Cuticle Accumulation of Petrogenic PAHs on Spartina Alterniflora: A Novel Exposure Pathway for Marsh Biota

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CUTICLE ACCUMULATION OF PETROGENIC PAHS ON SPARTINA ALTERNIFLORA: A NOVEL EXPOSURE PATHWAY FOR MARSH BIOTA

A Thesis
Submitted to the Graduate Faculty of the
Louisiana State University and
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ABSTRACT

Louisiana coastal marshes were heavily impacted by the 2010 Deepwater Horizon oil spill. MC252 oil pollution in coastal marshes lead to the exposure of biota to polycyclic aromatic hydrocarbons (PAHs). PAHs are persistent organic pollutants that can reside and accumulate in marsh biota. The aim of this study was to investigate the uptake mechanisms of PAHs by Spartina alterniflora at heavily oiled Bay Jimmy marsh in Barataria Basin, Louisiana.

Study objectives were to quantify and investigate the uptake mechanisms of PAHs in the leaves of Spartina. The plant/air partitioning of PAHs was studied using a fugacity approach to determine their partitioning coefficients. Semipermeable membrane devices (SPMDs) were utilized in a parallel study of the uptake of PAHs from the marsh surface and air. Sampling was between June 2016 and February 2017.

A three-step sequential extraction procedure was applied for analysis of PAHs in Spartina leaves. At the field, particulates deposited on Spartina leaves were washed with EDTA solution followed by dichloromethane to dissolve the cuticle. Leaf tissues were extracted by the accelerated solvent extraction method. PAHs in SPMDs were extracted via dialysis with hexane. A laboratory scale fugacity meter was designed to study plant/air partitioning of PAHs. PAHs were quantified in selective ion monitoring mode connected with a mass selective detector.

Similar PAHs profiles in Spartina leaf tissues and SPMDs suggested the cycling of PAHs at Bay Jimmy. Naphthalenes accumulated twice more than phenanthrenes in Spartina leaves and SPMDs. PAHs were sequestered in leaf tissues than the cuticle. Statistically significant correlation (p <0.05) of PAHs in plant leaves and SPMDs were as high as 97%. Soil analysis revealed naphthalenes and phenanthrenes to total PAHs fraction of less than 20%. Results indicated that air-leaf-partitioning was the dominant uptake route of PAHs accumulation in Spartina. Lower
plant/air partition coefficients of naphthalenes than phenanthrenes from the fugacity experiment suggested the accumulation of lower molecular weight PAHs in greater quantities. Temporal trends revealed seasonal variability of PAHs accumulation in *Spartina* and SPMDs. This study demonstrated the efficiency of *Spartina* cuticle measurements as passive samplers for assessing the pace of natural recovery in marsh systems.
CHAPTER ONE

Introduction and Literature Review

1.1 Background

The U.S. Gulf coast is a great reservoir of natural gas and oil for the country. In the past two decades, oil drilling activities in the Gulf along Louisiana’s coast have increased extensively (Thibodeaux et al., 2011). On April 20, 2010, the Deepwater Horizon (DWH) oilrig located about 50 miles off the coast of Louisiana suffered a catastrophic explosion while drilling (Thibodeaux et al., 2011). For 84 days the Macondo well released into the Gulf of Mexico approximately 4.9 million barrels of crude oil before it was capped on July 15 2010, making it one of the worst marine disasters in U.S. history (Crone and Tolstoy, 2010; Liu et al., 2014; McNutt et al., 2012).

About 2 million gallons of dispersant was applied to minimize the spread of oil and harmful environmental impacts of the spill to the marine and coastal ecosystem (Liu et al., 2014). However, due to its lower density compared to water, a major fraction (> 65%) rose to the sea surface after the spill and was evaporated or washed to the coastal shoreline (Ryerson et al., 2012). Dynamic physical forces and movement of gulf currents may have enhanced oil emulsification and mixing of non-contaminated waters with oil (Liu et al., 2014). Consequently, the spread of DWH oil lead to adverse environmental impact on the gulf and coastal ecosystems. Some immediate consequences/impacts of the oil spill were: oxygen depletion, oil contact with biota, acute fish toxicity, vegetation oiling, dispersion and advection by wind and water onto beaches, wetlands, bays, harbors, and estuaries (Thibodeaux et al., 2011).

The Gulf of Mexico coastline is dominated by salt marsh ecosystems that are vital habitats for fish, crabs, shrimp, snails, varieties of marsh vegetation, and other marsh species. These
ecosystems were vulnerable to not only the immediate effects of oil spills but also to persistent pollutants residing within the marsh biota for years following cleanup efforts. This is consequently due to the fact that only the exposed marsh edge comes into contact with the daily cleaning effects of tidal surges sufficient to subsequently wash away pollutants (Burger, 1994).

The occurrence of the DWH spill raised concerns over the release of persistent organic pollutants (POPs) such as polycyclic aromatic hydrocarbons (PAHs). Enhanced levels of PAHs were detected along the shoreline without visible traces of oil (Allan et al., 2012). The estimated 4.9 million barrels of the MC252 oil that was released into the gulf during the DWH oil spill contained approximately 3.9% PAHs by weight, an estimate of $2.1 \times 10^{10}$ g of PAHs were released during the spill (Reddy et al., 2012).

PAHs into the aquatic environment have been shown to be carcinogenic to human beings and wildlife (Neff, 1979). Even after oil is no longer visible, pollutants of concern can persist in the environment and affect exposed organisms. The measure of PAHs in an oil-contaminated site has been an indicator of contamination levels and environmental recovery after an oil spill (Allan, 2012). Therefore, it is vital to attain a significant understanding of the environmental fate of released PAHs in the Louisiana gulf coast marsh ecosystem.

The DWH oil spill damaged more than 650 miles of the gulf coast habitats (Liu et al., 2014). Among the areas impacted by the oil spill include Bay Jimmy located on the northern end of the Barataria Basin where large quantities of oil impacted its marsh ecosystem, Figure 1.1.
The shorelines were categorized and identified for remediation according to the maximum extent of oiling. Shoreline “K” in Bay Jimmy is host to ongoing studies of shoreline remediation and recovery (Zengel 2013).

After an oil spill the dominant vegetation are among the most affected primary producers in a coastal ecosystem (Burger, 1994). Consequently, once exposed, plants have the ability to uptake persistent organic pollutants such as PAHs and transfer them to higher trophic levels. Along with other prevalent marsh vegetation such as *Juncus roemerianus*, these marsh plants were exposed to heavy oiling after the DWH oil spill, (Lin and Mendelssohn, 2012). Studies show the potential of *Spartina alterniflora*, (a marsh vegetation) to uptake and accumulate PAHs via various mechanisms (Watts et al., 2006; Mohammad, 2015). In addition, the potential for PAHs uptake via leaf cuticle and vascular tissues of plants is a well-studied phenomenon (Desalme et al., 2013; Rotola et al., 2006).
1.1 Purpose of the Study

The purpose of this study was to examine the cuticular accumulation of petrogenic PAHs in *Spartina alterniflora* as an evident exposure pathway to marsh biota. The study area was Bay Jimmy marsh site located in the northern end of the Barataria Basin. Previous work of this location has demonstrated evidence of uptake and deposition of petrogenic PAHs into the cuticle and leaf tissues of *Spartina* (Mohammad, 2015). The objectives of this study were to determine the PAHs concentration accumulated in *Spartina* leaves. To measure the *Spartina* plant/air partition coefficient coupled with PAHs air measurements via a fugacity model. The use of semipermeable membrane devices as passive samplers for PAH on marsh surface and air canopy. And to determine whether alkyl-PAHs translocate (or volatilize) through aerenchyma or xylem coupled with partitioning to the *Spartina* cuticle.

1.2 Literature Review

1.2.1 Polycyclic Aromatic Hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) are a large group of well-studied organic contaminants of environmental concern due to their toxic, mutagenic and carcinogenic nature once exposed to human beings (Neff, 1979). PAHs comprise of more than 100 semi-volatile lipophilic organic compounds composed of two or more fused aromatic (benzene) rings. Fusing of aromatic rings occurs when a pair of carbon atoms is shared resulting into a structure of carbon and hydrogen atoms lying in a single plane (Neff, 1979). There are two types of PAH compounds: petrogenic and pyrogenic. The incomplete combustion of fossil fuels and biomass produces pyrogenic PAHs while petrogenic PAHs are naturally present in crude oil and consists primarily of alkylated versions of the ring structures present in PAHs of pyrogenic origin (Zakaria et. al, 2002)
PAHs can be emitted into the atmosphere by the incomplete combustion of biomass or fossil fuels. They are also one of the most toxic constituents of light crude oil comprising up to 10% of the organic compounds (Sammarco et al., 2013; Vinas et al., 2010). As semi-volatile compounds, PAHs can partition in the atmosphere between the gaseous and particulate phase allowing transportation in the atmosphere over long distances making them persistent in the environment (Desalme et al., 2013). Another reason for the persistence of PAHs in the environment is accredited to their lipophilic nature. Because of lipophilicity, PAHs easily accumulate in fats and oils. The lipid-rich nature of plant cuticles makes the accumulation of persistent organic pollutants such as PAHs very favorable. In the occurrence of an oil spill, PAHs present in the atmosphere or water can deposit on plant leaf surfaces and accumulate due to their strong affinity to the plant cuticle. Therefore, potential cuticle uptake of PAHs by Spartina after the DWH spill is considerable.

1.2.2 Saltmarsh Cordgrass: Spartina alterniflora

The smooth cordgrass Spartina alterniflora (alternately known as saltmarsh cordgrass, oyster grass or saltwater cordgrass) is a native emergent grass species in the Louisiana salt marshes also found along tidal salt marshes of the Atlantic and Gulf coasts. Spartina tends to grow parallel to and continuous along shorelines, commonly found growing on open coastal mashes between low and high tides. This native grass serves as food and habitat to a number of marsh birds, mammals and prevalent species in saltwater marshes.

Spartina is a long-lived warm season perennial grass that typically grows from 2 to 7 feet tall. It spreads extensively by long hollow rhizomes that anchor soft and spongy stems of up to ½ inch diameter with flat leaf blades (12 to 20 inches long) that taper to a long inward-rolled tip. During the months of September and October this smooth cordgrass produces seed-heads (10 to
12 inches long) that emerge at the end of the stems. Each spike will hold 12 to 15 spikelets that are 2 to 3 inches long. With an abundance of about 175,000 seeds per pound the flowers on the leaf blades are wind pollinated. There are two varieties of Spartina available on the commercial market. The ‘Bayshore’ Spartina released by in 1992 for use on the Atlantic coastal areas and the ‘Vermilion’ Spartina released in 1989 for use in the Gulf Coast areas.

As a native perennial cordgrass in marshes, Spartina is extensively utilized for erosion control along shorelines, canal banks, levees, and other soil water interfaces. Its plays an important ecosystem role as an effective soil stabilizer in areas of loose or unconsolidated soils accompanying marsh restoration. On tidal marshes, vigorous and abundant growth of this grass will absorb vigorous wave energy and screen suspended solids from intertidal waters while taking up available nutrients from the sediments. As sediment accumulates progressively, the grass will slowly spread away from the banks. Spartina can tolerate regular tidal floods with 0 to 35 parts per thousand salinity. It also has the ability to grow in petroleum contaminated soils.

1.2.3 Uptake of PAHs by vascular plants

The aftermath of oil spills leads to the exposure of organic pollutants to marine and coastal ecosystems. PAHs are a primary pollutant of concern in post-oil spill remediation efforts. As oil deposits and contaminates saltmarsh sediments there is potential for the uptake of PAHs via the root system, the soil-to-root pathway (Lin et al., 2007). Pot experiments and field studies have established the root uptake of organic pollutants for various plants and species, showing that plant accumulation of PAHs correlates with soil PAHs concentration and plant composition (Gao and Zhu, 2004).

However, the air to leaf pathway is a dominant transfer route of SVOCs present in the atmosphere to aboveground vegetation. This is because the surface area of leaves in contact with
air is about 6-14 times greater than the land the vegetation is growing on. The large leaf area of plants provides an extensive surface for gaseous exchange with the atmosphere. When associated with vegetation SVOCs such as PAHs may undergo different environmental fates such as photolysis, cellular metabolism, seasonal uptake and storage, movement into terrestrial food chains, re-release back to the atmosphere or transport into soil after senescing. The movement and location of the compounds in the plant will likely influence their fate, Figure 1.2.

**Figure 1.2**: PAHs exposure pathways for in-situ vascular plants (Desalme, 2013)

The occurrence and relative importance of the fate of compounds is controlled by the location of the compound within the plant and environmental conditions. In vascular plants, the leaf surface is protected by a waxy cuticle layer that increase the potential for interactions with compounds present in the atmosphere due to its properties. The leaf cuticle has a hydrophobic lipid structure synthesized by the epidermal cells. There are five general compartments within a vascular plant leaf: the epicuticular wax, the cuticle proper, the cuticle layer, pectinous layer, and the cell wall. However, it is generally believed that SVOCs remain/partition within the lipid-rich portion
of the plant, the leaf cuticle. The plant cuticle serves as the main interface for the exchange of PAHs between the air and vegetation. The cuticle has a great capacity for accumulation of organic pollutants although lipophilicities of its components may vary. Plant cuticles are mainly hydrophobic comprising of waxes and cutin, which consequently retain PAHs. Depolymerized lipids (cutin) are the major reservoirs of organic pollutants such as PAHs while the extractable lipids (wax) act as the anti-plasticizer that suppress the uptake by cutin (Chen et. al., 2008). Wild et al., (2006) gives a detailed review of the possible pathway mechanisms of SVOCs in a plant leaf.

Once retained on the leaf, PAHs undergo two fates: adsorption into the cuticle surface or absorption within the inner tissues. The adsorption into the cuticle is a reversible process linked to hydrophobicity and is temperature dependent. Whereas the latter is an irreversible process involving diffusion through the cuticle and stomata followed by absorption into the tissue membranes. The absorption of PAHs into the inner tissues is limited by the diameter of the cuticle pores especially for larger PAHs. Light (gaseous) PAHs such as phenanthrene and anthracene can easily diffuse through the cuticle and eventually be absorbed (Wild et. al., 2006), whereas the heavier PAHs remain mostly embedded within the cuticle. Heavy PAHs (predominantly bound to particles) can be readily washed off from the leaf because they diffuse at slow rates through the leaf cuticle. The nature of PAHs (whether gaseous or particle bound) and related physicochemical properties are key in determining the subsequent fate of PAHs in leaf tissues. A common way to distinguish adsorbed PAHs in the cuticle and those absorbed into the leaf tissues is by sequential extraction procedures (Wang et.al., 2008; Bakker et.al., 2001).
1.2.4 **Semipermeable membrane devices**

Passive samplers are a scientifically sound and cost-effective way of quantifying or predicting bioavailable fraction of hydrophobic contaminants in the dissolved phase. They sequester and accumulate the freely dissolved and bioavailable fraction of hydrophobic contaminants such as PAHs. Such a phenomenon mimics the passive uptake and accumulation of these contaminants by bio-membranes and lipid tissues present in the environment (Huckins et al., 2006).

Semipermeable membrane devices (SPMDs) are passive samplers often used as bioaccumulators of lipophilic environmental contaminants in aqueous, sediments and atmospheric media. Unlike biological systems, semipermeable membrane devices do not metabolize the sequester compounds, are site-specific, are much easier to extract, are not affected by poor environmental conditions and are therefore more suitable for bio-monitoring (EST Inc, 2016).

Semipermeable membrane devices are composed of a flat, low density polyethylene tubing containing a thin film of a pure, high molecular weight lipid know as triolein. This polymer consists of transport corridors of less than 10Å in diameter which allows for selective diffusion of hydrophobic organic chemicals such as PAHs which are sequestered in the lipid phase. The typical field deployment time for these passive samplers is between two weeks to two months. However, depending on specific conditions and target objectives, these periods can be adjusted accordingly (EST Inc, 2016).

1.2.5 **Fugacity approach for foliage uptake of PAHs**

The atmospheric transport of pollutant to terrestrial ecosystems plays an important role on the fate of organic pollutants. As dominant species of most terrestrial ecosystems, plants have the
capability to uptake organic pollutants across the air-vegetation interface. As primary producers, plants provide the entry route to higher trophic levels therefore the atmospheric transfer of PAHs must be considered in understanding the transfer of PAHs through the food chain.

The transport of organic pollutants between gaseous and solid phase is a reversible partitioning process controlled by concentration gradients (Horstmann and McLachlan, 1992). The environmental partitioning of organic substances can be described by the fugacity concept (Mackay, 1979). The fugacity of a substance is related to its chemical potential and is described as the escaping tendency of a compound from a particular compartment (Mackay et.al., 1986). A high fugacity equals a high tendency for escape, thus compounds will diffuse across boundaries from areas of high fugacity to areas of low fugacity. The fugacity approach express concentrations in any medium as the thermodynamic equivalent of vapor pressure which can easily be compared between different matrices. This approach makes fugacity a useful concept in determining the state of pollutants contamination in environmental compartments (Horstmann and McLachlan, 1992).

Horstmann and McLachlan (1992) studied the fugacity of semivolatile organochlorine compounds on solid surfaces using spruce needles. The needle surface fugacities were determined indirectly using the concentration of the compound in a gas phase equilibrated with the needle surface. A fugacity meter was developed for studying the transport of the semivolatile contaminants into the plants and investigation of the fate of contaminants in solid phases. Other studies by Komp and McLachlan (1997) used the fugacity meter approach to investigate the plant/air partition coefficients at different temperatures. The fugacity meter measured the concentration of semivolatile organic compound in air in equilibrium with a solid phase. This is a big advantage of the fugacity meter method over other classical uptake experiments where plants
must be exposed for a prolonged period of time before a partitioning equilibrium is reached (Komp and McLachlan 1997).

In different models of the fugacity meter the core is usually a glass column where the plant materials are packed (Komp and McLachlan, 1997; Horstmann and McLachlan, 1992). An air stream is then passed through the column to establish an equilibrium between the plant surface and air. The air and the plants in the column are then collected and analyzed for semi volatile organic compounds. The quotient of the concentration in the plants and air gives the plant/air coefficient under conditions that a contaminant equilibrium between the surface and the interior of the plants was established.
CHAPTER TWO

Mechanisms of *Spartina* PAHs Cuticle Accumulation in Coastal Wetlands

2.1 Introduction

An estimate of 4.9 million barrels of MC252 crude oil was released into the Gulf of Mexico after the Deepwater Horizon blowout that occurred from April 20 – July 15, 2010. Among the most significant aftermath of this historical marine oil spill was the pollution of 1773 km of the Gulf of Mexico shoreline. 44.9% of the oil impacted shoreline were costal wetland and salt marshes. Immediate clean-up efforts were authorized on about 8.9% of impacted marshes and associated habitats (Michel et al. 2013). Depending on initial conditions of the ecosystem, the negative effects of oil spills from the introduction of petroleum hydrocarbons can persist for many years. As of June 2013, the average concentrations of total polycyclic aromatic hydrocarbons in these gulf shoreline marshes was 374 time pre-oiled conditions. Turner et al. 2014 suggests that it may take many decades for these concentrations to reach baseline levels if no additional oiling occurs.

Polycyclic aromatic hydrocarbons (PAHs) represent some of the most toxic constituents of light crude oil and has the potential to bio-accumulate into marine invertebrates and vegetation (Sammarco et al. 2013). With a bio-accumulation potential, exposed marsh vegetation can serve as reservoirs of PAHs from either atmospheric deposition (Desalme et al., 2013), transfer via soil to root pathways ((Lin et al., 2007; Gao and Zhu, 2004), or the direct deposition of crude oil on the plants leaf surfaces. Understanding the uptake of PAHs is vital since plants provide the exposure route to higher trophic levels as primary producers. Plants also serve as bio monitors of atmospheric concentrations of organic pollutants such as PAHs. Such use is derived from the fact
that vegetation accumulates atmospheric organic pollutants in their leaves generally in proportions that correlate with atmospheric concentrations (Desalme et al., 2013; Wang et. al., 2008; Collins et al., 2006).

The interest of this work was to investigate the foliage uptake of PAHs by coastal saltmarsh cordgrass, *Spartina alterniflora* after the Deepwater Horizon oil spill. For marshes impacted by the MC252 oil attention calls to the potential of this abundant vegetation specie to uptake and accumulate PAHs present in the oil. It is hypothesized that PAHs volatilized from oil depositions in the marsh soil can re-partition and accumulate into the waxy cuticle of *Spartina*. Also foliage uptake and accumulation of PAHs by *Spartina* can arise from the deposition of oil from tidal inundations.

Accumulation of semi-volatile organic compounds such as PAHs into the cuticle and tissues of plants is not a foreign phenomenon. PAHs can accumulate in leaf cuticles due to the lipophilic nature of the compounds. Studies show that plant cuticles are composed of waxes and cutin that are hydrophobic with the potential for PAHs retention. However, this retention capacity is largely controlled by leaf surface morphology, stomatal opening, and cuticle composition (Desamle et al. 2013).

To serve as surrogates in a parallel study of the accumulation and partitioning of PAHs in leaf of *Spartina*, semi-permeable membrane devices (SPMDs) were deployed in the marsh surface and canopy. SPMDs are passive samplers composed of a flat, low density polyethylene tubing containing a thin film of a pure, high molecular weight lipid know as triolein. Triolein, a lipid polymer, allows the selective diffusion of hydrophobic organic chemicals within which PAHs become “sequestered” in the lipid or solution phase.
The mechanisms of the polyethylene membrane to sequester hydrophobic organic chemicals such as PAHs is phenomenologically similar to the size limiting transport of hydrophobic contaminants through bio-membranes via respiration (Oppenhuizen et al., 1985). Studies show that SPMDs appear to simulate key portions of the respiratory uptake of chemicals. The utility of SPMD-derived data in providing bioavailability information has been suggested by (Huckins et al., 1990a and Huckins et al., 1990b; Petty et al., 2000).

The objectives of this study was to investigate the mechanisms of PAHs uptake and accumulation of *Spartina alterniflora* by quantification of PAHs concentrations in the leaf surface, cuticle and tissues. As a parallel study PAHs concentrations in semipermeable membrane device were determined for comparison. The purpose of incorporating semi-permeable membrane devices as passive samplers was to understand the dominant uptake mechanisms of PAHs in *Spartina*. An interesting suggestion of the study is that the uptake of PAHs in marsh wetlands impacted by an oil spill is from the volatilization of PAHs from oil deposits in soil or water as compared to oil deposition on the plant surfaces from recurrent tidal inundations, Figure 2.1.

**Figure 2.1:** Hypothesized exposure pathway of PAHs into *Spartina*
2.2 Materials and Methods

2.2.1 Site location and sampling

Sampling was conducted at Bay Jimmy marsh in Northern Barataria Basin, Louisiana. Plant and soil samples were collected from two adjacent sites, Figure 2.2. This marsh was among the areas impacted by the oil spill when the MC252 crude oil first reached coastal ecosystems. Oil carried over by tidal inundations in the 2010 spill event spread to this coastal marsh ecosystem leaving its traces behind till present day.

![Figure 2.2: Sampling sites at Bay Jimmy](image)

At Bay Jimmy, samples were collected from two adjacent sites assigned as site 1 and site 2. During the oil spill both sites were heavily impacted by oil, however during the sampling period there was not visible evidence of oil at site 1. On the other hand, visible evidence of oil at site 2 was asphalt and soil amalgam along the marsh edge and shoreline. From each site, samples were collected from three plots along the marsh shoreline. The plots were designated as C1, C2, C3 for site 1 and as O1, O2, O3 for site 2. Sampling was done in triplicates at each sampling plot. For each sampling event, nine *Spartina alterniflora* leaf samples were collected from the two sites,
therefore yielding a total of 18 samples. Sampling was between June 9, 2016 and Feb 3, 2017 at intervals of 19-72 days.

2.2.2 Field extraction of Spartina leaf cuticle

Baker et. al., (2000) performed a sequential extraction analysis on the deposition of PAHs in leaves of Plantago. A similar sequential extraction method was implemented for the analysis of PAHs in Spartina surface, cuticle, and tissue. The extraction process was initiated immediately after sampling at each location. Plant leaves were placed in glass jars and shaken with 50 ml of EDTA solution (0.03 M, pH = 5) for about 2 minutes. This initial washing of leaves with EDTA solution served to mobilize any high molecular weight PAHs on the surface of the leaves (Bakker et.al., 2000). Following which, leaves were taken out of the EDTA solution and placed in glass jars containing 50 ml of pesticide-grade dichloromethane (DCM) and shaken again for 2 minutes (Wang et. al., 2008). This served as the second phase of the sequential extraction, which dissolves the leaf cuticle that may contain PAHs localized within it. The leaves were then placed in Ziploc bags and transported to the lab where they were stored at 4 °C prior to the complete extraction of the leaf tissue. Once at the lab the EDTA and DCM solvent extractions were stored in a cool location away from direct sunlight to avoid the potential of photolysis breakdown of present PAHs.

2.2.3 Sequential extraction method

The EDTA solution was exchanged in a separatory funnel with cyclohexane. The EDTA solution was shaken for about 1 minute with 50 ml of cyclohexane and allowed to settle. Once the immiscible mixture settled, the bottom layer (EDTA solution) was separated from the top layer (cyclohexane). The EDTA solution was drained and discarded while cyclohexane was retained for further analysis (Bakker et. al., 2000). The cyclohexane solution was evaporated at 70 °C to 1.5 ml in a RapidVap N2 Evaporation System (Labconco, USA) and then exchanged with a 1:1 hexane
acetone solution. It was again evaporated at 70 °C to a final volume of 3 ml then stored in a scintillation vial following the use of 1 ml of the sample for gas chromatography-mass spectrophotometry (GC/MS) analysis for the presence of PAHs.

Similarly, the second extraction phase with the 50 ml of DCM was evaporated at 30° C to 1.5 ml in a RapidVap N2 Evaporation System (Labconco, USA) then exchanged with a 1:1 hexane acetone solution and once again evaporated at 70 °C to a final volume of 3 ml and stored in a scintillation vial. Following the use of 1 ml of the sample for gas chromatography-mass spectrophotometry analysis of the presence of PAHs.

The third extraction phase was the complete extraction of the plant leaf tissue using the accelerated solvent extraction method. This involved the use of an Accelerated Solvent Extractor (ASE) which extracts elements from the leaf tissues utilizing a 50:50 mixture of hexane acetone under 17000 psi of pressure at 100 °C. In preparation of accelerated solvent extraction, the leaves were chopped into pieces, weighed, placed in a beaker, and mixed with sodium sulfate and magnesium sulfate to dry out the moisture content. The mixture was loaded into stainless steel cells after which the cells were loaded onto the accelerated solvent extractor. The extracted solvent was evaporated to 3 ml at 70 °C and passed through silica gel columns for solid phase extraction (SPE) cleanup process.

2.2.4 PAH recovery experiment

Prior to executing solid phase extraction (SPE) cleanup process on the collected field samples, PAHs recovery experiments were conducted to test the method. A PAH standard solution of 5 ng/µL was prepared using 1:1 hexane acetone as a solvent at 3 ml. The standard solution was used to test the recovery of the SPE cleanup process. A silica gel column was first activated with 3 ml of 2:3 hexane acetone solution. Following which, the 3ml of the standard solution was loaded
through the silica gel column. 10 ml of the 2:3 hexane acetone solution was used to elute the sample. The eluted solution was evaporated at 70 ºC to 1.5 ml and exchanged with a 1:1 hexane acetone solution and concentrated to a final 3 ml. 1 ml was used for GC analysis of present PAHs.

2.2.5 Soil sampling and analysis

Soil samples were collected from both sites at Bay Jimmy on select dates. At the field the samples were collected and stored in class jars. Once at the laboratory the soil samples were extracted using the Accelerated Solvent Extractor (ASE) which extracts elements from the leaf tissues utilizing a 50:50 mixture of hexane acetone under 17000 psi of pressure at 100 ºC. About 10 grams of the soil was mixed with sodium sulfate and magnesium sulfate to dry out the moisture content. The mixture was loaded into stainless steel cells after which the cells were loaded onto the accelerated solvent extractor. The extracted solvent was evaporated to 3 ml at 70 ºC. 1 ml of the sample was used for GC/MS analysis of present PAHs.

2.2.6 Field deployment of semipermeable membrane devices

Semipermeable membrane devices purchased from Environmental Sampling Technologies were set up at both sampling locations at Bay Jimmy. These devices are composed of a lay flat, low density polyethylene tubing containing a thin film of pure, high-molecular weight lipid triolein (1,2,3-tri-[cis-9-octadecenoyl]glycerol) (Cranor et al. 2009). This polymer allows for the diffusion of hydrophobic organic chemicals such as PAHs which are consequently sequestered in the lipid phase, (EST Inc, 2016). Two devices were set up on the three designated plots at both sites; one as a passive air sampler and another on the marsh shoreline as a surface sampler, Figure 2.3. With three plots at each sampling location, a total of 12 devices were deployed for each deployment period. A field blank was kept as a control sampler.
The semipermeable membrane devices set on the marsh surface near the shoreline were deployed in a standard 91.4 cm stainless steel carrier fastened inside a 15 cm high x 16 cm wide stainless steel canister. Fixing the semipermeable membrane devices within the stainless steel canister allows for a fixed sampling location, thus eliminating problems from aquatic life disturbances. The air samplers were fastened inside perforated cans similar in size to the canisters to allow air entrance and circulation. These were mounted on steel rods standing 4 feet high. The incubation period for the samplers after deployment was set between three to six weeks.

**Figure 2.3:** Schematic of semi-permeable membrane devices deployment and setup

2.2.7 *Dialysis of semipermeable membrane devices*

After the incubation period, the semipermeable membrane devices were removed from the storage container and cleansed immediately and cleaned by scrubbing the surface with a gloved hand proceeded by sequential solvent rinsing. First, with dilute hydrochloric acid to remove any salts, followed by de-ionized water, then a quick surface rinse with acetone then a final rinse with hexane. The cleaned devices were then placed in a contaminant-free, air-tight glass jar of a sufficient volume of hexane. Once in the lab the dialysis containers were placed in an incubator at 18 °C for 24 hrs. After this initial dialysis, the hexane was transferred into a separate container and a second portion of hexane was added and incubated at 18 °C for another 24 hrs. Following which,
both volumes of hexane were combined and the semipermeable membrane devices discarded (Alvarez, 2010).

Following dialysis, the hexane was evaporated at 70 °C to 1.5 ml in a RapidVap N2 Evaporation System (Labconco, USA) and then exchanged with a 1:1 hexane acetone solution and once again evaporated at 70 °C to 3 ml and stored in a scintillation vial. Following the use of 1 ml of the sample for gas chromatography-mass spectrophotometry analysis of the presence of PAHs.

2.2.8 Gas Chromatography - Mass Spectrometry Analysis of PAHs

1 ml of all extracted samples were analyzed using a Hewlett Packard 6800 N gas chromatograph with a HP 6890 series autosampler, DB-5 capillary column (30m x 0.25mm X 0.25μm film), and HP 5973 mass selective detector. The injector temperature was set at 300°C, the detector at 280 °C, and oven temperature was at 45 °C for 3 minutes, and then the temperature was increased to 6 °C/minutes to 315°C, and the temperature was held for 15 min. The carrier gas utilized was helium at 5.7 mL/min. A selected ion-monitoring mode was used for quantification based on internal standards. Quality control was maintained by running blanks (1 mL hexane/acetone at 50:50, 5 μL internal standard) analyzed at the beginning and end of each run. Calibration standards of a known concentration of PAHs were also included in each run with samples. PAHs targeted from analysis include are listed in Table 2.1.

Table 2.1: Polycyclic aromatic hydrocarbons targeted for analysis

<table>
<thead>
<tr>
<th>naphthalene</th>
<th>acenaphthene</th>
<th>phenanthrene</th>
<th>dibenzothiophene</th>
<th>C1-pyrene/fluoranthene</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1-naphthalenes</td>
<td>anthracene</td>
<td>C1-phenanthrenes</td>
<td>C1-dibenzothiophenes</td>
<td>C1-chrysenes</td>
</tr>
<tr>
<td>C2-naphthalenes</td>
<td>fluorene</td>
<td>C2-phenanthrenes</td>
<td>C2-dibenzothiophenes</td>
<td>C2-chrysenes</td>
</tr>
<tr>
<td>C3-naphthalenes</td>
<td>C1-fluorene</td>
<td>C3-phenanthrenes</td>
<td>C3-dibenzothiophenes</td>
<td>C3-chrysenes</td>
</tr>
<tr>
<td>C4-naphthalenes</td>
<td>C2-fluorenes</td>
<td>C4-phenanthrenes</td>
<td>Fluoranthene</td>
<td>hopanes</td>
</tr>
<tr>
<td>acenaphthylene</td>
<td>C3-fluorenes</td>
<td>chrysene</td>
<td>pyrene</td>
<td></td>
</tr>
</tbody>
</table>
2.3 Results and Discussion
2.3.1 PAHs in Spartina leaves

PAHs were detected and measured as average concentrations in the Spartina leaf surface, cuticle, and inner tissues. The PAHs included naphthalene, C1-naphthalenes, C2-naphthalenes, C3-naphthalenes, C4-naphthalenes, phenanthrene, C1-phenanthrenes, C2-phenanthrenes, C3-phenanthrenes, C4-phenanthrenes. However, PAHs concentrations detected and measured in highest quantity on the leaf surface, cuticle, and inner tissue at both sites were C1-naphthalenes, C2-naphthalenes, and C1-phenanthrenes, Figure 2.4. Tables A.1 and A.2 in the Appendix presents the measured average PAH concentrations on surface, cuticle, and inner tissues of Spartina leaves from both sites for all sampling dates.

The temporal trends in average concentrations of C1- naphthalenes, and C2- naphthalenes on the surface, cuticle, and inner tissue of Spartina leaves at site 1 and site 2 are presented in Figure 2.5. Average concentrations of the alkylated PAHs were about an order of magnitude higher in tissues than on the surface and cuticle of Spartina leaves. The reasons for such an observation is PAHs concentrations on surface, cuticle and tissues were normalized to the total leaf mass. Challenges in the determination of the leaf cuticle mass resulted into normalizing the cuticle mass to total leaf mass, leading to subsequently lower concentrations in the cuticle compartment. Also, low recovery results of PAHs following solid phase extraction cleanup of leaf tissues was second contributor to higher tissue concentrations. The recoveries for naphthalenes and phenanthrenes after solid phase extraction cleanup of the leaf tissues were 13% and 30% respectively.
Figure 2.4: Profile of the sum of PAHs detected in surface, cuticle, and tissue of *Spartina* leaves at site 1 and site 2

Naphthalenes and phenanthrenes concentrations in plant tissues were about an order of magnitude higher than in cuticles. These results suggest that the interior of the *Spartina* leaf may serve as a significant seasonal sink for volatile lower molecular weight PAHs. Once deposited on the surface of the leaf PAHs can be adsorbed into the cuticle and subsequently diffuse into the leaf interior (Desalme, 2013). Diffusion into the leaf tissues is probable due to the small thickness (0.5 µm) of the *Spartina* leaf cuticle (Maricle et al., 2009).
Figure 2.5: Average PAH concentrations of C1-naphthalenes, C2-naphthalenes on the surface, cuticle, and inner tissue of *Spartina* leaves at site 1 and site 2 (error bars signify the standard error of the mean from nine samples from each site)
On average naphthalenes, accumulation in the inner leaf tissues appeared to be slightly higher in site 2 for all sampling dates. At site 2, temporal trends for C1-naphthalenes in Spartina tissues exhibited that concentrations in June and July were on average 7.52 ± 1.55 µg/g leaf and 6.2 ± 1.5 µg/g leaf respectively. A decrease to averages of about 5 µg/g leaf was observed between September and December following an increase to an average of 7.74 ± 1.9 µg/g leaf in February. Slightly lower C1-naphthalenes concentrations were observed at site 1, Figure 2.5. Generally, temporal trends at site 1 revealed an increase in C1-naphthalenes from warmer to cooler months. At both sites, lowest concentrations were observed in September. On average, C1-naphthalenes average concentrations were about twice greater than C2-naphthalenes in the cuticle and tissues of Spartina at both sites. Slightly higher concentrations were observed at site 2. Temporal variabilities were similar to those observed for C1-naphthalenes.

Phenanthrenes concentrations on Spartina leaf surface, cuticle and tissue are presented in Figure 2.6. Generally, cuticular accumulation of phenanthrenes was about half that of naphthalenes accumulation in Spartina cuticle. Phenanthrenes are higher molecular weight PAHs compared to naphthalenes, resulting to lower accumulation amounts in the leaves (De Nicola et al., 2005; Desalme et al., 2013). Also, an accumulation of about an order of magnitude greater was observed for C1-phenanthrenes (m.w. = 192) as compared to C2-phenanthrenes (m.w. = 206) at both sites. A slightly higher accumulation was observed at site 1 compared to site 2. Similar temporal variabilities of naphthalenes were observed with phenanthrenes accumulation.

Numerous factors can account for temporal variability in PAHs accumulation in plants. Variation in temperature can influence the volatilization and partitioning of PAHs from the air and into vegetation. Season changes in plant life cycle can also influence this variability. A thicker cuticle/lipid layer in mature plants can favor higher PAHs accumulation (De Nicola et al.,
Figure 2.6: Average PAH concentrations of C1-phenanthrenes, and C2-phenanthrenes on the surface, cuticle, and inner tissue of *Spartina* leaves at site 1 and site 2 (error bars signify the standard error of the mean from nine samples from each site).
However, these factors may not be sufficient to explain the temporal variability observed in PAHs accumulation by Spartina leaves at Bay Jimmy marsh. Dynamic marsh such as wet-then-dry processes from periodic flooding-then-dried conditions may be a contributing factor to these variations (Yates et al., 1997).

![PAHs profile in soil samples at site 1 and site 2.](image)

**Figure 2.7**: PAHs profile in soil samples at site 1 and site 2.

PAHs profile in the marsh soil are presented in Figure 2.7. Observed PAHs after soil analysis included naphthalenes and phenanthrenes at low quantities. Soil analysis was a means of determining the uptake mechanisms of PAHs in *Spartina*. The observed naphthalenes concentrations in the plant leaves are about an order of magnitude greater than in the soil. Naphthalenes and phenanthrenes fractions to total PAHs in soil and soil/asphalt mixture were determined at both sites. Low fractions of < 20% for naphthalenes and phenanthrenes were
observed at both sites. Such low fractions of these PAHs in soil as compared to plant leaves suggested that root uptake may not be a potential pathways for the accumulation of PAHs in *Spartina*.

### 2.3.2 PAHs in semipermeable membrane devices (SPMDs)

The measured average mass accumulation rates (ug/day) of PAHs by semipermeable membrane devices (SPMDs) are listed in Table A.3 in the Appendix. SPMDs were deployed in the marsh canopy and surface with the aim of comparing the PAH concentrations in air and marsh surface to PAHs in the surface, cuticle and inner tissues of smooth cordgrass *Spartina*. The PAHs observed include naphthalene, C1-naphthalenes, C2-naphthalenes, C3-naphthalenes, C4-naphthalenes, phenanthrene, C1-phenanthrenes, C2-phenanthrenes, C3-phenanthrenes, C4-phenanthrenes, Figure 2.8. The PAHs profile in air and surface SPMDs at both sites were similar to those observed in *Spartina* leaves. Naphthalenes were observed at highest quantities compared to phenanthrenes. C1-naphthalenes were highest in air SPMDs while in surface SPMDs naphthalene and C1-naphthalenes were in highest quantity. Similar PAH profile in SPMDs and plants suggested that PAHs uptake in *Spartina* leaves may be from the air phase.

Comparisons were made between average air and surface SPMD PAHs mass in µg per SPMD for each sampling date at both sites, Figure 2.9. C1-naphthalenes were about 5 times greater in quantity compared to C2-naphthalenes and phenanthrenes in air and surface SPMDs at both sites. Phenanthrenes were detected in the lowest amounts in the SPMDs. This observation was similar to concentrations in plant leaves. The SPMDs polyethylene membrane ability to sequester lipophilic compounds like PAHs is similar to uptake in leaf cuticular waxes. Lower molecular weight PAHs are likely to be accumulated than heavier PAHs. At site 1, C1-naphthalenes in air SPMDs were about three times greater than in surface SPMDs. Similar C1-naphthalenes
concentrations were observed at site 2. Observed comparisons between air and surface SPMDs showed little variability in PAHs concentrations.

![Graph showing PAHs concentrations in air and surface SPMDs](image)

**Figure 2.8**: Profile of PAHs detected in air and surface SPMDs deployed at site and site 2.

Comparisons were made between average PAHs (µg per SPMD) in air and surface SPMDs for each sampling date at both sites, Figure 2.9. C1-naphthalenes were about 5 times greater in quantity compared to C2-naphthalenes and phenanthrenes in air and surface SPMDs at both sites. Phenanthrenes were detected in the lowest amounts in the SPMDs. This observation was similar to concentrations in plant leaves. The SPMDs polyethylene membrane ability to sequester lipophilic compounds like PAHs is similar to uptake in leaf cuticular waxes. Lower molecular weight PAHs are likely to be accumulated than heavier PAHs. At site 1, C1-naphthalenes in air
SPMDs were about three times greater than in surface SPMDs. Similar C1-naphthalene concentrations were observed at site 2. Observed comparisons between air and surface SPMDs showed little variability in PAHs concentrations.

Higher naphthalene concentrations in air and surface SPMDs, plant cuticle and tissues suggested the cycling of naphthalenes within the marsh ecosystem. Observation of PAHs in air and surface SPMDs suggests that volatilization of PAHs from oil residuals and oiled marsh soil in close proximity is a likely source of PAHs cycling in the marsh sites. The similar observations of PAHs profiles in *Spartina* leaves and SPMDs suggests the use of SPMDs as surrogates to understanding the partitioning process of PAHs between air phase and vegetation (Amdany et al., 2014, Piccardo et al., 2010).
Figure 2.9: Comparisons between PAHs mass in air SPMD and surface SPMDs at site 1 and site 2, (error bars signify the standard error of the mean from three SPMDs at each site).
The temporal trends in the average mass per SPMD for naphthalene, C1- naphthalene, and C2- naphthalene in SPMDs deployed in air and marsh surface at site 1 and site 2 are presented in Figure 2.10. Trends of alkylated and parent naphthalenes exhibited a decrease in both air and surface SPMD mass from July to February at both sites. Similarly, Figure 2.11 presents the average mass per SPMD of the alkylated and parent phenanthrenes in SPMDs deployed at the marsh.
surface and air canopy at both sites. At site 1, C1-phenanthrenes in air and surface SPMDs exhibited a decreasing trend from July to Feb. However, trends at site 2 exhibited an increase in phenanthrenes towards February.

**Figure 2.11**: Average PAH mass per SPMD of phenanthrene, C1-phenanthrenes, and C2-phenanthrenes in SPMDs deployed in air and on marsh surface at site 1 and site 2 (error bars signify the standard error of the mean from three SPMDs at each site).

Generally, variability in temporal trends of PAHs accumulation in SPMDs were observed comparable to accumulation in *Spartina* leaves. Although accumulation in air SPMDs was slightly higher than in surface SPMDs, naphthalenes accumulation was similar in air and surface SPMDs.
This observation was also made for phenanthenes accumulation in air and surface SPMDs. The little variability between PAHs accumulation in air and surface SPMDs suggested the following: i.) surface SPMDs anchored on the marsh shoreline may have been exposed to periodic wet-then-dry processes where submersion in marsh surface water was less frequent. ii.) absorption on PAHs in surface SPMDs could be from colloidal particles deposited on the SPMDs. iii.) both air and surface SPMDs were able to sequester volatile PAHs present in oil residuals and oiled marsh soil.

2.3.3 Correlation relationship between plants and SPMDs PAHs

The relationship between PAHs in the leaf cuticle and inner tissues of Spartina and SPMDs was statistically determined for alkylated naphthalenes and phenanthenes with one and two methyl groups. A positive relationship between the plants and SPMDs supports the use of passive samplers as surrogates for studying the presence of PAHs in polluted systems. Figure 2.12 presents the relationships between C1 and C2 naphthalenes in Spartina leaf cuticle and air and surface SPMDs at both sites. High correlation relationships (R^2 = 0.96 and R^2 = 0.93 p<0.05) for C1 and C2 naphthalenes in Spartina leaf cuticle and air SPMDs were observed at site 1 and site 2 respectively. Statistically significant correlations were not observed for naphthalenes in Spartina cuticle and surface SPMDs.

Relationships were also determined for C1 and C2 naphthalenes in Spartina leaf tissue and in air and surface SPMDs at both sites, Figure 2.13. Highest correlations (R^2 = 0.91 p<0.05) were observed for naphthalenes in the tissues and air SPMDs at site 1. However, the alkylated naphthalenes were less correlated (R^2 = 0.70 p<0.05) in tissues and air SPMDs at site 2. High correlations (R^2 = 0.97 p<0.05), were observed for the alkylated naphthalenes in Spartina tissues and surface SPMDs at site 1 compared to site 2.
In addition, correlation relationships at p<0.05 were observed for C1 and C2 phenanthrenes in *Spartina* leaf cuticle and tissues in air and surface SPMDs, Figure 2.14 and Figure 2.15 respectively. Fewer correlations were observed for phenanthrenes compared to naphthalenes since phenanthrenes accumulation in plant leaves was lower than naphthalenes. Higher correlations were observed in cuticle and air SPMDs at site 2. Lowest correlation relationships were observed for phenanthrenes in *Spartina* tissues and SPMDs at both sites.

Generally, naphthalenes exhibited the best correlations than phenanthrenes accumulation in plant leaves and SPMDs. The presence of high correlation relationships of the same PAHs in plants interior to those in air and surface SPMDs confirmed the cycling of PAHs in the polluted marsh sites. High correlations of PAHs sequestered in air SPMDs to those in cuticle and tissue of *Spartina* leaf suggested that PAHs accumulation in *Spartina* leaf interiors is likely from the air phase form the volatilization of lower molecular weight PAHs.

A common application of SPMDs is in air sampling of lipophilic contaminants in the air phase. The challenges of using SPMDs as surface samplers in the marsh shoreline is the variability of their exposure to tidal inundations. It is speculated that surface SPMDs may have been exposed to marsh surface water and soils during periods of high tide then left to dry at low tide periods. This variability could be an influence in the lack of high correlations for PAHs in the plants leaves and surface SPMDs. In addition, the deposition of adsorbed PAHs in colloidal and soil particulates may suggest the higher PAHs accumulation in surface SPMDs compared to plant tissues. The lack of correlations can be influenced by a greater accumulation in surface SPMDs due to this factor (Amdany et al., 2014).
Figure 2.12: Correlations of C1 and C2 naphthalenes in air SPMDs versus Spartina leaf cuticle at site 1 (a) and site 2 (b). Correlations of C1 and C2 naphthalenes in surface SPMDs and Spartina leaf cuticle at site 1 (c) and site 2 (d). Positive correlations ($p < 0.05$)
Figure 2.13: Correlations of C1 and C2 naphthalenes in air SPMDs and Spartina leaf tissue at site 1 (a) and site 2 (b). Correlations of C1 and C2 naphthalenes in surface SPMDs and Spartina leaf tissue at site 1 (c) and site 2 (d). Positive correlations (p < 0.05)
Figure 2.12: Correlations of C1 and C2 phenanthrenes in air SPMDs and *Spartina* leaf cuticle at site 1 (a) and site 2 (b). Correlations of C1 and C2 phenanthrenes in surface SPMDs and *Spartina* leaf cuticle at site 1 (c) and site 2 (d). Positive correlations (p < 0.05)
Figure 2.15: Correlations of C1 and C2 phenanthrenes in air SPMDs and *Spartina* leaf tissue at site 1 (a) and site 2 (b). Correlations of C1 and C2 phenanthrenes in surface SPMDs and *Spartina* leaf tissue at site 1 (c) and site 2 (d). Positive correlations (p < 0.05)
2.4 Conclusion

The data showed that parent and alkylated naphthalenes and phenanthrenes accumulated in the cuticle and inner tissues of *Spartina* as well as in SPMDs deployed at both sites at Bay Jimmy. The PAHs included naphthalene, C1-naphthalenes, C2-naphthalenes, C3-naphthalenes, C4-naphthalenes, phenanthrene, C1-phenanthrenes, C2-phenanthrenes, C3-phenanthrenes, and C4-phenanthrenes. There was not a difference in the amount of PAHs detected in the plants and SPMDs between the site 1 and site 2. This suggests that although oil was not visible at site 1 as in site 2 there was the presence of a significant source of PAH volatilization into the air phase. These findings supported the hypothesis that even years after an oil spill has occurred the lack of visible evidence of oil does not dispute the presence of persistent organic pollutants within the ecosystem.

Good correlations of PAHs accumulation in *Spartina* leaves and air SPMDs at the marsh sites explained the cycling of the organic contaminants at the Bay Jimmy marsh almost 6 years post Deepwater Horizon oil spill. It was suggested that the likely source of PAHs is in the air phase. Analysis revealed that the lower molecular weight C1 naphthalenes (MW=142) and C2 naphthalenes (MW=156) were present in larger quantities in cuticle, tissue, and SPMDs as compared to the other PAHs. C1 and C2 naphthalenes were sequestered more in the tissues as compared to cuticles of *Spartina*. Soil analysis revealed that naphthalenes and phenanthrenes fractions in soil were less than 2% of total PAHs suggesting that accumulation in *Spartina* leaves is likely from the air phase by foliage uptake rather than via the roots.

Lower molecular PAHs have the ability to be accumulated in cuticle and tissues of plants as compared to high molecular weight PAHs (De Nicola et al. 2005; Wang et al. 2008; Desalme
et al. 2013). In addition, the greater accumulation of PAHs in the tissue of *Spartina* leaf can be attributed to the thin 0.5 µm cuticle of *Spartina* (Diercks et al., 2010). The thin cuticle allows the diffusion of low molecular weight lipophilic PAHs into the inner tissue of the leaf. Because of the thin *Spartina* cuticle, challenges in measurement of the cuticle mass led to normalization of PAHs concentration in cuticle to the total leaf mass. Hence, greater accumulation was observed in *Spartina* tissues than in cuticle.

Temporal trends of observed PAHs revealed great variability of accumulation in *Spartina* leaves and SPMDs. The changes can be attributed to factors such as variation of temperature, which can affect the volatilization into air phase, and uptake of PAHs by plants and passive samplers. In addition, changes in the *Spartina* leaf sizes and morphology depend on the stage of its life cycle, which subsequently determines the leaf cuticle characteristics. Although *Spartina* is a perennial plant, the leaf size differs depending on the life stage. The temporal/seasonal variation of PAHs accumulation can also be attributed to this factor.

There was a greater accumulation of PAHs in air SPMDs at site 1 compared to surface SPMDs, while similar concentrations between air and surface SPMDs were observed at site 2. Strong correlation relationships were observed between cuticle and leaves of *Spartina* and SPMDs deployed on marsh surface and canopy. Relationships were strongest for lower molecular weight PAHs in tissues and air SPMDs. This relationship suggests that SPMDs can be used as surrogates for studying the PAHs accumulation in marsh ecosystems.

The analysis of post oil spill PAHs concentrations in marsh biota, as passive sampler is effective for assessing the pace of natural recovery in these marsh systems. Comparisons made to
a previous study by Mohammad (2015) at the same sites, shows that the PAHs concentration in the *Spartina* leaves were about an order of magnitude lower than in the previous study. This study showed that the application of SPMDs and *Spartina* leaves as passive samplers serves as a feasible monitoring method of PAHs cycling in polluted marsh following an oil spill.

### 2.5 Recommendations

1. This research work was conducted between June 2016 and February 2017. To understand better the temporal trends of the PAHs in the marsh future studies can conduct research over a 12 month period.

2. Development of effective methods of measurement of the cuticular mass of *Spartina* leaf could be effective in determination of PAHs accumulation in the leaf cuticle.

3. PAHs are hydrophobic in nature with a low solubility in water (Amdany et al., 2014). Submersion of SPMDs into water by anchoring to the marsh edge could lead to frequent exposure of the SPMDs to PAHs that may be present in marsh water phase.

4. Comparison of PAHs concentrations in SPMD after deployment to performance reference compounds (PRCs) could be effective in understanding the temperature variations in SPMD sampling, (Piccardo et al., 2010).
CHAPTER THREE

Partitioning of PAHs between air and *Spartina* leaf

3.1 Introduction

The transport process of contaminants between the environment and vegetation plays an important role in understanding the fate of pollutants. The transport of organic pollutants between the gaseous and solid phase is thought to be a reversible partitioning process that is controlled for concentration gradients (Horstman and McLachlan, 1992). The fugacity concept can be used to describe the partitioning of organic compounds (Mackay 1976). The fugacity of a compound is related to its chemical potential and can be described as the escape tendency of the compound. The fugacity concept has been applied to study the partitioning of semivolatile organic compounds from the air to vegetation such as Welsh ray grass (Tolls and MacLachlan, 1994), in rye grass (Komp and MacLachlan, 1997a) in grass and herb species (Komp and MacLachlan, 1997b).

The uptake of semivolatile organic compounds such as PAHs has been shown to occur primarily from the atmosphere. The deposition can occur through various mechanisms depending on the physical-chemical properties of the compound; three basic deposition mechanisms include gaseous deposition, particle bound deposition (wet and dry) and wet deposition of dissolved chemicals. The first two mechanism are driven by the gas/particle partitioning of the compound (MacLachlan, 1999). With gaseous deposition, the equilibrium partitioning results when the vegetation/gas-phase partitioning coefficient \( (K_{PA}) \) is relatively small \( (\log K_{PA} < 8) \) since the vegetation needs to extract a relatively small amount of semivolatile organic compounds from the air to attain equilibrium. As the \( K_{PA} \) increases \( (8 < \log K_{PA} < 9) \) the amount of compound needed
in vegetation to reach equilibrium with air will increase. As the value gets larger, the transfer of the chemical from the gas phase to the vegetation may be too slow for an equilibrium to be reached during the lifetime of the plant. At this point deposition is said to be transport limited therefore at high \( K_{PA} \) values (\( \log K_{PA} > 9 \)), partitioning of particles in the air is so large that the dominant uptake process by vegetation is particle-bound deposition (Poon et al. 2005; MacLachlan 1999).

Studies show that the vegetation/gas-phase equilibrium partition coefficient \( K_{PA} \) of many semivolatile organic compounds can be described as a function of \( K_{OA} \) where a linear relationship between the \( \log K_{PA} \) and \( K_{OA} \) was observed (Tolls and MacLachlan, 1994; Komp and MacLachlan, 1997a; MacLachlan 1999). The octanol-air partition coefficient (\( K_{OA} \)) is the ratio of the concentration of a semivolatile organic compound in octanol to its concentration in air. Hence, \( K_{OA} \) values can be used as a good predictor of the behavior of chemicals between the air phase and environmental compartments such as soil or vegetation.

The objective of this study was to quantify the concentration of PAHs in air and \textit{Spartina} leaf in the fugacity meter. To study the partitioning of PAHs between the plant and air phase using the fugacity meter by determining the plant/air partition coefficients \( K_{PA} \). And to compare the plant air/partition coefficients with octanol/air partition coefficients from literature.

\section*{3.2 Materials and Methods}

\subsection*{3.2.1 Fugacity meter}

A small laboratory scale fugacity meter was designed to studying the portioning of PAHs between the air phase and field samples of \textit{Spartina} leaves. The fugacity meter design was adopted from Horstmann and McLachlan (1992). The fugacity meter was set up inside a low temperature
(Thermo Electron Corporation Precision Model) incubator for temperature regulation. A schematic of the design is presented in Figure 3.1. At the heart of the fugacity meter was a 2 liter glass column where *Spartina* leaf samples were loosely packed. Clean air was first passed through a 6 g florisil trap to remove any contaminants. To maintain 100% relative humidity, the air stream was passed through a wash bottle filled with water. The air stream was subsequently passed through the glass column allowing an equilibrium between the air and plant surface. After leaving the column the air stream was passed through a final 6 g florisil trap to collect any PAHs that may have diffused from the plants to the air. A flowmeter was attached to measure the airflow through the system.

Two fugacity experiments were conducted at a temperature of 20 °C. For each experiment field samples were collected at Bay Jimmy oiled site and loosely packed into the glass column. Once at the lab, the fugacity meter was set up and air was passed through the system at a flowrate of 3.5 L/min. The airflow through the system and water in the glass wash bottle was frequently monitored to insure proper operation of the system. The first experiment was run for a total of 42 days. 28 days from the start date the florisil was removed for PAH analysis and new florisil was exchanged. The experiment was left to run for another 14 days before termination. The second experiment was set to run for 31 days. 19 days after the start date the florisil was removed and exchanged with new florisil. The experiment was left to run for another 12 days before termination. The plant leaves were removed at the end each experiment for analysis of PAHs.
Figure 3.1: Schematic of the fugacity meter for Spartina field samples setup inside an incubator

3.2.2 PAHs analysis in florisil and plant leaves

PAHs were analyzed in the florisil and plant leaves to detect concentrations in the air phase and in plant tissues. Once removed the florisil was shaken with about 100 mL of hexane to aid the exchange and transfer of PAHs (Horstmann and McLachlan, 1992). The hexane was evaporated at 70 °C to 1.5 ml in a RapidVap N2 Evaporation System (Labconco, USA) and then exchanged with a 1:1 hexane acetone solution and once again evaporated at 70 °C to a final volume of 3 ml and stored in a scintillation vial. Following the use of 1 ml of the sample dosed with 5µL of internal standards for gas chromatography-mass spectrophotometry analysis of the presence of PAHs.

At the end of the experimental run the plants were removed for the fugacity chamber and analyzed for presence of PAHs. The complete extraction of the plant leaf tissue was by the
accelerated solvent extraction method. This involved the use of an Accelerated Solvent Extractor (ASE) which extracts elements from the leaf tissues utilizing a 50:50 mixture of hexane acetone under 17000 psi of pressure at 100 °C. In preparation of accelerated solvent extraction, the leaves were chopped into pieces, weighed, and placed in a beaker mixing them with sodium sulfate and magnesium sulfate to dry out the moisture content. The mixture was loaded into stainless steel cells after which the cells were loaded onto the accelerated solvent extractor. The extracted solvent was evaporated to 3 ml at 70 °C and passed through silica gel columns for solid phase extraction (SPE) cleanup process similar to that discussed in Chapter 2. The final SPE cleanup volume was 10 ml of a 2:3 hexane acetone plant solution mixture. The solution was evaporated at 70°C to 1.5 ml and exchanged with a 1:1 hexane acetone solution and concentrated to a final 3ml. 1ml of the sample dosed with 5 µL of internal standards was used for gas chromatography-mass spectrophotometry analysis of the presence of PAHs.

3.2.3 Gas Chromatography - Mass Spectrometry Analysis of PAHs

1 ml of all extracted samples were analyzed using a Hewlett Packard 6800 N gas chromatograph with a HP 6890 series autosampler, DB-5 capillary column (30 m x 0.25 mm X 0.25 µm film), and HP 5973 mass selective detector. The injector temperature was set at 300°C, the detector at 280 °C, and oven temperature was at 45 °C for 3 minutes, and then the temperature was increased to 6 °C/minutes to 315 °C, and the temperature was held for 15 min. The carrier gas utilized was helium; at 5.7 mL/min. A selected ion-monitoring mode was used for quantification based on internal standards. Quality control was maintained by running blanks (1 mL hexane/acetone at 50:50, 5 µL internal standard) analyzed at the beginning and end of each run.
Calibration standards of a known concentration of PAHs were also included in each run with samples. PAHs targeted from analysis are listed in Table 3.1.

**Table 3.1**: PAHs targeted for analysis is fugacity experiment

<table>
<thead>
<tr>
<th>naphthalene</th>
<th>acenaphthene</th>
<th>phenanthrene</th>
<th>dibenzothiophene</th>
<th>C1-pyrene/fluoranthene</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1-naphthalenes</td>
<td>anthracene</td>
<td>C1-phenanthrenes</td>
<td>C1-dibenzothiophenes</td>
<td>C1-chrysenes</td>
</tr>
<tr>
<td>C2-naphthalenes</td>
<td>fluorene</td>
<td>C2-phenanthrenes</td>
<td>C2-dibenzothiophenes</td>
<td>C2-chrysenes</td>
</tr>
<tr>
<td>C3-naphthalenes</td>
<td>C1-fluorene</td>
<td>C3-phenanthrenes</td>
<td>C3-dibenzothiophenes</td>
<td>C3-chrysenes</td>
</tr>
<tr>
<td>C4-naphthalenes</td>
<td>C2-fluorenes</td>
<td>C4-phenanthrenes</td>
<td>Fluoranthene</td>
<td>hopanes</td>
</tr>
<tr>
<td>acenaphthylene</td>
<td>C3-fluorenes</td>
<td>chrysenes</td>
<td>pyrene</td>
<td></td>
</tr>
</tbody>
</table>

### 3.3 Results and Discussion

PAHs detected in the florisil and plant tissues are same to those detected in the cuticle and tissue of *Spartina* leaves. The plant-air partition coefficients ($K_{PA}$ in $\mu$g g$^{-1}$ /$\mu$gL$^{-1}$) for *Spartina* at 20 °C were calculated for naphthalene, C1-naphthalenes, C2-naphthalenes, C3-naphthalenes, C4-naphthalene, phenanthrene, C1-phenanthrenes, C2-phenanthrene, C3-phenanthrenes, and C4-phenanthrenes, Table 3.2. Parnis et al. (2015) estimated the octanol-air partition coefficient, $K_{OA}$ for these compounds at 25°C. $K_{OA}$ can be used to predict the partitioning of PAHs between the air and plant phase. The estimated $K_{OA}$ ranged between 4.81 (naphthalene) and 8.4 (C4-phenanthrene).

In both experiments the calculate log $K_{PA}$ values from the two fugacity experiments were similar for naphthalene, C1-naphthalenes, C2-naphthalenes, C3-naphthalenes, C4-naphthalene, and phenanthrene. However, values for C1-phenanthrenes, C2-phenanthrene, C3-phenanthrenes, and C4-phenanthrenes were much lower for the first experiment. The log $K_{PA}$ values for the presented PAHs were less than 8 an indication that PAHs were being extracted from the air phase.
and into to the plant tissues as equilibrium was attained in the fugacity meter. The estimated log $K_{OA}$ values appeared to be similar with the calculated log $K_{PA}$ values for naphthalene, C1-naphthalenes, and C2-naphthalenes, however, the trend is lost for the other PAHs.

**Table 3.2:** Calculated log $K_{PA}$ from fugacity meter experiments at 20 °C

<table>
<thead>
<tr>
<th>Compound (m.w.)</th>
<th>1st experiment (42 days)</th>
<th>2nd experiment (31 days)</th>
<th>log $K_{OA}$&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene (128)</td>
<td>4.3</td>
<td>4.2</td>
<td>4.81</td>
</tr>
<tr>
<td>C1-naphthalenes (142)</td>
<td>4.4</td>
<td>5.3</td>
<td>5.2</td>
</tr>
<tr>
<td>C2-naphthalenes (156)</td>
<td>5.0</td>
<td>5.5</td>
<td>5.6</td>
</tr>
<tr>
<td>C3-naphthalenes (170)</td>
<td>3.3</td>
<td>4.1</td>
<td>5.9</td>
</tr>
<tr>
<td>C4-naphthalenes (184)</td>
<td>3.7</td>
<td>3.5</td>
<td>6.4</td>
</tr>
<tr>
<td>Phenanthrene (178)</td>
<td>4.0</td>
<td>4.1</td>
<td>6.7</td>
</tr>
<tr>
<td>C1-phenanthrenes (192)</td>
<td>2.3</td>
<td>4.2</td>
<td>7.1</td>
</tr>
<tr>
<td>C2-phenanthrenes (206)</td>
<td>2.7</td>
<td>2.6</td>
<td>7.6</td>
</tr>
<tr>
<td>C3-phenanthrenes (220)</td>
<td>2.7</td>
<td>3.7</td>
<td>8.0</td>
</tr>
<tr>
<td>C4-phenanthrenes (234)</td>
<td>2.7</td>
<td>4.2</td>
<td>8.4</td>
</tr>
</tbody>
</table>

<sup>1</sup>Estimated log $K_{OA}$ at 25 °C (Parnis et al. 2015)

The second experiment that ran almost 10 days shorter than the first experiment gave more expected partition coefficients results for the PAHs with log $K_{PA}$ similar to the estimated log $K_{OA}$. The fugacity meter experiment performed well in studying the transport mechanism of PAHs between the air and plant leaves for naphthalenes. The behavior of these lower molecular weight PAHs was as expected for both experiments.
Partitioning experiment using the fugacity meter approach was effective in understanding the transport of PAHs from plant to air phase. Experimental results are key to help model the fate of PAHs in polluted marsh ecosystems. The partitioning process selectively discriminates against compounds with low hydrophobicity ($K_{OA}$) or low volatility ($K_{AW}$). Results showed that naphthalenes had the lowest $K_{OA}$ and $K_{PA}$ values indicating that transport of these compounds between the plant and air phase took place during the fugacity experiment. In addition, the low $K_{PA}$ values indicated that naphthalenes were accumulated in *Spartina* tissues.

$K_{PA}$ values for phenanthrenes were lowest in both experiments indicating that partitioning between the vegetation and air phase was irreversible. Once the compounds partitioned from the *Spartina* cuticle entry back to into the vegetation phase was hindered. Factors that may have contributed to hindered partitioning between the plant tissues and air phase include long experimental periods and low humidity in the fugacity meter.

The fugacity experiments were conducted in for a period of 31-42 days. Within this period, the plant leaves in the fugacity meter dried and died out. The low moisture content in the plant leaves reduced the re-entry and partitioning of PAHs released in the air phase. PAHs are lipophilic compounds that can be adsorbed into the waxy cuticle of plant leaves. The absence of the adoption media results into hindered transport and cycling of PAHs between the air and vegetation phase (Desalme et al. 2013). Previous studies on plant/air partitioning of semivolatile organic compounds were conducted at shorter periods with fresh leave samples (Tolls and MacLachlan, 1994; Komp and MacLachlan, 1997a; Komp and MacLachlan, 1997b).
3.4 Conclusion

The tendency of PAH to partition was described using a fugacity approach. The fugacity meter was a feasible method of studying the transport and partitioning of PAHs between the plant and air phase. The plant/air partitioning of lower molecular weight and volatile naphthalene was observed for in both fugacity experiments. However, partitioning of phenanthrenes was hindered due to low humidity and prolonged operations of the fugacity meter. Improved operations of the fugacity meter using field samples could help understand the partitioning behaviors of PAHs in polluted marsh systems. of the partitioning and transport of PAHs between vegetation and air can be used to understand the fate and changing behaviors of PAHs concentrations in the atmosphere following marsh oil spill, providing an effective measure of marsh recovery.

3.5 Recommendations

In this work, the fugacity meter was designed for studying transport of PAHs between air and Spartina leaves sampled from the field with experiments running at longer interval of days. Other studies have used the fugacity meter at much shorter periods (in hours) with artificially contaminated plants (Tolls and MacLachlan, 1994; Komp and MacLachlan, 1997a; Komp and MacLachlan, 1997b). Future operations of the fugacity meter using field samples at shorter time intervals and varying temperature can be an improvement to studying the fugacity capacities. In addition, control of humidity in the fugacity meter can help main a high moisture content in the leave therefore improving the transportation efficiency of PAHs between the plant and air phase.
REFERENCES


**APPENDIX**

Table A.1: PAH concentrations in µg/g of leaf in surface, cuticle, and inner tissue of *Spartina* at site 1 for each sampling date (average ± standard error)

<table>
<thead>
<tr>
<th>Compound (m.w.)</th>
<th>Jun 9/16</th>
<th>Jul 15/16</th>
<th>Sep 11/16</th>
<th>Sep 30/16</th>
<th>Dec 11/16</th>
<th>Feb 3/17</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Surface</td>
<td>Cuticle</td>
<td>Tissue</td>
<td>Surface</td>
<td>Cuticle</td>
<td>Tissue</td>
</tr>
<tr>
<td>naphthalene (128)</td>
<td>0.02 ± 0.01</td>
<td>0.29 ± 0.09</td>
<td>0.17 ± 0.08</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>1.23 ± 0.2</td>
</tr>
<tr>
<td>C1-naphthalenes (142)</td>
<td>0.06 ± 0.03</td>
<td>0.02 ± 0.01</td>
<td>2.55 ± 0.53</td>
<td>0.06 ± 0.03</td>
<td>0.02 ± 0.01</td>
<td>2.55 ± 0.53</td>
</tr>
<tr>
<td>C2-naphthalenes (156)</td>
<td>0.03 ± 0.01</td>
<td>0.02 ± 0.01</td>
<td>0.96 ± 0.2</td>
<td>0.02 ± 0</td>
<td>0.04 ± 0.01</td>
<td>0.84 ± 0.14</td>
</tr>
<tr>
<td>C3-naphthalenes (170)</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0.32 ± 0.14</td>
<td>0 ± 0</td>
<td>0.01 ± 0</td>
<td>0.32 ± 0.13</td>
</tr>
<tr>
<td>C4-naphthalenes (184)</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0.05 ± 0.01</td>
<td>0 ± 0</td>
<td>0.01 ± 0</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>Phenanthrene (178)</td>
<td>0.01 ± 0.01</td>
<td>0 ± 0</td>
<td>1.42 ± 0.28</td>
<td>0.01 ± 0</td>
<td>0.07 ± 0.02</td>
<td>1.48 ± 0.2</td>
</tr>
<tr>
<td>C1-phenanthrenes (192)</td>
<td>0.06 ± 0.03</td>
<td>0 ± 0</td>
<td>2.08 ± 0.45</td>
<td>0.03 ± 0</td>
<td>0.17 ± 0.03</td>
<td>1.79 ± 0.44</td>
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<tr>
<td>C2-phenanthrenes (206)</td>
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<td>0 ± 0</td>
<td>0.06 ± 0.03</td>
<td>0 ± 0</td>
<td>0.02 ± 0.01</td>
<td>0.08 ± 0.02</td>
</tr>
<tr>
<td>C3-phenanthrenes (220)</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0.79 ± 0.3</td>
<td>0 ± 0</td>
<td>0.09 ± 0.07</td>
<td>0.83 ± 0.19</td>
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<tr>
<td>C4-phenanthrenes (234)</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0.01 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0.01 ± 0</td>
</tr>
</tbody>
</table>

56
Table A.2: PAH concentrations in µg/g of leaf in surface, cuticle, and inner tissue of Spartina at site 2 for each sampling date

<table>
<thead>
<tr>
<th>Compound (m.w.)</th>
<th>Jun 9/16</th>
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<th></th>
<th>Jul 15/16</th>
<th></th>
<th></th>
<th>Sep 11/16</th>
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<th>Sep 30/16</th>
<th></th>
<th>Dec 11/16</th>
<th></th>
<th>Feb 3/17</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Surface</td>
<td>Cuticle</td>
<td>Tissue</td>
<td>Surface</td>
<td>Cuticle</td>
<td>Tissue</td>
<td>Surface</td>
<td>Cuticle</td>
<td>Tissue</td>
<td>Surface</td>
<td>Cuticle</td>
<td>Tissue</td>
<td>Surface</td>
<td>Cuticle</td>
<td>Tissue</td>
</tr>
<tr>
<td>Naphthalene (128)</td>
<td>0.01 ± 0</td>
<td>0.55 ± 0.21</td>
<td>0.52 ± 0.2</td>
<td>0.01 ± 0</td>
<td>0.04 ± 0.04</td>
<td>1.9 ± 0.66</td>
<td>0.01 ± 0</td>
<td>0.01 ± 0</td>
<td>0.14 ± 0.07</td>
<td>0.01 ± 0</td>
<td>0.01 ± 0</td>
<td>0.14 ± 0</td>
<td>0.01 ± 0</td>
<td>0.01 ± 0</td>
<td>0.14 ± 0</td>
</tr>
<tr>
<td>C1-naphthalenes (142)</td>
<td>0.06 ± 0.03</td>
<td>0.37 ± 0.07</td>
<td>7.52 ± 1.55</td>
<td>0.06 ± 0.03</td>
<td>0.37 ± 0.07</td>
<td>6.2 ± 1.5</td>
<td>0.2 ± 0.03</td>
<td>0.9 ± 0.18</td>
<td>0.91 ± 0.2</td>
<td>0.07 ± 0.01</td>
<td>0.27 ± 0.04</td>
<td>0.36 ± 0.06</td>
<td>0.07 ± 0.01</td>
<td>0.27 ± 0.04</td>
<td>0.36 ± 0.06</td>
</tr>
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<td>C2-naphthalenes (156)</td>
<td>0.05 ± 0.01</td>
<td>0.15 ± 0.03</td>
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<td>0.12 ± 0.03</td>
<td>0.12 ± 0.02</td>
<td>3.08 ± 0.49</td>
<td>0.07 ± 0.01</td>
<td>0.27 ± 0.04</td>
<td>0.36 ± 0.06</td>
<td>0.07 ± 0.01</td>
<td>0.27 ± 0.04</td>
<td>0.36 ± 0.06</td>
<td>0.07 ± 0.01</td>
<td>0.27 ± 0.04</td>
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<td>0.01 ± 0</td>
<td>1.16 ± 0.9</td>
<td>0.01 ± 0</td>
<td>0.07 ± 0.03</td>
<td>0.99 ± 0.77</td>
<td>0.01 ± 0</td>
<td>0.08 ± 0.03</td>
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<td>0.01 ± 0</td>
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<td>0.01 ± 0</td>
</tr>
<tr>
<td>C4-naphthalenes (184)</td>
<td>0.01 ± 0</td>
<td>0.01 ± 0</td>
<td>0.08 ± 0.02</td>
<td>0.01 ± 0</td>
<td>0.01 ± 0</td>
<td>0.06 ± 0.01</td>
<td>0.01 ± 0</td>
<td>0.01 ± 0</td>
<td>0.01 ± 0</td>
<td>0.01 ± 0</td>
<td>0.01 ± 0</td>
<td>0.01 ± 0</td>
<td>0.01 ± 0</td>
<td>0.01 ± 0</td>
<td>0.01 ± 0</td>
</tr>
<tr>
<td>Phenanthrene (178)</td>
<td>0.01 ± 0</td>
<td>0.15 ± 0.03</td>
<td>1.02 ± 0.21</td>
<td>0.03 ± 0.01</td>
<td>0.16 ± 0.04</td>
<td>2.88 ± 0.65</td>
<td>0.07 ± 0.01</td>
<td>0.63 ± 0.12</td>
<td>0.18 ± 0.04</td>
<td>0.21 ± 0.04</td>
<td>0.63 ± 0.13</td>
<td>0.54 ± 0.12</td>
<td>0.21 ± 0.04</td>
<td>0.63 ± 0.13</td>
<td>0.54 ± 0.12</td>
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<tr>
<td>C1-phenanthrenes (192)</td>
<td>0.27 ± 0.09</td>
<td>0.51 ± 0.15</td>
<td>2.59 ± 0.62</td>
<td>0.19 ± 0.05</td>
<td>0.44 ± 0.09</td>
<td>2.72 ± 0.8</td>
<td>0.21 ± 0.04</td>
<td>0.63 ± 0.13</td>
<td>0.54 ± 0.12</td>
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<td>0.01 ± 0</td>
<td>0.01 ± 0</td>
<td>0.03 ± 0.01</td>
<td>0.01 ± 0</td>
<td>0.02 ± 0.01</td>
<td>0.15 ± 0.12</td>
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</tr>
<tr>
<td>C3-phenanthrenes (220)</td>
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<td>0.27 ± 0.12</td>
<td>0.01 ± 0</td>
<td>0.02 ± 0.01</td>
<td>1.64 ± 0.33</td>
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</tr>
<tr>
<td>C4-phenanthrenes (234)</td>
<td>0.01 ± 0</td>
<td>0.01 ± 0</td>
<td>0.03 ± 0.01</td>
<td>0.01 ± 0</td>
<td>0.01 ± 0</td>
<td>0.02 ± 0</td>
<td>0.01 ± 0</td>
<td>0.01 ± 0</td>
<td>0.01 ± 0</td>
<td>0.01 ± 0</td>
<td>0.01 ± 0</td>
<td>0.01 ± 0</td>
<td>0.01 ± 0</td>
<td>0.01 ± 0</td>
<td>0.01 ± 0</td>
</tr>
</tbody>
</table>

(average ± standard error)
Table A.3: PAH mass/SPMD day for site 1 and site 2 for each sampling date.

<table>
<thead>
<tr>
<th>Site 1</th>
<th>Date</th>
<th>Jul 15/16</th>
<th>Sep 11/16</th>
<th>Sep 30/16</th>
<th>Dec 11/16</th>
<th>Feb 3/17</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Deployment period (days)</td>
<td>36</td>
<td>58</td>
<td>19</td>
<td>72</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>Compound (m.w.)</td>
<td>Air</td>
<td>Surface</td>
<td>Air</td>
<td>Surface</td>
<td>Air</td>
</tr>
<tr>
<td>Naphthalenes (128)</td>
<td>0.12 ± 0.07</td>
<td>0 ± 0</td>
<td>0.01 ± 0.01</td>
<td>0.13 ± 0.04</td>
<td>0.44 ± 0.26</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>C1-naphthalenes (142)</td>
<td>0.63 ± 0.19</td>
<td>0.04 ± 0.02</td>
<td>0.3 ± 0.1</td>
<td>0.05 ± 0.02</td>
<td>0.57 ± 0.17</td>
<td>0.13 ± 0.04</td>
</tr>
<tr>
<td>C2-naphthalenes (156)</td>
<td>0.05 ± 0.1</td>
<td>0.02 ± 0.01</td>
<td>0.03 ± 0.01</td>
<td>0.01 ± 0</td>
<td>0.03 ± 0.01</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>Phenanthrenes (178)</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>C1-phenanthrenes (192)</td>
<td>0.15 ± 0.06</td>
<td>0.06 ± 0.02</td>
<td>0.01 ± 0</td>
<td>0.02 ± 0.01</td>
<td>0.07 ± 0.04</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>C2-phenanthrenes (206)</td>
<td>0.06 ± 0.03</td>
<td>0.01 ± 0</td>
<td>0.01 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Site 2</th>
<th>Date</th>
<th>Jul 15/16</th>
<th>Sep 11/16</th>
<th>Sep 30/16</th>
<th>Dec 11/16</th>
<th>Feb 3/17</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Deployment period (days)</td>
<td>36</td>
<td>58</td>
<td>19</td>
<td>72</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>Compound (m.w.)</td>
<td>Air</td>
<td>Surface</td>
<td>Air</td>
<td>Surface</td>
<td>Air</td>
</tr>
<tr>
<td>Naphthalenes (128)</td>
<td>0 ± 0</td>
<td>0.02 ± 0.02</td>
<td>0.03 ± 0.02</td>
<td>0.09 ± 0.05</td>
<td>0.12 ± 0.04</td>
<td>0.42 ± 0.28</td>
</tr>
<tr>
<td>C1-naphthalenes (142)</td>
<td>0.07 ± 0.03</td>
<td>0.06 ± 0.03</td>
<td>0.18 ± 0.07</td>
<td>0.16 ± 0.05</td>
<td>0.43 ± 0.14</td>
<td>0.25 ± 0.1</td>
</tr>
<tr>
<td>C2-naphthalenes (156)</td>
<td>0.02 ± 0.01</td>
<td>0.03 ± 0.01</td>
<td>0.03 ± 0.01</td>
<td>0.01 ± 0</td>
<td>0.05 ± 0.02</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>Phenanthrenes (178)</td>
<td>0.02 ± 0.01</td>
<td>0.04 ± 0.04</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>C1-phenanthrenes (192)</td>
<td>0.04 ± 0.02</td>
<td>0.24 ± 0.08</td>
<td>0.02 ± 0</td>
<td>0.03 ± 0.02</td>
<td>0.08 ± 0.03</td>
<td>0.04 ± 0.02</td>
</tr>
<tr>
<td>C2-phenanthrenes (206)</td>
<td>0.05 ± 0.04</td>
<td>0.33 ± 0.12</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0.08 ± 0.07</td>
</tr>
</tbody>
</table>

(average ± standard error)
VITA

Joyce Gabriel Kassenga, daughter of Ritha and Gabriel Kassenga, was born (May 20, 1990) and raised in Dar es Salaam, Tanzania. She is the oldest of one sibling, a younger sister. Joyce received her Bachelor's of Science degree in Environmental Engineering from Ardhi University, Dar es Salaam in 2014. In the following year, Joyce joined Louisiana State University (LSU) to pursue her interest in environmental studies. Having previously worked on uptake mechanisms of heavy metals in native garden plants at Ardhi University, she chose to work with Dr. Pardue to study the cuticular accumulation of polycyclic aromatic hydrocarbons in oil-polluted marshes. Joyce anticipates to earn her Masters of Science degree in Civil and Environmental Engineering. She hopes to apply the knowledge acquired to help find solutions to global environmental challenges.