (Semple *et al.*, 2004). This fraction differs from the “bioavailable” fraction; bioavailability more specifically denotes the contaminant fraction that is available to cause deleterious effects to organisms when the organism is in contact with the contaminated media. This system is further complicated by dynamic inputs of new plant derived organic carbon. PdOC may enhance rates of PAH degradation by stimulating microbial activity and/or by increasing PAH bioavailability in the rhizosphere (Binet *et al.*, 2000).

1.2 PAHs in Soils and Sediments

Soils and sediments are highly heterogeneous with respect to organic matter composition, carbon structures, and sorptive properties. Several researchers have evaluated the binding affinities of functionally different carbon containing compartments of sediments to non-ionic contaminants (Luthy *et al.*, 1998; Hundal *et al.*, 2001; Chen *et al.*, 2005; Rockne *et al.*, 2002). To understand the risks created by PAH contamination in soils and sediments, it is necessary to characterize the potential accessibility of PAHs in various sediment fractions.

Many components of sediment may have a significant impact on PAH sorption and therefore affect bioavailability and bioaccessibility. For example, black carbon (BC) is

ubiquitous in many types of sediment and may compose a significant portion of lighter density fractions of sediments. BC is a type of OC created from the incomplete combustion of organic matter. Because of the highly condensed, aromatic nature of BC, it may significantly decrease bioavailability of PAHs in sediments that have high BC contents. Black carbon has been shown to have a much greater affinity for PAH sorption than sedimentary organic matter in general (Accardi-Dey and Gschwend, 2001).

Another dominant set of compounds, as pertains to PAH accessibility in sediment fractions, is derived from PdOC. Plant detrital material has been shown to be a strong sorbent for PAHs (Chen *et al.*, 2005; Sachleben *et al.*, 2004; Chefetz *et al.*, 2000). Although PdOC is an excellent sorbent for aromatic hydrophobic contaminants, it is also most available for rapid turnover by microorganisms. PdOC may enable unavailable PAHs to diffuse into more available, labile sediment compartments. PAHs may adsorb to labile plant exudates and other plant derived compounds. Many of these compounds are rapidly metabolized by sediment microorganisms. Consequently, PAHs associated with these rapidly cycling compounds may be accessible for microbial degradation.

Interactions between sedimentary sorbates such as BC or PdOC can further complicate the dynamics of PAH sorption. Hong *et al.* (2003) analyzed sorption of PAHs in soil contaminated with lampblack and oil. BC containing low levels of PAHs demonstrated sorption similar to BC models while lampblack with high levels of PAHs demonstrated sorption similar to that expected in a coal tar mixture. When oil was added to the BC, sorption sites were coated and sorption of PAH to the BC surface structure was diminished. This same behavior has been observed when natural organic matter is added to BC. Carter *et al.* (1994) found that the absorptive capacity of BC, when exposed to natural

organic matter, was significantly reduced. This implies that a variety of competing sorbates can effectively “coat” the BC particle and reduce the number of potentially available sorptive sites for PAHs. Because BC reduces bioavailability and bioaccessibility, blocking access to BC sorptive sites would therefore result in increased PAH availability.

PdOC may also make BC-sorbed PAHs more bioaccessible. When PdOC and BC come into contact in sediments, it is reasonable to expect that PAHs may diffuse from BC into more labile PdOC. It is also possible that enhanced carbon cycling caused by labile PdOC inputs may cause BC mineralization. The mechanisms by which BC is transformed in sediments is poorly understood, but it is possible that overall increases in carbon cycling in sediment due to PdOC inputs may include BC mineralization. These circumstances would explain how older, more recalcitrant PAHs may be mobilized by inputs of PdOC and made more accessible for biodegradation.

1.3 Mediators of PAH Dissipation; the rhizosphere and associated microbial populations

The area where plant roots, plant exudates, and contaminated media physically interact is termed “the rhizosphere” (Grayston *et al.*, 1996). Rhizosphere soil is a zone of enhanced microbial activity that is fueled by labile carbon from plant exudates. Rhizosphere soil also has enhanced microbial populations due to the physical support that root surfaces provide. Microbial populations in the rhizosphere are often 5 to 100 times that of surrounding soils (Liste and Prutz, 2006). Rhizosphere microbial populations may be responsible for enhanced degradation of PAHs in vegetated sediments. Microorganisms living in the rhizosphere that can attack and metabolize aged residues may be a primary means by which plant communities are able to dissipate weathered organic contaminants. Recent work has shown that some

microorganisms are able to desorb and biodegrade aged contaminants from less available compartments by producing surfactants and other compounds that enhance the accessibility of weathered compounds (Park *et al.*, 2001). PdOC may therefore enhance bioaccessibility of PAHs in the rhizosphere in 2 ways: first by acting as a more labile sorbent for PAHs, and second, by fueling the production of microbial compounds that enhance accessibility.

Some studies have shown a significant effect on PAH mitigation specifically in the rhizosphere (Anderson *et al* 1993, Shimp *et al* 1993). In one particular study, PAH was sprayed on a quartz sheet in a monolayer and a root was allowed to grow over the treated surface of the sheet (Thoma *et al.*, 2005). Fluorescence measurements quantitatively determined that PAH concentrations declined in the area of the quartz plate adjacent to the root over time. These findings suggested that the root directly contributed to PAH removal; however, whether PAH depletion occurred by physical diffusion into labile plant derived components or microbial degradation by root-associated microorganisms was unclear.

Proposed mechanisms by which PdOC may decrease levels of extractable contaminant are (1) enhanced sorption onto labile PdOC leading to accelerated sequestration, or (2) greater microbial density and activity along the root surfaces. Alternatively, a combination of these two hypotheses may be responsible; PAHs may diffuse into new labile organic carbon from more recalcitrant, mineralized compartments and become more available to microbial communities for metabolism. White *et al* (2003) found that addition of organic acids considerably enhance desorption of DDE from weathered soils. Low molecular weight organic acids, some of which are exuded from plant root systems to enhance mineral availability, disrupted the SOM and mediated a temporary increase in availability of organic compounds

that were previously unavailable. Thus, release of PdOC, including organic acids, into sediments may increase PAH bioaccessibility (Brogan *et al.*, 2003; Liste and Alexander, 2000).

Plant-derived materials demonstrate different sorptive affinities for PAHs (Salloum *et al.*, 2002; Chen *et al.*, 2005; Chefetz *et al.*, 2000). Plant cuticle, an aliphatic biopolymer, has greater organic carbon sorption coefficients (K_{oc} values) for PAHs than lignin or cellulose (Salloum *et al.*, 2002; Chefetz *et al.*, 2000). Mao *et al.* (2002) observed that the sorption capacity of some cuticular, aliphatic plant materials correlated well with PAH sorption capacity in soils. Kukkonen *et al.* (2003) observed a positive correlation between rapid PAH desorption from sediment and the presence of plant materials such as lipids and lignin. Rockne *et al.* (2002) observed that sediment fractions containing coarse plant detrital material had greater log K_{oc} values for PAHs and desorbed more PAHs than other sediment fractions. Interestingly, greater desorption was observed for larger PAH analytes such as pyrene, chrysene, and benzo[a]pyrene as well as two and three ring PAHs in sediments containing plant detrital material (Shor *et al.*, 2003).

1.4 Enhanced PdOC flux into PAH contaminated sediments in response to root stress.

PdOC loss to sediments and its effect on rhizomal bacterial growth is a probable mechanism by which vegetation attenuates impacts organic contaminants in sediments. Numerous factors can have an affect on plant exudation rates, which can therefore mediate the overall impact of vegetation. For example, both plant species and plant age dramatically impact exudation by plant roots. Mature specimens of several species of dominant or co-dominant northern hardwood trees showed dramatic variations in variety and quantity of carbohydrate and organic acid exudation between species (Smith, 1976). A study by Leyval *et al.* (1993) also found significant

differences in carbon exudation in vegetation depending on age. Older trees were found to exude less soluble organic carbon than trees that were less than one year old. Environmental factors can also influence the exudation rates of PdOC (Grayston 1996). Dehydration stress (Reid, 1974, 1977) also caused greater soluble carbon exudation rates. In this study, ^{14}C labeled vegetation released more carbon when their root systems were exposed to water stress. ^{14}C labeled plant compounds were also translocated to the roots in greater quantities in roots undergoing water stress. Several studies have shown that modulating temperature above and below a normal growth range can also cause enhanced exudation of carbon from roots (Husain *et al.*, 1963; Meharg *et al.*, 1998). Therefore, enhanced translocation and exudation of PdOC may occur as a stress reduction method to respond to non-specific root stress.

Liste *et al.* (2006) and Kmentová and Kummerová (2004) showed significant root stress on a wide variety of seedlings in weathered PAH contaminated soil. As PAHs cause a non specific stress on the root system, one would expect that PAH contaminated sediments would enhance exudation of PdOC. Increased PdOC could stimulate the microbial community and enhanced co-metabolism of PAHs or accelerate binding activities that may decrease PAH bioaccessibility in the SOM. Either of these outcomes would have important ramifications for phytoremediation technologies and treatments in the future. This response must be shown definitively in field relevant trials. Many studies have explored the PAH-plant relationship and demonstrated that PAHs induce plant stress (Ryan *et al.*, 1988; Liste *et al.*, 2006). However, it is unclear if PAH contamination causes a specific response that enhances PdOC flux rather than non-specific root stress.

Microorganisms may also increase plant exudation by consuming plant exudates in the rhizosphere (Barber *et al.*, 1977). Plants benefit from microbial turnover of root exudates and

subsequent co-metabolism of soil organic matter, which releases nutrients and enhances soil fertility. Benefits from microbial biomass on root systems include mycorrhizal associations that cause enhanced uptake of trace elements and rhizomal bacterial that inhibit the growth of some plant pathogens (Raaijmakers. 1997).

1.5 Field Studies of Phytoremediation of PAHs

Field and laboratory studies of polycyclic aromatic hydrocarbon (PAH) phytoremediation have yielded mixed results with regards to PAH degradation. Sediment contaminated with petroleum sludge is often remediated with tillage and nutrient addition. This technology, called landfarming, is designed to promote microbial degradation of hazardous compounds. During landfarming, the presence of plants can cause a marked decrease in contaminant levels (Schwab and Banks, 1994). Most short term studies evaluated PAH attenuation within a single growing season after vegetated and non-vegetated soils were amended with specific PAH analytes. Some of these studies showed that vegetation did not significantly degrade PAHs relative to non-vegetated controls (Lalande *et al.*, 2003; Mueller *et al.*, 2006). Other studies did show significant reductions in PAH concentrations within a few months (Aprill and Sims 1990; Spriggs *et al.*, 2005; Maila *et al.*, 2005; Alvarez-Bernal *et al.*, 2007; Jouneau *et al.*, 2005). Some greenhouse studies showed that dissipation of PAHs was greater in the presence of plants than in their absence and that the uptake of PAH into plant tissues did not account for PAH attenuation. Over a four week growing period, Corgie *et al.* (2004) observed lower concentrations of pyrene in soil directly adjacent to plant roots relative to soil away from vegetation. Banks *et al.* (1999) also observed significant reductions in labeled PAH concentrations in planted soils versus non-planted soils; their tracer studies also showed that loss of PAH was not the result of abiotic sorption to root structures. Miya and

Firestone (2001) proposed that enhanced PAH degradation in soils impacted by root exudates and root debris resulted from rhizo-stimulated heterotrophic, bacteria populations capable of PAH degradation. However, Jouanneau *et al.* (2005) observed less pyrene mineralization in soils amended with both plants, *Phragmites australis*, and a pyrene-degrading *Mycobacterium* bacteria compared to soils amended with only pyrene-degrading *Mycobacterium* bacteria.

A long-term study reported significant PAH reductions in the presence of mixed vegetation after 12 years exposure in field lysimeters (Doick *et al.*, 2005). Significant declines in PAH concentrations were also observed in creosote-contaminated soils after three to four years of hybrid poplar growth at a former creosote facility field site (Marr *et al.*, 2006; Widdowson *et al.*, 2005; Pitterle *et al.*, 2005). Declines in PAH concentrations were observed for only the two and three-ring PAH analytes at the creosote site (Widdowson *et al.*, 2005). In another long term study, when mulberry root extracts were added to sediments aged with ^{14}C -phenanthrene for 518 days, root-amended soils mineralized more phenanthrene and sequestered more ^{14}C label in humin relative to sterile controls (Kamath *et al.*, 2005). The authors proposed that continuous addition of organic matter (mulberry exudates) enhanced phenanthrene dissipation by two possible mechanisms: (1) enhanced microbial populations that enhance biotic degradation and/or (2) increased phenanthrene partitioning into more available OM compartments via destabilization of the humic matrix.

1.6 Chemical Fractionation in PAH studies

Humic fractionation is a fractionation method that relies on separation of sediment constituents based on chemical behavior to probe sediment carbon structure and functional

properties (Gregory *et al* 2005, Kögel-Knabner and Zeigler 1993). Brogan *et al.* (2003) showed that increasing total organic carbon content leads to decreased PAH bioavailability for microbial degradation in phenanthrene spiked sediments. Over the course of the experiment, availability also showed some correlation with fulvic acid content. Spiking sediments with fulvic acids enhanced microbial mineralization of PAHs, albeit, temporarily; mineralization increased when fulvic acid was added to soils with low fulvic acid content. Liste and Alexander (2000) found that PdOC enhanced extractable concentrations of PAH when added to sterilized soils. These findings suggest that chemically labile biogenic carbon fractions like humic and fulvic acids can alter soil/sediment organic matter and enhance PAH bioaccessibility.

Previous work has used chemical fractionation to investigate PdOC impact on PAH attenuation in labile and recalcitrant humic fractions of sediments (Neiman *et al.*, 1998; Guthrie *et al.*, 1999), particularly sediment and soil humin. Humin is generally defined as recalcitrant sediment material that remains in an insoluble form after alkaline treatment that removes the humic and fulvic organic acid fractions (Stevenson 1982). Humin accounts for a large portion of PAH sorption (Pan *et al.* 2006, Kohl and Rice. 1997), and is generally considered to desorb PAHs slower than other fractions (Dexter and Pavlou, 1978; Murphy *et al.*, 1990, 1992).

1.7 Using Carbon Isotopic techniques to probe for PdOM sediment modification

Using the known natural abundances of radioactive carbon isotopes, it is possible to use carbon isotopic signatures as a probe for biological activity. Petrogenic PAH contamination is composed of plant derived carbon that has been buried for geologically significant timeframes in order to undergo the catagenetic processes involved in petroleum hydrocarbon production. These

compounds are therefore highly depleted in unstable carbon isotopes. ^{14}C levels in petroleum hydrocarbons are extremely low and can therefore be used as a reverse tracer. Cheng *et al.* (1993) demonstrated that pulses of ^{14}C labeled CO_2 were successfully incorporated into plant structures and the ^{14}C signature was tracked into root exudates and other PdOC in the rhizosphere. Furthermore, this study demonstrated that, following incorporation into plant structures, evolved $^{14}\text{CO}_2$ was a by-product of microbial respiration caused by microbial ingestion and metabolism of labeled PdOC in the rhizosphere. Isotopic information on vegetated contaminated and pristine sediments are of interest when determining to what extent plants release increased amounts of PdOC when exposed to PAH contamination.

1.8 *Phragmites australis*

Phragmites australis is of particular interest in this study for several reasons. First, *Phragmites* is able to withstand harsh conditions and stress (Lissner *et al.*, 1997). This species has been shown to be tolerant of high metal, phenolics, and azo dye concentrations (Davies *et al.*, 2005). *Phragmites australis* has also been found at PAH-contaminated sites, and is responsible for much of the natural vegetation of these sites (Windham *et al.*, 2001; Ye *et al.*, 2003). *Phragmites* has also been shown to produce significant amounts of PdOC in sediments where it has become established (Brix *et al.* 2001). Ravit *et al.* (2006) found that *Phragmites australis* does not have a significant difference in microbial community diversity and functional ability when compared to *Spartina alterniflora*, even though there are significant differences in root structure and fine root biomass between these two wetland plants. Bacterial communities in wetlands sampled in this study were mainly a function of the sediment type and anthropogenic disturbance than plant species.

We previously reported that the presence of plant carbon in *Phragmites* distillate waste sediment was associated with significant reductions of total PAH concentrations (TPAH) and with increased PAH weathering indices (Gregory *et al.*, 2005). Reductions of [TPAH] and inputs of plant, or modern, carbon were as great if not greater in *Phragmites* humin fractions as *Phragmites* bulk sediments. At this particular site, several decades of *Phragmites* colonization and propagation altered the sediment organic matter (SOM) composition of distillate waste, reduced total PAH concentrations, and increased PAH weathering. Would similar changes occur after only two years of plant growth in petrogenic-contaminated sediments?

The purpose of this study was to better understand changes to PAH attenuation in vegetated sediments after only a few years of plant growth. We collected sediments from vegetated and non-vegetated areas at a site with historic fuel-oil contamination. PAH concentrations, PAH weathering indices, and organic matter composition in sediments colonized by *Phragmites*, cattails, or willow trees were compared to the same indices for non-vegetated sediments. We hypothesized that sediment fractions with measurable increases in plant organic matter (POM) content would likewise show measurable changes to sediment organic matter composition and PAH attenuation as indicated by more weathered PAH diagnostic ratios or reduced PAH concentrations. We hypothesize that enhanced flux of PdOC in sediment fractions is the primary mediator of PAH dissipation and should be detectable after only 2 years.

CHAPTER 2. MATERIALS AND METHODS

2.1 Site Description and Sampling Methods.

The Indiana Harbor Canal (IH) is located in a heavily industrialized section of Gary, Indiana. Total petroleum hydrocarbon (TPH) concentrations range from 2% to 43% of sediment mass; eight samples of oiled-shore sediments collected in 2001 had mean PAH concentrations of 4,100 ppm (Sand Creek Consultants, Inc., 2002). Diesel fuel is the predominant petrogenic contaminant source at the site, and, for decades, workers have noticed a persistent petroleum sheen in canal waters. In 2002, several varieties of hybrid poplars and willows trees (*Salix exigua*) were planted in near shore sediments as part of a phytoremediation treatability study. Disturbance of the site resulted in colonization by *Phragmites australis*, the common reed plant, and *Typha latifolia*, cattails. Sediment analyses in 2004 showed that PAH contamination for oiled-shore sediments was greatest in the first 5 inches of sediment (personal communication, Steve Rock, USEPA).

Sediment samples were collected by removing approximately 10 cm of sediment by hand trowel or shovel within a 2 m diameter of vegetated or non-vegetated sediments. Sediment samples were collected at 6 to 9 locations for each plant species and 3 locations of non-vegetated sediments. Non-vegetated sediments were composited at the site prior to storage and transport. In vegetated areas, plant litter and debris were removed from the sediment surface after which sediments with plants roots were removed *in toto*. Visible plant roots and plant material were removed from sediments prior to mixing sediments together for each plant species. Sediments were transported in plastic bags at 0 °C. Prior to

chemical fractionation and analyses, small visual root residuals were removed, and sediments were sieved (2mm) and homogenized again in ceramic bowls.

2.2 Sediment Fractionation.

Aliquots of bulk sediment (10-15 g.) were first freeze-dried and weighed to obtain sediment masses, then chemically fractionated to obtain bulk sediment, humin, and de-ashed humin fractions. Chemical fractionation proceeded as follows: First, sediments were weighed and lipid extracted with 20 mL of a 4:1 mixture of dichloromethane: acetone on a shaker table for 24 hours. Solvent was decanted and sediments were again extracted with 20 mL of a 4:1 mixture of dichloromethane: acetone for 24 hours. Solvent fractions were combined and this extract is referred to as the bulk sediment extract. Sediments were then extracted with a 0.1 N sodium hydroxide (NaOH) solution for 24 hours, with the headspace of the tubes purged with N₂(g). This treatment extracted the humic and fulvic acids from the sediment and destabilized the sediment matrix. NaOH was decanted from the sediments after 24 hours and sediments were lipid extracted with 20 mL of dichloromethane for 24 hours. Solvent was decanted and sediments were again extracted with 20 mL of dichloromethane for 24 hours. Solvent fractions were combined and this extract is referred to as the bulk humin or humin extract. Sediments were then extracted with a 10% solution of hydrogen fluoride: hydrochloric acid (HF:HCl) solution for 7 days, with the headspace of the tubes purged with N₂(g). This treatment dissolves the mineral matrix of the sediment and further destabilized the sediment matrix. The HF:HCl solution was decanted from the sediments after 24 hours and sediments were lipid extracted with 20 mL of dichloromethane for 24 hours. Solvent was decanted and sediments were again extracted

with 20 mL of dichloromethane for 24 hours. Solvent fractions were combined and this extract is referred to as the HF humin or de-ashed humin extract. Preferential loss of larger alkylated PAH homologues due to the chemical fractionation procedure of sediment has been evaluated and discussed previously (Gregory et al., 2005). Because sediments also contain significant metal contamination (Sand Creek Consultants, Inc. 2002), conductivity measurements of *Phragmites* and non-vegetated bulk sediments were determined using extracts of sediment/water ratios (Rhoades, J.D., 1996) and a YSI Model 85 multi probe meter (Yellow Springs, OH).

2.3 OM Composition.

Replicate aliquots of bulk sediment, humin, and de-ashed humin fractions were analyzed for percent organic carbon (% OC) using an SOP NC 2100 CHN Analyzer (Thermo Electron S.p.A., Milan, Italy). Sediment samples were sent to The Lawrence Livermore National Laboratory (Livermore, CA) for standard radiocarbon dating analysis. Sediment fractions were combusted to CO₂ then reacted with a Fe/H₂ catalyst to form graphite. Samples were analyzed by Accelerator Mass Spectrometry (AMS) with standards (NBS Oxalic Acid I and II (NIST-SRM-4990, 4990c) and blanks for organic carbon samples (Johnson-Mathey 99.9999% graphite powder). Splits of combusted samples were taken for ¹³C analysis to correct AMS values (Stuiver and Polach, 1997). Data is expressed as the percent modern carbon (¹⁴C PMC) determined from the measurement of fraction modern that is the deviation of the ¹⁴C/C ratio of a sample from “modern” (Stuiver and Polach). Petroleum contains very little ¹⁴C activity (¹⁴C PMC ~ 0%) because of its

ancient origin; whereas, modern plant carbon and recent humification of modern plant carbon will reflect modern ^{14}C activity (^{14}C PMC~100%) from atmospheric $^{14}\text{CO}_2$.

2.4 Fourier Transform Infra-Red Spectroscopy.

Approximately 1mg of freeze dried, fractionated sediment was ground with approximately 0.1g of Potassium Bromide (KBr) and pressed into a pellet using a manual press. Spectra were then collected on a Nicolet Impact 400 FTIR (Nicolet, Madison, Wisconsin). Spectra were recorded by co-addition of 32 spectra with a resolution of 4 cm^{-1} .

2.5 Gas Chromatography/Mass Spectroscopy with Select Ion Monitoring.

Extracts of bulk sediment, humin, and de-ashed humin were first loaded onto neutral activated alumina columns prior to analysis by Gas Chromatography/Mass Spectroscopy (GC/MS). Extracts were concentrated to 5 mL, split for archival purposes, and then spiked with deuterated phenanthrene (d_{10} , 500 ng/mL) and benzo[*a*]pyrene (d_8 , 505 ng/mL) as internal standards. Extracts were analyzed for 42 PAHs using a modified method of EPA 8270 EPA 8270 (US EPA, 1986b) similar to that employed by Luellen and Shea (2002). GC/MS select ion monitoring (SIM) mode analyses were conducted on a HP5890 Series II GC equipped with electronic pressure control connected to an Agilent 6890 using a Restek 30 m $\times$ 0.25 mm Rtx-5 (film thickness 0.25 μm) MS w/Integra-Guard column. PAH homologues that were quantified using this method are listed in Table 1.

2.6 Statistical Analyses.

Statistical significance was determined by Dunnett's ANOVA ($p < 0.05$) (Dunnett, 1955, 1964) by which multiple means of vegetated data sets were compared against non-vegetated controls. Student t-tests were also performed to determine statistical significance between different vegetated sites. Unless otherwise noted, triplicate samples were used to determine the averaged recoveries of PAHs in sediment extracts and total organic carbon present in sediment samples. Values in tables represent the mean of measurements $\pm$ one standard deviation.

Table 1. Inventory of 42 Alkylated and Non-alkylated PAHs

analyte	abbrev	no. of rings	analyte	abbrev	no. of rings
naphthalene ^a	N0	2	fluoranthene ^a	FL	4
biphenyl	BP	2	pyrene ^a	PY	4
acenaphthylene ^a	ACL	3	C1-fluoranthenes/pyrenes	FP1	4
acenaphthylene ^a	ACE	3	retene	Re	4
dibenzofuran	Dfu	3	benz[<i>a</i>]anthracene ^a	BaA	4
C1-naphthalenes	N1	2	chrysene ^a	C0	4
C2-naphthalenes	N2	2	C1-chrysenes	C1	4
C3-naphthalenes	N3	2	C2-chrysenes	C2	4
C4-naphthalenes	N4	2	C3-chrysenes	C3	4
fluorene ^a	F0	3	C4-chrysenes	C4	4
C1-fluorenes	F1	3	benzo[<i>b</i>]fluoranthene ^a	BbF	5
C2-fluorenes	F2	3	benzo[<i>k</i>]fluoranthene ^a	BkF	5
C3-fluorenes	F3	3	benzo[<i>e</i>]pyrene	BeP	5
dibenzothiophene	D0	3	benzo[<i>a</i>]pyrene ^a	BaP	5
C1-dibenzothiophene	D1	3	perylene	Pryl	5
C2-dibenzothiophene	D2	3	indeno[1,2,3- <i>cd</i>]pyrene ^a	IOP	6
C3-dibenzothiophene	D3	3	dibenz[<i>a,h</i>]anthracene ^a	DA	5
anthracene ^a	AN	3	benzo[<i>g,h,i</i>]perylene ^a	BgP	6
phenanthrene ^a	P0	3	coronene	Co	6
C1-phenanthrenes/anthracenes	P1	3			
C2-phenanthrenes/anthracenes	P2	3			
C3-phenanthrenes/anthracenes	P3	3			
C4-phenanthrenes/anthracenes	P4	3			

^a EPA priority pollutant.

CHAPTER 3. RESULTS

The original composite samples are referred to as bulk sediment fractions. Humin represents the sediment organic matrix after extraction with dichloromethane (DCM) and alkaline salts to remove lipids and humic/fulvic acids, respectively. De-ashed humin represents humin after extraction with HF/HCl to remove the mineral matrix.

3.1 Impact of Vegetation on Sediment Composition.

Data from FTIR (Figure 1.) show very little difference in the bulk sediment between vegetated and non-vegetated samples. Both vegetated and non vegetated samples show stretching in the $3000\text{--}2700\text{ cm}^{-1}$ region. This region is characterized as stretching of methyl and methylene groups, indicating alkane content (Michalian, K.H. and Freisen, W.I. 1990; Painter, P.C. et al, 1981; Silverstein, R.M. et al, 1991; Ledesma, E.B. et al. 2000). As expected, moving into bulk humin that has been lipid extracted, these peaks disappear. All sediments also show the characteristic mixture of peaks in the $900\text{--}650\text{ cm}^{-1}$ region indicating polycyclic aromatic compounds are still present in the sediment fractions. In bulk sediments, there appears to be less of a contribution due to C-H stretching and bending in the vegetated zone as evidenced by peaks near 2900 and 1400 cm^{-1} respectively and slightly more contribution from the more polar C-O stretch at 1000 cm^{-1} (Silverstein, R.M. et al, 1991), but the high levels of petroleum contamination mask any major shifts. This is not the case in the bulk humin where much of the available petroleum residue has been lipid extracted from the sediment. The effects of vegetation are much more obvious in the bulk humin. A significantly larger peak encompassing the C-O stretching zone around 1100

cm⁻¹ is present in vegetated sediments vs. the non-vegetated control. The effects of vegetation on total organic carbon (TOC) are visible in figure 2, with little deviation between sites.

3.2 PAH Concentrations in Sediment Fractions.

Individual PAH concentration data for Bulk Sediment, Humin, and De-ashed humin can be seen in tables 2-4. Mean concentrations of 42 alkylated and non-alkylated PAHs [TPAH] and the sum of the 16 EPA priority PAHs do not significantly decline between non-vegetated and vegetated bulk sediments (Table 5.) (Dunnett's ANOVA, $p < 0.05$). Humin and de-ashed humin fractions also showed no significant declines in TPAH concentrations between non-vegetated and vegetated sediment fractions (Table 6) (Dunnett's ANOVA, $p < 0.05$). In fact, TPAH concentrations for *Phragmites* and cattails humin fractions increased relative to non-vegetated humin fractions.

Humin fractions of cattails had significantly greater TPAH concentrations than the other humin fractions analyzed (t -test, $p < 0.05$), and *Phragmites* de-ashed humin had greater, though not significantly greater, TPAH concentrations than all other de-ashed humin fractions (t -test, $p < 0.05$). These findings could be attributed to site heterogeneity and variant PAH concentration distributions. However, Rockne *et al.*, 2002 observed that 85% of TPAHs in an estuarine sediment were found in sediment fractions that contained plant detrital material even though these fractions only contained 4% of the total sediment mass. Thus, the presence of *Phragmites* or cattail plant material in humin fractions may explain increased TPAH concentrations in these fractions.

In order to account for lower PAH recovery due to carbon dilution from plant carbon inputs, TPAH concentrations were carbon-normalized in each sediment fraction (Table 5). Carbon-normalized [TPAH] significantly decreased in all vegetated bulk sediments relative to non-vegetated bulk sediments (Dunnett's ANOVA, $p < 0.05$; Table 6). However, carbon-normalized [TPAH] for humin and de-ashed humin fractions diverged from non-normalized TPAH data. Specifically, carbon-normalized [TPAH] for *Phragmites* and cattails humin were much greater than non-vegetated and willow tree humin. Also, *Phragmites* and willow tree de-ashed humin fractions had much greater carbon-normalized [TPAH] than cattails and non-vegetated de-ashed humin fractions.

3.3 PAH Weathering and Sediment Composition.

Weathering is a phenomenon by which the chemical composition of PAH contamination changes and dissipates over time. In general, more alkylated homologues are more stable and resistant to degradation. Examples of weathered vs. unweathered PAH distributions can be seen in PAH histograms of the 4 study sites shown in figures 3-6. Unweathered alkylated PAH distributions are seen in figure 3 (non vegetated sediment). More weathered distributions are present in figure 4 (*Phragmites* colonized sediment). Weathering patterns are useful only qualitatively. Alkylated PAH ratios provide a quantitative measure of PAH weathering and can be used (1) to describe the degree of weathering and biodegradation of spilled oil and (2) to predict and monitor the effectiveness of remediation activities (Douglas *et al.*, 1996). The ratio of C₃-naphthalenes and C₂-phenanthrenes (N₃/P₂) is used for diesel fuel and crude oil in the early stages of

weathering as the tri-alkylated naphthalenes have a shorter lifespan in relation to many other PAHs. C₃-dibenzothiophenes vs. C₃-chrysenes (D₃/C₃) ratios can be used to detect later stages of weathering for diesel and crude oils (Douglas *et al.*, 1996). We did not observe more weathered D₃/C₃ ratios for any vegetated sediment fractions relative to the non-vegetated sediment fractions. This outcome is not unexpected given the short exposure of sediment to vegetation (2 years).

Figure 7 shows cross plots of N₃/P₂ weathering ratios to percent total organic carbon (%TOC) for all sediment fractions. More weathered ratios are observed for *Phragmites* bulk sediment and humin fractions and willow tree humin relative to non-vegetated fractions. N₃/P₂ ratios for all samples in de-ashed humin were similar; this is not unexpected given the refractory nature of de-ashed humin and the short period of exposure to vegetation. Relationships between % TOC and N₃/P₂ ratios for non-vegetated and *Phragmites* bulk sediment and humin fractions are not apparent. In fact, there is no consistent pattern between N₃/P₂ ratios and sediment fractions for either non-vegetated or vegetated sites. Previously, we observed increased N₃/P₂ weathering with increased % TOC in distillate refinery waste colonized by *Phragmites* and other trees and shrubs after 30 years of plant growth (Gregory *et al.*, 2005). This trend is not evident in these sediments after only two years of vegetative growth. Organic carbon content of sediment fractions does not appear related to PAH dissipation nor even to the presence of vegetation.

Figure 8 shows a more consistent relationship between N₃/P₂ weathering ratios and percent modern carbon (PMC) measurements. For bulk sediment and humin fractions, the most weathered ratios are observed in *Phragmites* fractions that contain more ¹⁴C activity, or modern carbon, than all other sediment fractions (Figure 8). Willow trees also had more

weathered N_3/P_2 ratios in humin fractions and more modern carbon than non-vegetated humin. All three vegetated de-ashed humin fractions were more weathered than non-vegetated de-ashed humin but only cattails and *Phragmites* contained more modern carbon than non-vegetated de-ashed humin.

3.4 *Phragmites*' ability to colonize contaminated sediment

Visual differences between plant colonization of Indiana Harbor Canal (IHC) sediments are presented in Figure 4 (photos courtesy of Steve Rock). IHC sediments were planted with either cattails or *Phragmites* that had been harvested from the IHC site during sediment collection. Plants were rooted in clean soil on top of IHC sediment; the two sediments (clean and IHC) were separated by burlap material. As is evident in Figure 9, *Phragmites* roots grew around the burlap material and into the IHC sediment. Cattails roots did not penetrate IHC sediment and remained in the clean soil. Such distinct differences may readily explain the observed differences in plant carbon accumulation and PAH weathering between *Phragmites* and cattail sediment fractions in our study.

Figure 1. Selected Fourier Transform Infrared (FTIR) spectra of Indiana Harbor Sediment Fractions.

Figure 2. Total Organic Carbon (% TOC) and Percent Modern Carbon (% PMC) for bulk sediment and humin fractions of vegetated and non vegetated sediments at the Indiana Harbor Canal site. Values are means $\pm$ one standard deviation.

Figure 3. Individual PAH concentrations from the non vegetated zone from bulk sediment, humin, and de-ashed humin. Error bars indicate one standard deviation with $n = 3$. * indicated a non weathered alkylated PAH distribution.

Figure 4. Individual PAH concentrations from the Phragmites dominated zone from bulk sediment, humin, and de-ashed humin. Error bars indicate one standard deviation with $n = 3$. * indicated a weathered alkylated PAH distribution.

Figure 5. Individual PAH concentrations from the Cattails dominated zone from bulk sediment, humins, and de-ashed humin. Error bars indicate one standard deviation with $n = 3$.

Figure 6. Individual PAH concentrations from the Willow dominated zone from bulk sediment, humins, and de-ashed humins. Error bars indicate one standard deviation with n = 3.

Figure 7. Cross plots of C₃-naphthalenes to C₂-phenanthrenes (N₃/P₂) alkylated PAH homologues to percent Total Organic Carbon (% TOC) for sediment and humin fractions of vegetated and non vegetated sediments at the Indiana Harbor Canal site. Values are means $\pm$ one standard deviation.

Figure 8. Cross plots of C₃-naphthalenes to C₂-phenanthrenes (N₃/P₂) alkylated PAH homologues to. Percent Modern Carbon (PMC) for sediment and humin fractions of vegetated and non vegetated sediments at the Indiana Harbor Canal. Values are means $\pm$ one standard deviation

Phragmites

Cattails

Courtesy of Steve Rock, USEPA

Figure 9. *Phragmites australis* and cattails (*Typha angustifolia*) growth in greenhouse containers. Plants were rooted in clean soil that was placed on top of Indiana Harbor Canal sediment. *Phragmites* grew around and into the oiled sediment, where as cattails did not.

Table 2. PAH concentrations for parent compounds and alkylated homologues from Bulk Sediment extracts. Concentrations are given in µg/g sediment with n = 3.

		Non-Vegetated		<i>Phragmites australis</i>		Cattails		Willow	
		Ave	Std dev	Ave	Std dev	Ave	Std dev	Ave	Std dev
Naphthalene	N0	0.67	0.05	1.08	0.24	1.14	0.34	0.59	0.11
Biphenyl	BP			0.29	0.02	0.34	0.15	0.15	0.03
Acenaphthylene	ACL	1.63	1.29	0.96	0.13	1.81	0.46	1.37	0.24
Acenaphthene	ACE	2.87	1.66	1.41	0.20	6.68	1.15	1.82	0.29
Dibenzofuran	Dfu	1.27	0.51	0.88	0.06	1.21	0.30	0.65	0.06
C1 - Naphthalenes	N1	4.26	4.46	1.73	0.25	3.28	1.02	1.32	0.17
C2 - Naphthalenes	N2	30.67	29.47	5.14	0.48	17.27	2.51	4.33	0.34
C3 - Naphthalenes	N3	100.60	39.32	50.28	2.90	105.21	6.54	32.74	4.87
C4 - Naphthalenes	N4	112.57	98.50	105.64	5.98	272.81	38.87	119.82	10.23
Fluorene	F0	31.82	13.43	6.39	0.21	18.56	2.29	0.88	1.03
C1 - Fluorenes	F1	116.53	18.78	58.69	2.65	140.60	15.27	46.72	9.27
C2 - Fluorenes	F2	282.06	45.92	199.71	11.26	349.69	34.37	182.50	33.64
C3 - Fluorenes	F3	250.75	41.40	153.21	8.67	297.89	30.30	169.80	42.06
Dibenzothiophene	D0	38.23	11.15	31.50	1.77	15.90	3.96	4.95	0.97
C1 - Dibenzothiophene	D1	189.09	42.11	137.61	8.43	201.80	27.27	76.99	15.38
C2 - Dibenzothiophene	D2	307.07	42.08	243.31	13.29	376.09	55.29	219.82	42.92
C3 - Dibenzothiophene	D3	250.99	40.96	181.84	13.89	313.46	62.16	196.61	29.85
Anthracene	AN	15.34	3.12	7.53	0.42	17.90	2.59	6.26	1.49
Phenanthrene	P0	8.19	0.51	7.22	0.58	14.18	4.81	5.45	0.52
C1 - Phenanthrenes/Anthracenes	P1	118.33	21.84	105.39	7.92	105.29	13.24	46.81	12.26

Table 2 continued

		Non-Vegetated		<i>Phragmites australis</i>		Cattails		Willow	
		Ave	Std dev	Ave	Std dev	Ave	Std dev	Ave	Std dev
C2 - Phenanthrenes/Anthracenes	P2	429.90	75.42	390.22	17.15	460.10	41.10	235.08	42.14
C3 - Phenanthrenes/Anthracenes	P3	346.34	27.17	229.30	19.07	461.22	59.75	233.62	36.58
C4 - Phenanthrenes/Anthracenes	P4	109.61	11.86	43.41	4.03	142.99	51.97	55.53	5.16
Fluoranthene	FL	124.42	16.86	104.34	3.04	152.87	21.81	95.63	18.37
Pyrene	PY	457.20	36.74	361.30	23.56	494.21	60.67	352.72	69.12
C1 - Fluoranthenes/Pyrenes	FP1	524.47	56.63	439.06	18.10	582.64	66.09	409.91	73.47
Retene	Re								
Benz[a]anthracene	BaA	104.90	17.19	101.39	6.75	120.48	14.51	90.25	14.45
Chrysene	C0	229.13	33.91	205.49	15.71	270.21	25.57	229.98	37.69
C1 - Chrysenes	C1	188.29	20.02	173.30	14.32	226.73	11.81	213.68	28.99
C2 - Chrysenes	C2	83.56	6.42	67.44	5.07	106.64	7.58	85.71	10.23
C3 - Chrysenes	C3	31.21	3.31	22.98	2.25	45.71	5.16	31.27	3.06
C4 - Chrysenes	C4	6.64	1.12	5.25	0.82	14.21	2.63	8.31	2.16
Benzo[b]fluoranthene	BbF	56.96	5.93	50.28	3.90	68.88	11.82	63.94	7.70
Benzo[k]fluoranthene	BkF	17.26	0.76	14.45	1.20	21.26	3.79	18.82	1.56
Benzo[e]pyrene	BeF	109.73	10.08	94.58	7.59	126.48	19.74	125.62	14.83
Benzo[a]pyrene	BaF	42.46	6.06	40.38	2.87	47.70	9.19	38.41	4.01
Perylene	Pryl	2.36	0.48	2.00	0.33	4.31	0.79	2.35	0.61
Indeno[1,2,3-cd]pyrene	IOP	17.63	2.89	17.00	1.31	24.86	3.47	19.88	4.47
Dibenz[a,h]anthracene	DA	17.58	2.17	15.95	1.14	20.68	2.94	18.64	3.38
Benzo[g,h,i]perylene	BgP	31.42	6.84	31.29	2.39	44.05	6.01	37.00	11.23
Coronene	Co	2.04	2.10	3.87	0.59	4.36	0.94	3.73	3.08

Table 3. PAH concentrations for parent compounds and alkylated homologues from humin extracts. Concentrations are given in µg/g sediment with n = 3.

		Non-Vegetated		<i>Phragmites australis</i>		Cattails		Willow	
		Ave	Std dev	Ave	Std dev	Ave	Std dev	Ave	Std dev
Naphthalene	N0	0.07	0.01	0.09	0.03	0.09	0.02	0.05	0.01
Biphenyl	BP			0.02	0.00	0.04	0.01	0.03	0.00
Acenaphthylene	ACL	0.02	0.01	0.02	0.01	0.03	0.00	0.02	0.01
Acenaphthene	ACE	0.01	0.00	0.01	0.00	0.03	0.01	0.01	0.00
Dibenzofuran	Dfu	0.04	0.00	0.05	0.01	0.06	0.01	0.03	0.00
C1 - Naphthalenes	N1	0.08	0.00	0.09	0.03	0.12	0.02	0.07	0.01
C2 - Naphthalenes	N2	0.16	0.11	0.11	0.03	0.23	0.09	0.07	0.01
C3 - Naphthalenes	N3	0.14	0.03	0.15	0.03	0.36	0.11	0.10	0.01
C4 - Naphthalenes	N4	0.23	0.08	0.25	0.02	0.71	0.24	0.15	0.02
Fluorene	F0	0.10	0.04	0.07	0.01	0.12	0.02	0.05	0.02
C1 - Fluorenes	F1	0.20	0.03	0.20	0.04	0.53	0.15	0.15	0.03
C2 - Fluorenes	F2	0.38	0.09	0.41	0.18	1.18	0.38	0.31	0.05
C3 - Fluorenes	F3	0.37	0.12	0.37	0.24	1.27	0.35	0.38	0.10
Dibenzothiophene	D0	0.21	0.07	0.32	0.07	0.26	0.04	0.12	0.03
C1 - Dibenzothiophene	D1	0.41	0.07	0.49	0.15	0.98	0.26	0.29	0.04
C2 - Dibenzothiophene	D2	0.59	0.21	0.64	0.32	1.61	0.57	0.50	0.09
C3 - Dibenzothiophene	D3	0.43	0.21	0.52	0.38	1.39	0.58	0.37	0.08
Anthracene	AN	0.18	0.16	0.25	0.32	0.14	0.02	0.26	0.03
Phenanthrene	P0	0.32	0.02	0.49	0.15	0.51	0.07	0.27	0.03
C1 - Phenanthrenes/Anthracenes	P1	0.44	0.01	0.81	0.31	0.96	0.27	0.39	0.05

Table 3 continued

		Non-Vegetated		<i>Phragmites australis</i>		Cattails		Willow	
		Ave	Std dev	Ave	Std dev	Ave	Std dev	Ave	Std dev
C2 - Phenanthrenes/Anthracenes	P2	0.67	0.14	1.81	0.60	1.94	0.65	0.58	0.09
C3 - Phenanthrenes/Anthracenes	P3	0.49	0.23	1.26	0.82	1.75	0.61	0.46	0.10
C4 - Phenanthrenes/Anthracenes	P4	0.11	0.03	0.25	0.24	0.43	0.16	0.14	0.01
Fluoranthene	FL	0.27	0.06	0.35	0.20	0.62	0.16	0.25	0.05
Pyrene	PY	0.97	0.33	0.99	0.48	2.11	0.66	0.80	0.06
C1 - Fluoranthenes/Pyrenes	FP1	1.07	0.46	1.64	0.09	2.60	0.85	0.92	0.06
Retene	Re								
Benz[a]anthracene	BaA	0.30	0.07	0.44	0.29	0.71	0.25	0.29	0.06
Chrysene	C0	0.90	0.29	1.14	0.27	2.09	0.73	0.79	0.04
C1 - Chrysenes	C1	0.71	0.33	0.63	0.32	1.87	0.72	0.65	0.11
C2 - Chrysenes	C2	0.34	0.17	0.27	0.15	1.08	0.40	0.32	0.07
C3 - Chrysenes	C3	0.11	0.08	0.08	0.06	0.43	0.16	0.11	0.04
C4 - Chrysenes	C4	0.02	0.01	0.01	0.01	0.11	0.09	0.01	0.01
Benzo[b]fluoranthene	BbF	0.36	0.22	0.29	0.20	0.61	0.18	0.28	0.02
Benzo[k]fluoranthene	BkF	0.13	0.06	0.16	0.17	0.34	0.13	0.17	0.06
Benzo[e]pyrene	BeF	0.87	0.53	0.59	0.28	1.61	0.48	0.68	0.07
Benzo[a]pyrene	BaF	0.40	0.35	0.22	0.22	0.59	0.20	0.21	0.03
Perylene	Pryl	0.01	0.01	0.02	0.03	0.11	0.06	0.01	0.01
Indeno[1,2,3-cd]pyrene	IOP	0.26	0.21	0.16	0.09	0.54	0.18	0.23	0.03
Dibenz[a,h]anthracene	DA	0.15	0.13	0.08	0.04	0.37	0.15	0.14	0.03
Benzo[g,h,i]perylene	BgP	0.65	0.44	0.36	0.17	1.15	0.32	0.54	0.12
Coronene	Co	0.16	0.06	0.08	0.06	0.33	0.08	0.15	0.03

Table 4. PAH concentrations for parent compounds and alkylated homologues from De-ashed humin extracts. Concentrations are given in µg/g sediment with n = 3.

		Non-Vegetated		<i>Phragmites australis</i>		Cattails		Willow	
		Ave	Std dev	Ave	Std dev	Ave	Std dev	Ave	Std dev
Naphthalene	N0	0.082	0.091	0.140	0.200	0.040	0.032	0.039	0.043
Biphenyl	BP			0.134	0.027	0.013	0.008	0.012	0.010
Acenaphthylene	ACL	0.000	0.000	0.010	0.007	0.004	0.002	0.005	0.001
Acenaphthene	ACE	0.002	0.002	0.023	0.027	0.004	0.002	0.005	0.003
Dibenzofuran	Dfu	0.003	0.005	0.063	0.050	0.024	0.017	0.025	0.020
C1 - Naphthalenes	N1	0.050	0.040	0.111	0.150	0.044	0.036	0.057	0.040
C2 - Naphthalenes	N2	0.041	0.038	0.166	0.197	0.068	0.052	0.109	0.035
C3 - Naphthalenes	N3	0.051	0.039	0.182	0.127	0.056	0.022	0.087	0.052
C4 - Naphthalenes	N4	0.062	0.032	0.037	0.064	0.068	0.008	0.044	0.046
Fluorene	F0	0.022	0.039	0.065	0.036	0.031	0.011	0.027	0.024
C1 - Fluorenes	F1	0.042	0.024	0.179	0.059	0.077	0.009	0.057	0.046
C2 - Fluorenes	F2	0.072	0.064	0.253	0.099	0.118	0.016	0.081	0.058
C3 - Fluorenes	F3	0.083	0.074	0.224	0.059	0.117	0.016	0.085	0.055
Dibenzothiophene	D0	0.067	0.059	0.400	0.100	0.141	0.024	0.096	0.090
C1 - Dibenzothiophene	D1	0.135	0.029	0.408	0.100	0.190	0.030	0.135	0.098
C2 - Dibenzothiophene	D2	0.162	0.086	0.309	0.202	0.227	0.020	0.173	0.089
C3 - Dibenzothiophene	D3	0.139	0.122	0.224	0.101	0.101	0.020	0.100	0.049
Anthracene	AN	0.272	0.044	0.067	0.013	0.039	0.006	0.031	0.014
Phenanthrene	P0	0.061	0.054	0.821	0.311	0.249	0.054	0.191	0.153
C1 - Phenanthrenes/Anthracenes	P1	0.095	0.113	0.639	0.194	0.208	0.039	0.195	0.085

Table 4 continued

		Non-Vegetated		<i>Phragmites australis</i>		Cattails		Willow	
		Ave	Std dev	Ave	Std dev	Ave	Std dev	Ave	Std dev
C2 - Phenanthrenes/Anthracenes	P2	0.159	0.095	0.481	0.128	0.250	0.028	0.213	0.089
C3 - Phenanthrenes/Anthracenes	P3	0.131	0.115	0.277	0.109	0.193	0.019	0.163	0.077
C4 - Phenanthrenes/Anthracenes	P4	0.076	0.067	0.044	0.039	0.042	0.002	0.033	0.018
Fluoranthene	FL	0.098	0.144	0.176	0.035	0.103	0.010	0.071	0.050
Pyrene	PY	0.134	0.052	0.438	0.131	0.270	0.030	0.188	0.130
C1 - Fluoranthenes/Pyrenes	FP1	0.239	0.014	0.511	0.195	0.302	0.020	0.232	0.127
Retene	Re								
Benz[a]anthracene	BaA	0.188	0.054	0.188	0.026	0.107	0.003	0.079	0.038
Chrysene	C0	0.200	0.195	0.466	0.087	0.382	0.010	0.262	0.143
C1 - Chrysenes	C1	0.424	0.294	0.340	0.098	0.287	0.002	0.305	0.150
C2 - Chrysenes	C2	0.129	0.048	0.137	0.049	0.132	0.006	0.295	0.321
C3 - Chrysenes	C3	0.036	0.032	0.023	0.040	0.028	0.010	0.243	0.355
C4 - Chrysenes	C4	0.003	0.005	0.007	0.007	0.000	0.000	0.089	0.077
Benzo[b]fluoranthene	BbF	0.027	0.047	0.144	0.024	0.110	0.007	0.090	0.080
Benzo[k]fluoranthene	BkF	0.152	0.192	0.023	0.003	0.023	0.002	0.099	0.140
Benzo[e]pyrene	BeF	0.105	0.079	0.265	0.076	0.228	0.006	0.214	0.198
Benzo[a]pyrene	BaF	0.121	0.098	0.100	0.046	0.055	0.007	0.050	0.061
Perylene	Pryl	0.052	0.072	0.004	0.004	0.005	0.002	0.004	0.007
Indeno[1,2,3-cd]pyrene	IOP	0.059	0.053	0.072	0.014	0.043	0.005	0.080	0.050
Dibenz[a,h]anthracene	DA	0.033	0.031	0.038	0.007	0.025	0.003	0.073	0.071
Benzo[g,h,i]perylene	BgP	0.075	0.019	0.153	0.048	0.132	0.017	0.207	0.144
Coronene	Co	0.006	0.005	0.026	0.008	0.011	0.003	0.028	0.016

Table 5. PAH Concentrations^{a,b} ($\pm$ 1 Standard Deviation), Percent Total Organic Carbon (% TOC)^b, and Percent Modern Carbon^b (¹⁴C PMC) Content for Vegetated and Non-Vegetated Indiana Harbor Sediment Fractions.

	Non- Vegetated	<i>Phragmites</i>	Cattails	Willow Trees
Bulk Sediment				
[TPAH] ($\mu\text{g/g soil}$) ^a	4,796 $\pm$ 602.4	3,713 $\pm$ 185.1	5,701 $\pm$ 613.2	3,490 $\pm$ 555.5
[16 PAH] ($\mu\text{g/g soil}$)	1,205 $\pm$ 124.7	942 $\pm$ 62.94	1,421 $\pm$ 51.89	890 $\pm$ 53.5
[TPAH] ($\mu\text{g/g OC}$)	33,500 $\pm$ 6,450	26,900 $\pm$ 1,610	16,600 $\pm$ 2,340	16,000 $\pm$ 3,554
Humin				
[TPAH] ($\mu\text{g/g soil}$) ^a	13.3 $\pm$ 4.50	16.2 $\pm$ 4.47	38.6 $\pm$ 3.20	11.4 $\pm$ 0.811
[16 PAH] ($\mu\text{g/g soil}$)	4.22 $\pm$ 1.25	3.81 $\pm$ 0.602	12.0 $\pm$ 0.645	4.47 $\pm$ 0.163
[TPAH] ($\mu\text{g/g OC}$)	253 $\pm$ 96.3	482 $\pm$ 143	640 $\pm$ 75.9	159 $\pm$ 17.5
De-Ashed Humin				
[TPAH] ($\mu\text{g/g soil}$) ^a	3.88 $\pm$ 0.337	8.37 $\pm$ 2.30	4.54 $\pm$ 0.320	4.57 $\pm$ 1.76
[16 PAH] ($\mu\text{g/g soil}$)	1.61 $\pm$ 0.790	2.54 $\pm$ 0.304	1.66 $\pm$ 0.165	1.76 $\pm$ 0.511
[TPAH] ($\mu\text{g/g OC}$)	81.2 $\pm$ 9.88	192 $\pm$ 2.92	95.5 $\pm$ 18.7	146 $\pm$ 27.3

^a Total alkylated and non-alkylated PAH (n=42) concentrations of lipid extracts from sediment fractions analyzed at NCSU.

^b Values are mean $\pm$ one standard deviation (n=3).

4. DISCUSSION

A variety of field and greenhouse studies have explored the role of vegetation in PAH dissipation, with a variety of results. Previously, we discussed that PdOC may enhance rates of PAH degradation by stimulating microbial activity and/or by increasing PAH bioavailability in the rhizosphere. In this study, actual dissipation of PAH is difficult to detect strictly by PAH contaminant levels. This is not surprising given the extremely high concentrations of PAH and the short timeframe of vegetation colonization. In this case, the best indicators of PAH dissipation and the impacts of vegetation and PdOM flux come from weathering indices and radiocarbon PMC measurements.

The divergence of mass sediment and carbon normalized [TPAH] data in humin fractions from bulk sediments TPAH data does not reflect greater organic carbon content in these fractions. The % TOC for the different sediment fractions is shown in Figure 2. Greater carbon-normalized TPAH concentrations for humin fractions of *Phragmites*, cattail, and willow trees do not occur in conjunction with notable increases in % TOC.

Greater organic carbon content did not explain increased TPAH concentrations in sediments fractions containing plant detrital material (Rockne *et al.*, 2002). Equilibrium aqueous-solid partitioning data showed that sediment fractions with plant detrital material were stronger, preferential sorbents for PAH sequestration and partitioning than sediment fractions without plant materials. The authors speculated that as PAHs partition from various organic matter-rich regions in sediments aggregates, they reversibly partitioned into sediment material rich in plant, vascular debris. Thus, greater PAH concentrations in cattail

humins and *Phragmites* de-ashed humins may result from PAH sequestration and partitioning into plant derived materials in these sediment fractions.

Rockne *et al.* (2002) and Shor *et al.*, (2003) observed that sediment fractions with plant detrital material and high PAH concentrations disproportionately impacted high molecular weight PAH desorption and more extensively desorbed all PAHs than sediment fractions without detrital material. These authors stressed the important role of plant material as a preferential sorbent in sediments where other geosorbents such as soot are not the primary source of PAHs to sediments.

Carbon isotopic techniques have been used to trace PdOC cycling in rhizosphere sediment (Reid, 1974, 1977; Cheng *et al.* 1993) and can be used to determine if correlations exist between enhanced PAH attenuation and PdOC inputs. ^{14}C measurements quantitatively track new plant carbon inputs into both refractory and labile sedimentary compartments, as both low molecular weight exudates and high molecular weight root structures have modern carbon signatures. Interestingly, all three vegetated humin fractions contain more modern carbon than non-vegetated humin (Figure 2; (Dunnnett's ANOVA, $p < 0.05$). This trend is not observed in bulk sediment fractions and is less apparent in de-ashed humin fractions. Increased modern carbon content for *Phragmites* and cattails humin (Figure 2) is related to greater carbon-normalized TPAH concentrations (Table 6), but only *Phragmites* humin shows a relationship between increased modern carbon and more weathered N_3/P_2 ratios.

Phragmites australis is considered an invasive species, but it is also a useful model plant to study because of its tolerance to anthropogenic contamination and physiological characteristics. *Phragmites australis* is known for its translocation of oxygen and carbon

from plant shoots/leaves to sediments via its roots (Minchinton, 2002). Nearly 50% of its litter becomes substrate material for soils and sediments and the majority of its biomass is non-grazed (Asaeda et al, 2002). Total organic acid concentrations in pore water of both deep water and land stands of *Phragmites australis* average 1,100-1,600 $\mu\text{mol/L}$, and are predominantly acetic, citric, tartaric, and malic acids (Cizkova *et al.*, 1999). These attributes, no doubt, contribute to *Phragmites*' success and tolerance of saturated (Armstrong and Armstrong, 2001), metal-contaminated (Yea *et al.*, 2003), saline (Chambers *et al.*, 1998), and eutrophic environments (Lin *et al.*, 2002). Likewise, these characteristics may explain the increased amounts of plant carbon present in *Phragmites* sediments.

5. CONCLUSION

Plants both aerate the soil, encouraging volatilization, and release PdOM, which modifies the sediment matrix. Both of these processes are likely to have an impact on PAH contamination, but untangling the role of PdOC inputs and fluxes in sediments is not a trivial matter. This study shows alteration of sediment composition in bulk and humin sediment fractions after 2 years of plant growth in fuel-oiled sediments. Mass normalized TPAH concentrations did not decline in vegetated sediments relative to non-vegetated sediments. Carbon-normalized TPAH concentrations were significantly lower in vegetated bulk sediments but higher in humin fractions. Percent TOC data could not be used to estimate PAH concentrations, modern carbon (plant carbon) accumulation, or PAH weathering in vegetated sediments fractions relative to non-vegetated sediments. Significant increases in percent modern carbon measurements did relate to greater N_3/P_2 weathering in vegetated bulk and humin fractions. These data show that *Phragmites* accumulates more plant carbon in sediment fractions than cattails or willow trees and that this accumulation results in greater weathering of two and three ring PAHs. The fact that modern carbon is present in humin and de-ashed humin at such measured levels after 2 years of plant growth is important to PAH carbon turnover and biogenic carbon cycling processes.

6. FUTURE WORK

Fractionation of sediments into functionally different carbon pools has been done several times in recent studies (Grunewald et al., 2006; Sollins et al., 1996; Helfrich et al. 2006; Oren and Chefetz, 2005) in order to probe the characteristics and functionality of sediment components. Density fractionation, in particular, allows the separation of carbon pools in a simple manner (Swanston et al., 2005; Ghosh et al., 2001; Golchin et al., 1997); whereas, chemical fractionation separates the most recalcitrant SOM from mineral fractions. Light fractions of sediments appear to be the most responsive to new organic carbon inputs and least well protected in terms of sequestering carbon. Heavy density fractions are associated with mineral matrices and tend to be lower in organic carbon and contain the oldest, most recalcitrant carbon pool. There is some evidence that the heavy density fraction contains a small pool of more labile carbon (Chotte et al., 1998), but the bulk of the rapidly cycling carbon resides in the light density fraction.

Radiocarbon analyses show that light density fractions are younger and less recalcitrant than heavy density fractions; heavy density fractions tend to have a lower proportion of biogenic new carbon and are associated with inorganic fractions. PAH degradation varies among sediment fractions and particle types. Bioslurry experiments with density fractions from PAH-contaminated sediments showed that PAH availability and associated degradation rates were greatest in heavier density fractions (Talley et al., 2002) where the primary organic particle type was coal derived and highly aromatic (Ghosh, et al., 2003; Talley et al., 2002). Conversely, degradation rates were shown to be greatest in lighter fractions when the primary particle type was a pitch mixture.

Physical separation of carbon pools can provide important information on the PAH content and sorptive properties of some compartments. This information is useful to understand the behavior of PAH carbon in SOM; however, physical separations do not directly measure the impact of PdOC on sedimentary carbon cycling. Though the majority of recent PdOC inputs will reside in lighter density fractions, these fractions also contain other sediment constituents as well. In order to more directly explore PdOC, it is necessary to use chemical fractionation to separate known fractions of PdOC. Physical and Chemical fractionation of sediments in conjunction with desorption and K_{oc} experiments would give a greatly enhanced understanding of the impact of vegetation on PAH contaminated sediments.

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