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Uptake and Deposition of Pyrogenic and Petrogenic PAHs on Spartina Alterniflora

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UPTAKE AND DEPOSITION OF PYROGENIC AND PETROGENIC PAHs ON
SPARTINA ALTERNIFLORA

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Masters of Science

in

The Department of Civil and Environmental Engineering

by
Yasmin Mohammad
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Abstract

Saline marshes dominated by *Spartina alterniflora* were impacted by MC252 crude oil along Louisiana's shoreline. Two locations were evaluated in this study; a lightly oiled, saline marsh near Port Fourchon, LA and a less saline, heavily oiled marsh near Bay Jimmy in the Barataria Basin, LA. Objectives of this study were to develop a method to extract polycyclic aromatic hydrocarbons (PAHs) from *Spartina* leaves and to compare and contrast the relative uptake and deposition of petrogenic and pyrogenic PAHs in *Spartina* leaves.

A three-step sequential extraction procedure was developed for the extraction and analysis of PAHs on *Spartina* leaves. In the field, particles deposited on the leaf were dissolved in EDTA solution, and the leaf cuticle was dissolved in dichloromethane. PAHs were quantified in selective ion monitoring mode using a gas chromatograph connected with a mass selective detector. A controlled experiment was also performed in a greenhouse to determine the partition coefficients between air and *Spartina* plant surfaces.

Samples collected from Fourchon Beach were extracted, and results demonstrated the presence of both naphthalenes and phenanthrenes in the *Spartina* leaves. In general, Fourchon Beach samples, where the water was, more saline and displayed a lower concentration of PAHs in the *Spartina* leaves. Bay Jimmy samples, where the water was less saline included a higher concentration of PAHs.

The concentration of naphthalenes was higher than that of the phenanthrenes. Samples from Bay Jimmy were collected three times, Summer 2013, Winter 2013, and Summer 2014. The samples from summer 2013 included a high
concentration of phenanthrenes. Naphthalenes were present but in low concentrations. The Bay Jimmy winter 2013 samples were consistent with the summer 2013 samples in that the phenanthrenes were present, however, the naphthalenes were present in a higher concentration. The winter 2014 samples continued to show a high concentration of phenanthrenes and an even higher concentration of naphthalenes.

In both sampling locations, the PAHs present were dominantly in the cuticle and the tissue of the Spartina leaves. These results were consistent with those recovered in the control experiment. The plant leaves in the experiment displayed the same results.
Chapter 1  Introduction

1.1 Introduction and Purpose of Study

On April 20, 2010 an explosion occurred, sinking the Deepwater Horizon rig in the Gulf of Mexico. Remote underwater cameras revealed the riser pipe was leaking gas and oil onto the ocean floor, about 42 miles off the coast of Louisiana. The well was capped 87 days later, at which point, approximately 4.9 million barrels of oil had leaked into the Gulf of Mexico. (http://ocean.si.edu/gulf-oil-spill)

Spilled oil eventually reached the Gulf shoreline, impacting beaches and marshes. Saline marshes dominated by Spartina alterniflora were impacted by MC252 crude oil along Louisiana’s shoreline. One of the areas that were heavily impacted by the spill was Fourchon Beach, a 9-mile coastal headland beach located near Port Fourchon in southern Barataria Basin and part of the Caminada Headlands beach system. Washover tidal events carried oil from the subtidal and intertidal portion of the beach to the supratidal mudflats and marsh areas of the beach barrier. A floating water and oil mixture of volatile petroleum components washed over the headland beach during a tropical storm, contaminating the Fourchon Beach headlands and mangrove sites [1, 2]. At the northern end of the Barataria Basin, oil was carried in large quantities to Bay Jimmy and Bay Baptiste, impacting the surrounding marshes [3, 4].

Vegetation along the marsh shoreline consisted of healthy and severely degraded oil impacted sites. Oil impacted sites along the marsh consisted of oil residues on the marsh. The oil residue caused die-offs in previous marsh communities. Various levels of polycyclic aromatic hydrocarbons (PAHs), one of the
most toxic (to vegetation, macrophytes) fractions of crude oil, were found in the surface sediment at impacted locations along the marsh shoreline. Even though oil is readily observed in the sediments of these marshes, the role of marsh vegetation such as *Spartina alterniflora* as a reservoir for PAHs is not well characterized. PAHs can be taken up by *Spartina* through various mechanisms and particularly the partitioning of semivolatile organics like PAHs to the waxy cuticle of plants is a well-established phenomena.

The purpose of this study was to quantify the uptake of PAHs into *Spartina* from field samples at two sites, a light oiled, more saline marsh near Port Fourchon, LA and a less saline, more heavily oiled marsh near Bay Jimmy in the Barataria Basin, LA. The objectives of this research included the determination of the relative uptake and deposition of petrogenic and pyrogenic PAHs onto *Spartina* leaves in areas impacted by the Macondo spill; measurements of the relative uptake of PAHs onto marsh periwinkle snails occupying *Spartina* leaf surfaces; and the measure of the *Spartina*/PAH partition coefficients under more controlled conditions in a greenhouse.

1.2 Literature review

1.2.1 Polycyclic aromatic hydrocarbons (PAHs)

A variety of petroleum compounds comprise MC252 crude oil spilled from the Macondo well, located in the Gulf of Mexico in 2010. Polycyclic aromatic hydrocarbons (PAHs) are semivolatile organic compounds that often result from incomplete combustion. PAHs consist of two or more fused benzene rings. There are two types of PAHs, petrogenic and pyrogenic. The combustion of fossil fuels
petroleum) and biomass produce pyrogenic PAHs. These compounds are released into the environment in the form of exhaust and solid residues from combustion sources. Petrogenic PAHs are naturally present in crude oil and consist primarily of alkylated versions of ring structures present in PAHs of pyrogenic origin. They are introduced to water bodies through oil spills, municipal runoff, or discharge from tanker operations [8]. PAHs represent a small percentage of the overall crude oil composition, however, they are one of the most toxic (to vegetation and macrophytes) components present in crude oil [9]. When PAHs are released to the coastal environment (i.e., from oil spills), they often become associated with particles such as the marsh sediments. PAHs are classified by their molecular weight, the higher the molecular weight, the slower the rate of microbial degradation processes [10]. PAHs are insoluble, they retain the characteristics of being able to stay in sediment for an extensive time period; therefore, PAHs are a persistent, toxic substance that can be harmful to the coastal environment.

1.2.2 Spartina alterniflora

*Spartina alterniflora* (smooth cordgrass) is the dominant emergent grass species in U.S. Gulf Coast salt marshes [11]. The plant is found growing along the tidal salt marshes of the Atlantic and Gulf coats. *Spartina* is a long-lived plant that favors warmer seasons. It is a dominant species that grows from 2 to 7 feet tall and spreads significantly by elongated hollow rhizomes. Stems that are soft and spongy, 0.5 inch in diameter, emerge from the rhizomes. *Spartina* have flat lead blades that are usually 12 to 20 inches long and are diminished to a long inward-roll. In the months of September and October seedheads, which are 10 to 12 inches long,
develop from the end of each stem. Each stem spike will hold twelve to fifteen spikelets (2 or 3 inch long spikelets). The flowers on the tips of the blades are wind pollinated. [12]

Smooth *Spartina* families have a tendency to grow parallel to and continuous along shorelines. The sizes of vegetative colonies are determined by a number of site-specific circumstances (elevation, shoreline slope, and frequency, depth and duration of flooding events). *Spartina* is usually found growing in open coastal marshes, between high and low tides from Newfoundland south to Florida, Louisiana, and Texas. This plant can be found on sandy, aerobic or anaerobic soils, with pHs ranging from 3.7-7.9. *Spartina* can tolerate regular flooding with 0 to 35 parts per thousand of salinity. [12]

Shoreline plantings of *Spartina* help with erosion. The best water depths for starting plant communities are 1 to 18 inches. Plantings are usually started as a single or double row of *Spartina*, running parallel to the shoreline. The plant spacing is dependent of the desired coverage of *Spartina* on the shoreline. To enhance the growth of shoreline *Spartina*, commercial fertilizers are suggested. Fertilizers help stimulate the growth and development of young *Spartina* plants. Pests that may be found on *Spartina* include rust-like fungi, beetles, and the sugar cane borer. However, none of these pests are threatening to the population. [12]

There are two varieties of *Spartina* on the commercial market; the *Bayshore* *Spartina* was introduced, in 1992, for use on Atlantic coastal areas by the Cape May Plant Material Center in Cape May Court House, New Jersey. In 1989, the Golden
Meadows Plant Materials Center, in Galliano, Louisiana, released *Vermilion Spartina* for application in the Gulf coastal area. [12]

*Spartina* is utilized for erosion control along canal banks, shorelines, levees, and many other areas of soil water interface. *Spartina* is a highly effective soil stabilizer that is used on interior tidal mudflats, dredge-fill sites, and many other areas with loose, unconsolidated soils associated with marsh restoration. [12]

Under normal circumstances on tidal marshes, dynamic stands of this plant will absorb wave energy and shelter suspended solids from intertidal waters [11]. Simultaneously, the plants will uptake nutrients in the sediments. With the accumulation of sediment, the *Spartina* will begin to spread away from the water bank. The salt-water plant provides food as well as shelter to numerous mammals as well as marsh birds. *Spartina* also has the ability to tolerate soils that are contaminated with petroleum. [12]

1.2.3 PAH uptake into plants

PAHs in soils and sediment threaten environmental, ecological, and human health. They are a principal concern when pursuing remediation for polluted sites. In the vegetated environment, plant roots produce enzymes, amino acids, and growth hormones, which maintain root activity and aid in the PAH degradation, removal, and mineralization [13]. Therefore, the mechanisms by which PAHs interact with vegetation are key. The uptake mechanisms of PAHs by vegetation include the air-to-leaf pathway and the soil-to-root pathway. In the air to leaf pathway, the plant cuticle and the epicuticle wax are important components of a plant’s storage and re-release of PAHs [14]. The PAHs come into contact with the
plant from the atmosphere. The cuticle itself is a nonliving lipid surface layer. It acts as a barrier between the atmosphere and the plant, protecting it from water loss and the invasion of pathogens. [15] [16]

The cuticle consists of a polymeric matrix enclosed with plant waxes; these two components, the polymeric matrix and the plant waxes, form a barrier for diffusion into the plant leaves. Gas phase atmospheric PAHs come into contact with the plant after turbulent transport via diffusion through the atmospheric boundary layer (which is above the surface of the leaf) [17]. The PAHs then partition into the leaf's surface on the impermeable cuticular skin [18]. The PAHs can then diffuse further into the cuticle ultimately reaching the plant tissue or they will partition back into the air, dependent on environmental conditions. [19]. PAHs are predisposed for accumulating on vegetation due to their strong affinity for lipids [20]. PAHs are lipophilic molecules; therefore, they have the ability to partition onto the lipid surface of cuticles. Due to this, PAHs with low molecular weights are more likely to penetrate the waxy leaf cuticle. [21]

PAHs may also be adsorbed into plants via the root cortical zones (lipophilic constituents) or absorbed by root cells and then transferred to the aerial parts of the plant via the transpiration stream [22]. PAHs are mostly adsorbed on but not absorbed by the plant roots. The low molecular weight PAHs may be transferred to shoots, whereas the higher molecular weight PAHs have only been found adsorbed on the root epidermis [23] [22, 24] [25].

In order to measure PAHs on plants to establish the pathway of PAH uptake into vegetation, a complete extraction method must be utilized. Efforts have been
put forth to improve extraction methods to help clarify the difference between adsorbed and absorbed PAHs on both the surface and tissue of the plant [13]. Schwab et al. (1998) researched the affinity of plant roots for naphthalene. Root samples were extracted utilizing CaCl$_2$ and methanol [26]. Binet et al. (2000) utilized chloroform to wash anthracene from ryegrass root surfaces, and followed with the extraction of PAHs from the root tissue with chloroform [27].

Other studies have explored different techniques to understand the uptake, storage, as well as metabolism of organic contaminants within the plant roots [13]. Wild et al. (2005) utilized a two-photon excitation microscopy to visualize and track the movement and uptake of anthracene and phenanthrenes from a contaminated medium into living maize and wheat roots [28]. Most of the studies performed in this field have been focused on PAH accumulation in the roots of farm vegetables (lettuce, carrots, potatoes, and corn. Studies on aquatic plants have rarely been analyzed. Jiao et al. (2006) performed an analysis on the roots of paddy rice. Root samples were analyzed in a 3-step extraction procedure. The procedure was adapted from Schwab et al. (1998). PAHs in the free space of the roots were first assessed using CaCl$_2$. Next, the root adsorbed PAHs were analyzed though the use of methanol and a liquid-liquid extraction, and finally, the PAHs absorbed in the root tissue were assessed utilizing an accelerated solvent extraction (ASE) [28].

Wang et al. (2008) studied the PAHs in leaf cuticles and inner tissues of six species of trees urban Beijing; Wang also utilized a sequential extraction procedure, modified from that of Binet et al. (2000). Leaf samples were initially subjected to dichloromethane (DCM) for 2 minutes of gentle shaking. The leaf extract was then
filtered and used for measurements of both cuticle wax and PAHs in the cuticle of the leaf. The extracted leaf samples were then carried to step two of the extraction, the samples were freeze-dried and pulverized and passed through a sieve. Dried samples were extracted using ASE technology (DCM and acetone as a solvent). [29]

Similarly, Kaupp et al. (2000) performed a sequential extraction to analyze the deposition of PCDD/Fs and PAHs on a maize canopy under ambient conditions. Samples were initially subjected to water, which served to remove large metal particles, assumed to be mobile. Second, the samples were subjected to an aqueous solution of EDTA (effective in removing metal particles from the sample). The third extraction consisted of dipping the sample in chloroform, which removed small particles that had become embedded into the cuticle of the plant. [30]

Bakker et al. (2000) also carried out a sequential extraction; the analysis was performed on the deposition of PAHs in leaves of *Plantago*. Samples were washed twice by shaking with 100 mLs of an EDTA solution (pH = 5), following, the EDTA solution was extracted by refluxing with 100 mL of cyclohexane for 10 minutes. Leaves from the EDTA solution were then immersed in 45 mL of DCM for 30 seconds and 10 seconds, respectively; this procedure served to extract the cuticular wax of the leaves. The DCM was concentrated to 61 mL (under a nitrogen flow), and 45 mL of methanolic potassium hydroxide (KOH) was added. Following, the DCM-KOH mixture was refluxed for 30 minutes. Cyclohexane was then added to the mixture and refluxed for 10 minutes. This served for the extraction of the cuticular wax of the leaf. Subsequently, the leaves were ground with mL of liquid nitrogen and
45 mL of KOH was added and the procedure of step 2 of the sequential extraction was repeated [31].

1.2.4 Modeling plant exposure and uptake

PAHs in contaminated soils are often associated with the organic matter fraction; therefore, they are not predicted to be highly susceptible to plant uptake and translocation [32],[33]. Therefore, PAHs in above-ground plant tissues are likely derived from the atmosphere as a result of the deposition of compounds and the retention vapor-phase on the leaf cuticle [34],[33],[35].

The key accumulation pathway for PAHs is from the air to the leaf surface [36]. Bacci et al. (1990) studied the plant-air partitioning phenomenon and developed a quantitative model. The “Azalea Model” can be used to describe plant uptake [35]. The Azalea Model was created to compute the bioconcentration of non-polar and low-volatile compounds by Azalea leaves.

The model assumes that the main mode of chemical displacement and transport of chemicals is through vapor movements from contaminated soil to the air. This phenomenon most likely occurs by means of global gas chromatographic process: in terrestrial systems, the soil acts as the support, the organic matter contained in the soil is the stationary phase, and the air acts as the carrier phase [33]. In the air, partitioning between the gas and particulate phases is determined by the subcooled liquid vapor pressure, \( P_L \), of the substance. Higher \( P_L \) values indicate an increase in the fraction in the vapor phase.

The uptake of contaminants by plant leaves can be quantified by means of the equilibrium leaf/air bioconcentration factor (BCF) [7]. A correlation was
developed between key intrinsic properties of the chemicals, including the 1-octanol/water partition coefficient ($K_{ow}$) and the air/water partition coefficient ($K_{aw}$). Bacci et al. (1990) reported significantly high uptake rates compared to the release rates; the BCF was also of high value for the high $K_{ow}$ samples sampled.

A important part of the evaluation of the environmental fate of chemicals is the estimation of the amount to which chemicals will achieve concentrations in vegetation phases [37]. The bioconcentration factor, BCF, is a dimensionless ratio of the concentration in the vegetation to that in the specific medium. It is often viewed as a balance between two kinetic processes, the uptake and release rate. The BCF is correlated with the octanol-water partition coefficient, $K_{ow}$ [35].

According to Trapp et al. (1990) bioconcentration in the plant is contingent on the transfer rates of contaminants and the ratio of $K_{ow}$ and $K_{OC}$ values. Uptake into vegetation is primarily from the air for chemicals with a high $K_{ow}$ and a high Henry’s law constant (high enough for volatilization). [38] Analysis done by McKay et al. (1982) revealed that the bioconcentration phenomenon, from a physical-chemical perspective can be modeled as a partition coefficient between a vegetation and water. If the dominant phase of the contaminant is a lipid, like the cuticle waxes, a proportional relationship between the BCF and $K_{ow}$ is predicted [37].

Bacci et al. (1992) performed an analysis of chlorinated dioxins and their volatilization from soils and bioconcentration into plant leaves. The study assumed that the air-to-leaf transfer of gaseous organics is the key process for plant uptake. This was the case particularly for less water-soluble compounds that are characterized by low mobility in water and subsequently have negligible
translocation through the roots from contaminated soils. The “Azalea Model” was used to quantify the BCF value as well as partition coefficients. Azalea leaves were exposed to 1,2,3,4-tetrachloro dibenzo-p-dioxin (1,2,3,4-TCDD). Results showed that the concentration of contaminant in the Azalea leaf continually increased until hour 400, at which point, the concentration of 1,2,3,4-TCDD in the leaf began to linearly decrease. At equilibrium, the following values were reported: $K_1 = 19,135 \, h^{-1}$, $K_2 = 0.0002 \, h^{-1}$, and $BCF = 9.1 \times 10^7$. $K_1$ and $K_2$ are the uptake and release rate constants, respectively. The study concluded that low-volatile chemicals can significantly move from soil into the air. High leaf/air bioconcentration is expected for conservative and non-polar compounds. [14]

Hiatt et al. (1999) studied leaves as indicators of exposure to airborne volatile organic compounds (VOCs). The amount of chemical that may be present in the leaves is related to the BCF. The BCF for lipophilic organic compounds was related to their partition coefficients (between air and octanol). The study observed the content of VOCs in leaves and air to explain the relationship between air and leaf concentrations [39]. The BCF values ranged from 200 - 39,000 (ng./kg dry leaf) (depending on the compound and leaf analyzed). Higher BCF values were attributed to the likely presence of organic matter (oils, lipids, and waxes) in the leaves that have the ability of dissolving greater amounts of VOCs. The uptake and release rate, $k_1$ and $k_2$, were also calculated. The release of VOCs from the leaves was observed to be at a much slower rate than their uptake [39].

Hiatt et al. (1998) also studied the bioconcentration factors for volatile organic compounds in vegetation. Samples of air and leaves (8 different species)
were taken from the University of Nevada, and the data was used to estimate the bioconcentration of volatile organic compounds (VOCs) and to characterize the VOCs between the leaves and air. The bioconcentration of the volatiles in the leaves can be estimated using partition coefficients between air and octanol ($K_{OA}$). BCF values reported ranged from 180-29,000 depending on the species of leaves and the compound. [40]

The kinetics and partitioning of semivolatile organic pollutants in forest litter were analyzed by Nizzetto et al. (2013). The study aimed to derive parameters for forest litter, including air equilibrium partition coefficient and uptake/release rate constants. Results indicated that the uptake rate constants were to be significantly higher than the release rate constants. The uptake rates also served as a guide for the concentration of pollutants in forest litter. The study did not utilize the BCF as a guide for the uptake of chemicals. [41]

Harner et al. (1998) measured the octanol-air partition coefficients for PAHs; the study utilizes the octanol air partition coefficient, $K_{OA}$, as the key description of the partitioning of semivolatile compound between the atmosphere and terrestrial phases. $K_{OA}$ is described as the ratio of the solute concentration in octanol ($C_O$) to the concentration in the air ($C_A$). The ratio is calculated when the system is at equilibrium. The study is performed based on $K_{OA}$ values as a function of temperature for PAHs; PAH are said to partition between the gas phase and vegetation according to their vapor pressure or $K_{OA}$ values. Log $K_{OA}$ values were reported for fluorene, phenanthrene, pyrene, and fluoranthene. Values ranges from log 6.79 to log 8.88, depending on the compound [42].
Paterson et al. (1991) performed a study on the correlation of the equilibrium and kinetics of leaf-air exchange of hydrophobic organic chemicals. The study utilizes the “Azalea Model” to develop a theoretical basis for correlating the equilibrium and kinetic processes of air—leaf partitioning of gaseous organic chemicals. The assumption was made that the primary site of chemical accumulation is in the cuticular waxes. The study suggests a simple expression that describes the dependence of these processes on chemical properties [7]. Paterson defines a Z value, which expresses the partitioning properties of chemicals in the environment (in terms of fugacity capacities). The study describes partition coefficients between air and water, $K_{AW}$ and octanol and water, $K_{OW}$, in terms of the Z value. Two BCF values were utilized; $BCF_V$ described the ratio of volumetric concentrations and $BCF_M$, the mass ratio as reported by Bacci et al. (1990). $BCF_M$ was the preferred ratio for reporting experimental results and $BCF_V$ was useful for the estimation of the amount of chemicals in the fresh leaves [7]. The uptake and release rate constants, $k_1$ and $k_2$, were also considered in the study. Results displayed that $BCF_V$ values in the range of $10^6$ - $10^7$ (g/m$^3$)/ (g/m$^3$); the release rate constants were in the $10^{-3}$ (h$^{-1}$) range. $K_i$ values were not reported [7].

Villeneuve et al. (1988) studied lichen samples from southern France for the existence of chlorinated hydrocarbons. Results were compared to those reported in the industrial regions of Italy and remote regions of Antarctica [43]. Concentrations were compared to atmospheric concentration in the same areas, and a BCF value of $2 \times 10^5$ (ng/g chemical in lichen dry weight)/(ng/g chemical in air) between lichen and air was reported[43].
McLachlan et al. (1997) studied the influence of temperature on the plant/air partitioning of semivolatile organic compounds. The temperature dependence of the octanol-air partition coefficient, \( K_{oa} \), was investigated under controlled laboratory conditions using polychlorinated biphenyls (PCBs). The results concluded that if a \( K_{oa} \) value is greater than 1, the compound is a less polar storage medium for lipophilic organic compounds than 1-octanol [44].

1.2.5 Exposure to higher organisms

The coastal environment includes salt-water plants that Petrogenic PAHs can be accumulated onto plant surfaces. *Spartina alterniflora* is the dominant macrophyte in salt marshes along the Gulf of Mexico. The plant is responsible for the increased levels of primary production in these ecosystems. *Spartina* hold a tall frame, typically 1-3 meters, and take habitat adjacent to the shore, where porewater turnover is high and the sediments are oxidized [45]. The sediment supporting the *Spartina* plants is water saturated, as those in most wetlands [17]. If the sediment becomes vulnerable to events, such as oil spills, the plant also becomes vulnerable [5].

Along the shoreline, *Spartina* serves as a food source for the marsh periwinkle snail [46]. The accumulation of PAHs may cause indirect human and animal exposure to PAHs through the consumption of vegetation [31]. PAHs first absorb into the surface of the plant and then continue to diffuse into the cuticle of the plant. Subsequently, the PAHs will continue to diffuse to the tissue of the plant.

The marsh periwinkle, *Littorina irrirata*, is a gastropod in the order of *Mesogastropoda*. It belongs to an important genus of the common marsh snails. The
snails can grow to a size of 1.25 inches (30 mm) long. It takes approximately two to three years for the snails to grow to full length. They range in color from bleached white to dark brown [47]. The marsh periwinkle is well known for their circumtidal migratory behavior on Spartina alterniflora stems. The snail feeds on the marsh substratum at low tide and climb grass stalks with tidal inundation [48].

The marsh periwinkle is an important part of the intertidal marsh community. Major challenges for the preservation of the salt marsh periwinkle include the loss of habitat and significant changes to predator population [49]. The marsh periwinkle is a valuable contributor in the decomposition of Spartina alterniflora, a wetland grass common in salt marshes. It holds a mutualistic relationship with Spartina leaf fungi; the periwinkle serves as an indicator species of the health of the salt marsh habitat as a whole. Therefore, it is important to maintain the health and existence of the species.

In a Louisiana marsh, an estimated 59% of snails feed on Spartina alterniflora, and the snails were estimated to consume 12% of all the annual Spartina alterniflora production [50]. Barlocher et al. (1993) experimented with the growth of salt marsh periwinkle Littoraria irrorata fungal and Spartina alterniflora diets. An experiment was done to analyze the affinity of the marsh periwinkle on various diets; seven of the diets consisted of Spartina alterniflora treatments [50]. The study revealed that the marsh periwinkle is sensitive to diets inoculated with bacteria [50]. Based on this study, it may be concluded that the marsh periwinkle does not tolerate changes or intrusions in its diet.
1.2.5.1 Periwinkle Snail and Spartina

Mendelssohn et al. (2012) studied the oil impacts on Coastal wetlands after the Deepwater Horizon oil spill. An analysis was done of the various effects of the oil spill on both vegetation and creatures in the area. The marsh periwinkle, \textit{Littoraria irrorata}, has an important grazing impact on \textit{Spartina alterniflora} \cite{51} \cite{52}. \textit{Spartina} grazing pressure would likely decrease due to the toxic effects on the marsh periwinkle; that may help in the post-spill recovery of \textit{Spartina}. Mendelssohn et al. (2012) concludes that the Deepwater Horizon oil spill did indeed affect (directly and indirectly) both the marsh periwinkle snail as well as \textit{Spartina alterniflora}; subsequent to the oil spill the population marsh periwinkle decreased\cite{51}.

Bennett et al. (1999) performed a study to analyze the effects of PAH contaminants and nutrient availability of micromicrophytobenthos in the sediments of the marshes in Port Fourchon, Louisiana. The study utilized the marsh periwinkle, \textit{Littorina irrorata}, as a model species; the marsh periwinkle is the dominant macro-epibenthic grazer in the \textit{Spartina alterniflora} dominated salt marsh \cite{53}. A portion of the study was a microcosm study with both high exposure PAHs and low exposure PAHs. Low molecular weight PAHs were found in the extraction of the \textit{Littorina irrorata} tissue. All of the \textit{Littorina irroratas} in the study experienced weight loss; this weight loss may be due to the health of the species. There was no significant difference between the high exposure and low exposure PAHs in the \textit{Littorina irrorata} tissue. However, the abundance of high molecular weight and alkylated PAHs in the high exposure treatments are symbolic of petroleum hydrocarbon contamination at the Port Fourchon Marsh \cite{53}. The high abundance
of high molecular weight PAHs displays the historical constant input of PAH at the produced-water site. On the contrary, low molecular weight PAHs are quickly removed by bacterial metabolism and weathering, however; they may still accumulate over time [53].
Chapter 2  Plant uptake, PAH deposition and partitioning onto *Spartina alterniflora* and Marsh Periwinkle Snails

2.1 Introduction

Saline and brackish marshes dominated by macrophytes such as *Spartina alterniflora* were impacted by MC252 crude oil from the Macondo spill in 2010. Of the 1,773 km of oiled shoreline, 50.8% was beach and 44.9% marsh [54]. Impacted marshes often included emulsified pools of oil that did not readily penetrate the marsh surface. Oil penetrated into the marsh at a maximum of 10-15 m due to the viscosity of the oil emulsion and the small tidal range (0-0.5 m) [54]. Bulk oiling along the shoreline and coating of the marsh vegetation with oil during high tidal events in 2010 (Hurricane Alex and T.S. Bonnie) caused plant die-off, which accelerated erosion, particularly in northern Barataria Bay [55]. Only 8.9% of marshes received any remedial response due to the poor number of remedial options for these environments [54]. As a result, natural recovery processes remain important for marsh recovery.

A potential reservoir of polycyclic aromatic hydrocarbons (PAHs) in marsh systems are those present in the macrophytes, either deposited as PAH-containing particles (i.e., soot) on the plant surface, partitioned into the waxy cuticle on the leaves or present in the plant tissues [6]. These reservoirs results from mechanisms that transfer PAHs from the soil to the plant including, uptake and translocation of PAHs in the transpiration stream, volatilization and re-deposition on leaf cuticle, and sorption from direct contact with soil particles [56]. Atmospheric deposition has also been identified as a pathway; PAHs from the soil volatilize into the atmosphere and are trapped in the cuticle of the leaf [6], [33]. For marshes impacted
by MC252 oil, one hypothesis is that PAHs can volatilize from oil remaining in the marsh soil including exposed emulsion pools, followed by partitioning into the waxy cuticle of the leaf. Uptake of PAHs into the transpiration stream is also possible but extremely difficult to measure in the field. As a result, it is usually calculated by eliminating all other mechanisms of uptake [56],[6]. Plant uptake of PAHs were analyzed to determine the mechanism of adsorption. External deposition and cuticle uptake of PAHs will be studied to determine the path of PAH by *Spartina*. [5], [21]

PAHs on plant surfaces can lead to exposure to organisms such as the marsh periwinkle snail (*Littoraria irrorata*), which spends a large portion of their lifecycle on *Spartina*. Michael Blum, director of the Tulane-Xavier Center for Bioenvironmental Research, identified this species of snail on Spartina, in Bay Jimmy. These organisms hold a mutualistic relationship with *Spartina* leaf fungi, serving as an indicator species of the health of the salt marsh habitat as a whole [47],[57]. PAHs partitioned into plant cuticles, for example, pose a potentially unique route of exposure, previously unquantified for the Macondo spill.

This study quantified the concentrations and distribution of PAHs on *Spartina* from field samples at two sites, a lightly oiled, more saline marsh near Port Fourchon, LA and a less saline, more heavily oiled marsh near Bay Jimmy in northern Barataria Basin, LA. The objectives of this study included the determination of the relative uptake and deposition of petrogenic and pyrogenic PAHs on *Spartina* leaves in the areas that were impacted by the Macondo spill, while measuring the uptake of PAHs into the marsh periwinkle snails who spend a large portion of their life cycle on *Spartina* leaf surfaces.
2.2. Materials and Methods

2.2.1 Sample locations

Samples were collected from two locations, a marsh near Port Fourchon, Louisiana and marsh adjacent to Bay Jimmy, Louisiana in northern Barataria Basin (Figures 2-1 and 2-2). The Port Fourchon marsh site is just north of Fourchon Beach, a 9-mile coastal headland beach was heavily impacted by the Deepwater Horizon oil spill [58-60]. At this site, washovers carried oil from the subtidal and intertidal portion of the beach to the supratidal mudflats and marsh areas north of the beach [61, 62]. A floating water and oil mixture of volatile petroleum components washed over the headland beach during a tropical storm, contaminating the Port Fourchon Beach headlands and mangrove sites [1, 2]. Further into the Basin, oil was carried in high quantities into the upper Basin to Bay Jimmy, Bay Baptiste, impacting the surrounding marshes [63],[64],[61].

At Port Fourchon, the sites from which samples were collected include an oiled plot (Oil 1, Oil 2, Oil 3, Oil 4, and Oil 5), an inland plot which was about 40 feet inland from the oiled plot (1C, 2C, 3C, 4C, and 5C), and a control site which was about 150 feet from the oiled plot (CS1, CS2, CS3, CS4, and CS5) in an area which received no oil (Figure 2-1). No response activities were conducted at this site and oiling occurred in a single washover event that occurred in summer of 2010.
Figure 2-1: Sampling locations for Port Fourchon marsh. Oil reached the marsh in May 2010, as depicted on bottom right. A boardwalk was built along the marsh area affected by the oil spill. Samples were taken of *Spartina* stems at three locations in the Port Fourchon marsh, an oiled plot, an inland plot, and a control site. The oiled plot represents the area which the oil breached the marsh, the inland plot is located 40 ft. from the oiled plot, and the control site is 150 ft. from the oiled plot. The control plot was not directly exposed to oil. Five sets of samples were taken from each of the three-sampling areas (all sampling was done in triplicate, yielding a total of 15 samples from each sampling area).
Figure 2-2: Sampling locations in Bay Jimmy. Colored areas refer to the NOAA/Incident Command remediation effort in the marsh areas. Following that, with the permission of NOAA, Tulane University planted varieties of *Spartina alterniflora* along the southern marsh shoreline. The numbers on the map correspond to the variety of *Spartina* planted in that area. Plant sampling was performed in 7 of the plots, 3A, 4B, 4C, 5A, 5D, 6A, and 6D (all sampling was done in triplicate, therefore, a total of 21 samples were collected from Bay Jimmy for each event).
At the Bay Jimmy site, samples were collected from a heavily oiled shoreline that received a variety of response activities and planting of various varieties of *Spartina alterniflora* [4]. Plot numbers coincide with each variety of plants in that area. Plot numbers and corresponding plant varieties are shown in Figure 2-2.

2.2.2 Field Sampling

Three leaves were sampled from each *Spartina* plant starting with the green leaf coming off of the stem nearest the root and working upward (Figure 2-3). The leaves were placed in a Ziploc bag and labeled with the date and sample location identification. Once all samples were collected, the initial steps of the sequential extraction were performed at the field site. The extraction was initiated with EDTA (1 M, pH = 5), which served to mobilize the particles from the surface of the leaves [65]. The leaves were placed in a glass container containing 100 mL of EDTA and shaken for 2 minutes [65]. Subsequently, the leaves were taken out of the EDTA solution, and placed in a glass container with 150 mL of pesticide-grade dichloromethane (DCM) and shaken for 2 minutes [27, 29]. The DCM served as the second phase of the extraction, which dissolved the cuticle of the leaves. Finally, the leaves were stored in a Ziploc bag and transported to the laboratory and stored at 4°C prior to complete extraction of the leaf tissue.

Once back in the lab, the EDTA solutions were extracted in a separatory funnel with cyclohexane as the solvent. Half of the EDTA solution sample was placed in a 100 mL separatory funnel with 50 mL of cyclohexane and shaken and inverted for about 1 minute, following which, the EDTA solution was drained from the funnel and the remaining sample added [65].
Figure 2-3: Sequential extraction method. In the field, three *Spartina* leaves were removed from the plant, extracted with 100 mL of EDTA for 2 minutes, followed by 150 mL of DCM for 2 minutes. The leaves were transported back to the lab, weighed, and extracted with hexane:acetone using accelerated solvent extraction. Samples were then concentrated and subjected to solid phase extraction (SPE) cleanup followed by GC/MS analysis. The EDTA extracts were extracted utilizing a separatory funnel with cyclohexane, subjected to a solvent exchange and analyzed using GC/MS. The DCM extracts were used to quantify the PAHs in the plant cuticle (100 mL) as well as to determine the weight of the cuticle of the leaf (50 mL). The 100 mL of DCM sample was first concentrated to 1 mL, subjected to SPE cleanup, followed by a solvent exchange and GC/MS analysis.
The separatory funnel was again shaken and inverted for about 1 minute, and then the EDTA was again allowed to drain from the funnel [65]. The cyclohexane was then evaporated (at 71°C) to 5 mL in a RapidVap N₂ Evaporation System (Labconco, USA), and stored in a scintillation vial. Subsequently, 1 mL was used for GC-MS analysis of the sample.

The DCM samples were similarly processed. Initially, 100 mLs of the DCM sample was evaporated (41°C) to 1 mL in the RapidVap as described above. The 1 mL volume was then subjected to solid phase extraction with a silica gel column. The cleanup allow for the elimination of any interference in the analysis of PAHs. The column was activated with 1 mL of water, following, the DCM sample was slowly loaded on the column and washed with 40 mL of 2:3 hexane:acetone. The samples were once again evaporated to 1 mL, then 50 mLs of 1:1 hexane acetone was added to the sample and once again evaporated to 5 mLs. The sample was stored in a glass scintillation vial and stored in the refrigerator. The samples were then analyzed by GC-MS as described below. This analysis allowed for the determination of the concentration of PAHs on the cuticle of the leaf.

The DCM samples were also utilized to determine the cuticle weight of the leaves. The RapidVac evaporation glassware cylinders were initially weighed on a calibrated scale. Following, the 50 mLs of DCM sample was placed in the cylinders and evaporated (41°C) until there was no liquid left in the cylinder. The cylinder was then weighed to determine the cuticle weight. [27, 29].

The third phase of the extraction was the complete extraction of the leaf samples using accelerated solvent extraction (ASE). The sample leaves were cut up
into small pieces and weighed. Following, the leaves were placed in a beaker and mixed with sodium sulfate and magnesium sulfate; these serve to dry the moisture from the samples. The leaves were loaded into stainless steel cells for accelerated solvent extraction. The cells were loaded and were then placed in a Dionex Accelerated Solvent Extractor (ASE) 350 (Thermo Scientific). The ASE extracts oil elements from the leaf samples utilizing a 50:50 mixture of hexane:acetone under 17000 psi of pressure at a temperature of 100°C. The extracted samples were then evaporated (at 70°C) and passed through SPE cleanup as described above. The samples were once again concentrated to 5 mLs and of that, 1 mL was utilized for GC-MS analysis. This allowed for the analysis of the concentration of PAHs in the tissue of the leaves.

All samples (1 mL) were analyzed utilizing a Hewlett Packard 6800 N gas chromatograph, equipped 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, and at 15 sample intervals. Calibration standards of a known concentration of PAHs were also included in each run with samples. PAHs targeted from analysis include: naphthalene (C₀N), C₁-naphthalenes (C₁N), C₂-
naphthalenes (C\textsubscript{2}N), C3-naphthalenes (C\textsubscript{3}N), C4-naphthalenes (C\textsubscript{4}N),
acenaphthylene (ACL), acenaphthene (ACE), fluorene (C\textsubscript{0}F), C1-fluorenes (C\textsubscript{1}F), C2-
fluorenes (C\textsubscript{2}F), C3-fluorenes (C\textsubscript{3}F) phenanthrene (C\textsubscript{0}P), C1-phenanthrenes (C\textsubscript{1}P),
C2-phenanthrenes (C\textsubscript{2}P), C3-phenanthrenes (C\textsubscript{3}P), C4-phenanthrenes (C\textsubscript{4}P),
dibenzothiophene (C\textsubscript{0}D), C1- dibenzothiophenes (C\textsubscript{1}D), C2- dibenzothiophenes
(C\textsubscript{2}D), C3- dibenzothiophenes (C\textsubscript{3}D), fluoranthene (FAN), pyrene (PY), C1-
pyrene/fluoranthene (C\textsubscript{1}-PY/FA), chrysene (C\textsubscript{0}C), C1-chrysenes (C\textsubscript{1}C), C2-chrysenes
(C\textsubscript{2}C), and C3-chrysenes (C\textsubscript{3}C). C\textsubscript{3}0-Hopane was also analyzed.

2.2.3 Marsh periwinkle sampling

Marsh periwinkle snails (*Littoraria irrorata*) were also sampled from field sites in Bay Jimmy on June 12th, 2014. Snails were collected from the same plots as *Spartina* leaf samples on the June 12 sampling event. Snails were collected from six of the seven plots in Bay Jimmy (there were no snails on one of the plots). Snails were hand collected off of *Spartina* leaves in each plot; there was no visible contamination on the snails. The snails were gathered in 50 mL centrifuge tubes (about 40 snails per tube), labeled, and stored in the freezer once in the lab. Three centrifuge tubes were filled with snails from each plot, allowing for analysis to be done in triplicates. The snail tissue was separated from their shells by breaking the shells while frozen and removing the snail tissue. The frozen snails were placed on clean paper towels and covered with another layer of clean paper towels; a sheet of aluminum foil was then placed on top of the paper towels (having no direct contact with the snails) and force was applied to the covered snails, by a hammer.
Subsequently, the snails were uncovered and the dismantled shells were separated from the snail tissue with a pair of tweezers (cleaned with hexane: acetone). Once the shells were removed, the remaining tissue was weighed and then extracted by means of ASE using 1:1 hexane acetone as the extraction solvent. Once the extraction was completed, the solvent was concentrated to 10 mL and analyzed by a GC/MS.

2.2.4 PAH Recovery Experiment

Prior to the execution of this method on field samples, a PAH recovery experiment was performed to ensure the reliability of the method. A PAH standard solution of 10 ng/μL was prepared using 1:1 hexane acetone as a solvent. The standard solution was subjected to the sequential extraction method as described above. One mL was first diluted to 30 mLs using 1:1 hexane acetone. The sample was then concentrated back down to 1 mL, diluted to 30 mL once again (1:1 hexane acetone), and concentrated to a final 1 mL for GC-MS analysis.

The 10 ng/μL PAH standard was also used to test the recovery of the SPE cleanup process. The column was activated with 1 mL of water, following, the standard solution was loaded on the column and washed with 40 mL of 2:3 hexane acetone. The standard solution was evaporated to 1 mL, 50 mLs of 1:1 hexane acetone was added to the standard and once again evaporated to 1 mL. The standard was then analyzed by GC-MS technology. The entire recovery experiment was done in triplicate, and a minimum average recovery of 70% was achieved (Table 2.1).
Table 2.1: Recovery of spiked PAHs (%) from the plant tissue extraction method. Standard error of the mean was computed from triplicate analyses.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Average % Recovery</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>76.4</td>
<td>1.8</td>
</tr>
<tr>
<td>Acenaphthylene</td>
<td>75.5</td>
<td>0.1</td>
</tr>
<tr>
<td>Acenpathene</td>
<td>73.0</td>
<td>2.3</td>
</tr>
<tr>
<td>C2-naphthalenes</td>
<td>72.9</td>
<td>1.6</td>
</tr>
<tr>
<td>Fluorene</td>
<td>73.5</td>
<td>2.3</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>82.0</td>
<td>3.9</td>
</tr>
<tr>
<td>Anthracene</td>
<td>77.9</td>
<td>3.3</td>
</tr>
<tr>
<td>Dibenzothiophene</td>
<td>75.4</td>
<td>1.6</td>
</tr>
<tr>
<td>C1-phenanthrene</td>
<td>76.8</td>
<td>1.3</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>75.9</td>
<td>0.7</td>
</tr>
<tr>
<td>Pyrene</td>
<td>76.5</td>
<td>0.5</td>
</tr>
<tr>
<td>C2-dibenzothiophenes</td>
<td>72.2</td>
<td>1.3</td>
</tr>
<tr>
<td>Chrysene</td>
<td>74.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Benzo(a)anthracene</td>
<td>74.9</td>
<td>2.8</td>
</tr>
<tr>
<td>Benzo(b)fluoranthene</td>
<td>77.5</td>
<td>1.1</td>
</tr>
<tr>
<td>Benzo(k)fluoranthene</td>
<td>83.0</td>
<td>6.6</td>
</tr>
<tr>
<td>Benzo(a)pyrene; perylene</td>
<td>76.7</td>
<td>4.1</td>
</tr>
<tr>
<td>Indeno(ghi-cd)pyrene</td>
<td>77.7</td>
<td>0.4</td>
</tr>
<tr>
<td>Benzo(ghi)perylene</td>
<td>77.1</td>
<td>1.6</td>
</tr>
<tr>
<td>Dibenz(a,h)anthracene</td>
<td>76.1</td>
<td>2.2</td>
</tr>
</tbody>
</table>

2.3. Results and Discussion

2.3.1 Port Fourchon Beach Samples

The PAHs quantified in both the surface and the tissue of all samples are normalized to the dry weight of the *Spartina* leaves, and the PAHs quantified in the cuticle of the leaves are normalized to the cuticle weight. Figures 2-4 – 2-6 displays the analysis of PAHs in Port Fourchon marsh *Spartina* samples collected in summer 2013. The PAHs that were detected were primarily alkylated naphthalenes and phenanthrenes, compounds that generally have a petrogenic rather than pyrogenic origin.
Figure 2-4: PAH concentrations on the *Spartina* leaf surface from the oiled Port Fourchon marsh site. PAHs concentrations are from the surface of the leaf (shown in white), the cuticle of the leaf (shown in purple), and the internal tissue of the leaf (shown in yellow). Data are presented from 5 locations in the oiled plot (Oil 1-5). Error bars signify the standard error of the mean from three replicates taken at each plot.
Figure 2-5: PAH concentrations on the *Spartina* leaf surface from inland Port Fourchon marsh sites. PAHs concentrations are from the surface of the leaf (shown in white), the cuticle of the leaf (shown in purple), and the internal tissue of the leaf (shown in yellow). Data are presented from 5 locations in the oiled plot (1C-5C). Error bar signify the standard error of the mean from three replicates taken at each plot.
Figure 2-6: PAH concentrations on the *Spartina* leaf surface in the control Port Fourchon marsh site. PAHs concentrations are from the surface of the leaf (shown in white), the cuticle of the leaf (shown in purple), and the internal tissue of the leaf (shown in yellow). Data are presented from 5 locations in the control area (CS1-5). Error bars signify the standard error of the mean from three replicates taken at each plot.
These compounds, along with alkylated chrysenes, were the primary PAHs observed in MC252 oil that reached this shoreline [58-60, 62]. Alkylated chrysenes were not detected in the plant samples. All samples displayed the majority of PAHs present in the cuticle or the tissue of the plant (Figures 2-4 – 2-6). Much lower concentrations were observed in particulates on the leaf surface, measured by extraction with EDTA.

In several cases, PAH concentrations in the oiled plots (Oil 1-5) (Figure 2-4) were higher than the concentrations in the inland (Inland 1-5) (Figure 2-5) and the control site (Control 1-5) (Figure 2-5). In the oiled plots, concentrations were dominated by the C2-naphthalenes, and concentrations were higher in the plant tissue than the cuticle in 4 of the 5 plant samples obtained. Concentrations of C2-naphthelenes were as high as 4000 ng/g of leaf tissue.

The plants in the inland locations also contained PAH concentrations dominated by C2-naphthalenes (Figure 2-5). At these locations, however, 4 of the 5 locations had higher concentrations in the cuticle rather than the plant tissue. Concentrations were of the same order of magnitude as the oiled plots. The control sampling sites (Figure 2-6) contained lower concentrations of PAHs than the plants in the oiled plots and the inland sites.

2.3.2 Bay Jimmy Samples

The PAHs quantified in both the surface and the tissue of all samples are normalized to the dry weight of the Spartina leaves, and the PAHs quantified in the cuticle of the leaves are normalized to the cuticle weight. Figure 2-7 portrays the results from the summer 2013 analysis of Spartina leaves at a marsh adjacent to Bay
Jimmy, LA. Samples collected in the summer of 2013 also displayed 2 and 3-ring petrogenic PAHs, namely the alkylated naphthalenes and phenanthrenes. The dominant PAHs detected at this event were the C2-phenanthrenes, ranging from the 25,000 to 65,000 ng/g of leaf weight. With exception of a detection of C4-phenanthrenes in plot 3A, samples had highest concentrations of PAHs in the cuticle.

Figure 2-8 displays the results of the winter 2014 sampling of Spartina leaves at Bay Jimmy. The winter 2014 samples showed an increased concentration of total PAHs, both alkylated naphthalenes and phenanthrenes, even if the maximum concentration was lower. C1-phenanthrenes were the dominant PAH detected in all 6 plots sampled.
Figure 2-7: PAH concentrations on the Spartina leaf surface from the Bay Jimmy marsh site in Summer 2013. PAHs concentrations are from the surface of the leaf (shown in white), the cuticle of the leaf (shown in purple), and the internal tissue of the leaf (shown in yellow). Data are presented from 5 plot locations (3A, 4B, 5A, 5D and 6A) oriented along the shoreline. Error bars signify the standard error of the mean from three replicates taken at each plot.
Figure 2-8: PAH concentrations on the *Spartina* leaf surface from the Bay Jimmy marsh site in Winter 2014. PAHs concentrations are from the surface of the leaf (shown in white), the cuticle of the leaf (shown in purple), and the internal tissue of the leaf (shown in yellow). Data are presented from 6 plot locations (3A, 4B, 4C, 5A, 5D, 6A and 6D) oriented along the shoreline. Error bars signify the standard error of the mean from three replicates taken at each plot.
In Figure 2-9, the results for the summer 2014 samples collected from Bay Jimmy, LA are plotted. These samples follow the trend of increasing PAH concentrations seen in the winter 2014 samples. These samples had a PAH profile that was more evenly distributed across the range of alkylated naphthalenes and phenanthrenes. The majority of the PAHs were found in the cuticle of the leaf.

Marsh Periwinkle Snail Analyses

Along with leaf samples, marsh periwinkle snails were collected from Bay Jimmy in Summer 2014. The marsh periwinkle spends a portion of its life cycle grazing on Spartina leaves therefore; it was hypothesized that the snail would also contain PAHs in its tissue. The same suite of alkylated naphthalenes and phenanthrenes were detected for snail samples (Figure 2-10) as for Spartina collected at the same sampling locations and times. Both naphthalenes and phenanthrenes were present in the snail and Spartina sample. For the snail samples it cannot be unequivocally concluded whether PAHs were in the snail tissue or whether it may have been observed from surface contamination.

2.3.3 Discussion and Conclusions

This studied quantified the concentration and distributions of PAHs on Spartina leaves as well as in the marsh periwinkle snail. Alkylated naphthalenes and phenanthrenes were quantified in both the cuticle and the tissue of Spartina leaves. MC252 crude oil is composed of alkylated naphthalenes, phenanthrenes, dibenzothiophenes, and chrysenes. The PAHs found included alkylated naphthalenes and phenanthrenes; no dibenzothiophenes or chrysenes were detected in any of the samples.
Figure 2-9: PAH concentrations on the Spartina leaf surface from the Bay Jimmy marsh site in Summer 2014. PAHs concentrations are from the surface of the leaf (shown in white), the cuticle of the leaf (shown in purple), and the internal tissue of the leaf (shown in yellow). Data are presented from 6 plot locations (3A, 4B, 4C, 5A, 5D, 6A and 6D) oriented along the shoreline. Error bars signify the standard error of the mean from three replicates taken at each plot.
Figure 2-10: Analysis PAH concentrations (ng PAH/g tissue) in marsh periwinkle snails from the Bay Jimmy marsh site in Summer 2014. Data are presented from 6 plot locations (3A, 4B, 4C, 5A, 5D, 6A and 6D) oriented along the shoreline. Error bars signify the standard error of the mean from three replicates taken at each plot.
Port Fourchon samples displayed the characteristics of a lightly oiled, more saline marsh. PAHs were quantified in the oiled and inland plots, however concentrations and distributions of PAHs were lower than those of the Bay Jimmy marsh. The Bay Jimmy samples indicated a more oiled, less saline marsh, with a wider distribution and higher concentrations of PAHs. The marsh periwinkle snail, collected from Bay Jimmy, also contained PAHs in its tissue. Analysis demonstrated similar PAHs in the snail tissue as those in the plant leaves.

According to the EPA’s IRIS database, the reference dose (RfD) for naphthalene is 0.002mg/kg body weight[66]. For a 70 kg body weight person, 1.4 g of naphthalene would be considered dangerous. The snail data indicated a max concentration of 325 ng/g of naphthalene; therefore, 4.3 kg of contaminated snail tissue must be ingested to reach the RfD.

As indicated by this study, the effects of the oil spill persist four years after the Macondo oil spill. The presence of both alkylated naphthalenes and phenanthrenes were detected, in or on both leaf samples and snail tissue.
Chapter 3  Air-plant partitioning between petrogenic PAHs and Spartina alterniflora leaf surface

3.1 Introduction

The potential mechanisms of plant uptake of PAHs includes (1) root uptake from contaminated soil, (2) adsorption by cuticle of volatilized PAHs from the surrounding air, or (3) uptake from contaminated soil though the transpiration stream followed by retention in plant leaf and other tissues [67, 68]. The key accumulation pathway for PAHs is from the air to the leaf surface [36]. The plant-air partitioning phenomenon has been studied and an was used to describe plant uptake [35].

After the Macondo oil spill in 2010, oil moving into the marshes placed PAHs in close proximity with macrophytes such as Spartina alterniflora and Avicennia germinans [3, 55, 69]. Experiments described in Chapter 2 of this thesis demonstrated that PAHs were detected in or on Spartina and the marsh periwinkle snails on these surfaces. While uptake of PAHs by Spartina has been studied [6], more fundamental information including the air:cuticle partition coefficients for alkylated PAHs on Spartina leaf surfaces are unknown. Understanding these processes could aid in understanding contaminated fate in spill-impacted marshes. Numerous mechanisms may account for the transfer of PAHs from contaminated soil to plant tissue, including uptake in the transpiration stream, volatilization and consequent redeposition on leaves, and sorption from direct contact with soil particles [6],[56]. Atmospheric deposition (air to leaf) has been identified as a pathway in many studies [6], [67]. The air to leaf route may be considered as the main contamination for higher plant leaves [35].
This study quantified the *Spartina*/petrogenic PAH partition coefficients. A controlled greenhouse experiment was performed using a chamber that allowed exposure of live *Spartina* surfaces to volatilizing crude oil containing petrogenic PAHs. PAH air concentrations and plant measurements allowed for calculation of partition coefficients.

### 3.2 Methods and Materials

#### 3.2.1 Chamber Experiment

A greenhouse experiment was performed to determine the PAH partition coefficients between the air and *Spartina* leaf surfaces. Intact *Spartina* plants were sampled from marshes near Port Fourchon, LA and planted in pots in a greenhouse. *Spartina* was planted in potting soil, fertilized with Miracle Grow and kept submerged. Once new growth was observed from underground rhizomes, plants were deemed ready for chamber experimentation. Fourchon Beach, located adjacent to Port Fourchon, LA was heavily impacted by the Macondo spill [58-60, 62]. Oil reached the area in 2010 and active cleanup ceased in late 2013. The plant samples were taken in March 2014 in a marsh area that did not receive direct oiling. PAHs on plant surfaces were below detection limits for alkylated PAH in the initial samples taken in the chamber experiment described below.

Two potted plants were placed in a 45-gallon hexagonal aquarium tank, each in a tray of tap water (Figure 3-1). A small fan was also placed in the tank to circulate the air.
Figure 3-1: Experimental layout for air:plant partitioning study consisting of three *Spartina* in a 45-gallon hexagonal aquarium tank, a small fan to circulate the air, and a 50 mL glass beaker containing 25 mLs of weathered south Louisiana crude oil.
The tank was closed with a glass top, which had three holes; two for air circulation allowing external air to passively replenish air in the chamber, and one to serve as an air sampling port. A “time zero” series of plant samples was taken prior to the initiation of the study using the techniques described below. Following this sampling event, the study was initiated by placing a round, 50 mL glass beaker containing 25 mL of weathered south Louisiana crude oil into the aquarium adjacent to the plants.

Air sampling was performed using a Buck Libra L-4 air-sampling pump equipped with silica sorbent tubes. Air sampling was initiated on Day 1 of the experiment. The pump was left to sample for 5 days, at a flow rate of 0.00201 gallons/minute. The silica sorbent tube was removed and replaced with a new, identical tube on day 5 and 20. The silica sorbent was extracted using accelerated solvent extraction (ASE) methods, utilizing hexane: acetone (50:50) as the solvent as described below. This allowed calculation of separate time weighted average gas-phase PAH concentrations for time intervals of 0-5 days (silica tube 1), 5-20 days (silica tube 2), and 20-25 days (silica tube 3).

Leaf samples were taken on days 0, 5, 10, 15, 20, and 25. Leaf samples were taken by removing the chamber top and snipping 3 leaves from the Spartina in the chamber. These samples were subjected immediately to the sequential extraction method described below.

3.2.2 Chemical Analysis

In order to determine the concentration of PAHs in the plant leaves, a sequential extraction was performed on samples collected during the chamber
experiment. Dichloromethane (DCM) served as the first phase of the extraction, which dissolved the cuticle of the leaves. Leaf samples were placed in 150 mL of pesticide-grade DCM for 2 minutes in a clean glass container. The leaves were taken out of the DCM and stored in a Ziploc bag and transported to the laboratory for further extraction.

The DCM samples were used for the determination of the concentration of PAHs on the cuticle of the leaf as well as the cuticle weight of the leaf. Initially, 100 mL of sample DCM was evaporated (41°C) to 1 mL in a RapidVap N₂ Evaporation System (Labconco, USA). The 1 mL was then subjected to solid phase extraction (SPE) with a silica gel column. The cleanup allow for the elimination of any interference in the analysis of PAHs. The column was activated with 1 mL of water, following, the sample was slowly loaded on the column and washed with 40 mL of 2:3 hexane acetone. The samples were once again evaporated to 1 mL, 50 mL of 1:1 hexane acetone was added to the sample and once again evaporated to 5 mL. The sample was placed in a glass scintillation vial and stored in the refrigerator. The samples were then analyzed by GC-MS. This analysis allowed for the determination of the concentration of PAHs on the cuticle of the leaf.

The DCM samples were also utilized to determine the cuticle weight of the leaves. The RapidVap evaporated cylinders were initially weighed on a calibrated scale. Following, the 50 mLs of DCM sample was placed in the cylinders and evaporated (41°C) until there was no liquid left in the cylinder. The cylinder was then weighed to estimate the cuticle weight.
The second phase is the exhaustive extraction of the leaf samples using ASE (Dionex, Model 350) with hexane:acetone (1:1) as an extraction solvent. The sample leaves were cut up into small pieces and weighed. Following, the leaves were placed in a beaker and mixed with diatomaceous earth. The leaves were then loaded into the ASE cells, where they underwent a hexane:acetone extraction. The ASE extracts oil elements from the leaf samples utilizing a 50:50 mixture of hexane:acetone under 17000 psi of pressure at a temperature of 100°C. The extracted liquid was then evaporated (at 70°C) and passed through SPE cleanup. The samples were once again concentrated to 5 mLs and of that, 1 mL was utilized for GC-MS analysis. This allowed for the analysis of the concentration of PAHs in the tissue of the leaves.

Once air sampling was completed, for the time intervals ending on days 5, 20, and 25, the sorbent tubes were taken to the lab. The silica and glass wool were carefully separated from the glass and mixed with diatomaceous earth. The mixture was then extracted utilizing the ASE. Once extracted, samples were concentrated and tested in the GC-MS as described below.

All samples (1 mL) were analyzed utilizing a Hewlett Packard 6800 N gas chromatograph, equipped 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, and at 15 sample intervals. Calibration standards of a known concentration of PAHs were also included in each run with sample

3.2.3 Partition Coefficients

The octanol – air partition coefficient ($K_{oa}$) is a ratio of the concentration of a chemical in octanol to the concentration of that chemical in the air [70]. $K_{oa}$ is useful in predicating the behavior of chemicals between the air and the environmental conditions (i.e., soil or vegetation) [70, 71]. $K_{oa}$ may be estimated (Equation 1) based on the octanol – water partition coefficient, $K_{ow}$, Henry’s law constant, $H$, the universal gas constant, $R$, and temperature, $T$ [70, 72-74].

$$K_{oa} = \frac{K_{ow} (RT)}{H} \quad (1)$$

Equation one can be written in similar form utilizing the unitless Henry’s law constant, $K_{AW}$: [70, 75].

$$K_{oa} = \frac{K_{ow}}{K_{AW}} \quad (2)$$

Equation (2) can be used to estimate the octanol - air partition coefficient, which is the ratio of the observed air – water partition coefficient to the dimensionless Henry’s law constant[70, 75].

The plant-air partition coefficient, $K_{pa}$, is a ratio of the concentration of PAHs in the plant leaf to the concentration of PAHs in the air. $K_{pa}$ may be used to understand the uptake of PAHs form the air into the *Spartina* leaves [76]. The coefficient can be used solved for using equation (3)

$$K_{pa} = \frac{C_L}{C_a} \quad (3)$$
Where, \( C_L \) is the concentration of PAHs in the leaf and \( C_a \) is the concentration of PAHs in the air.

### 3.3 Results and Discussion

The average concentration of PAHs in the plant leaf increased with time reaching a maximum concentration after 10-15 days before decreasing (Figure 3-2). Results quantified include PAHs in the cuticle only, PAHs in the tissue of the plant leaves were also analyzed, however, they were not detected

PAHs found in the cuticle of the leaf include naphthalene, C1- naphthalenes, C2- naphthalenes, C3- naphthalenes, C4- naphthalenes, C1- phenanthrenes, and C2-phenanthrenes. The PAHs found are all present in MC252 crude oil. These compounds coincide with PAHs detected in the \textit{Spartina} leaves analyzed in field locations, reported in Chapter 2 of this thesis. In the chamber experiment, a maximum concentration of 6,000 \( \mu \)g PAH/ kg plant cuticle was quantified. Experiments described in Chapter 2 revealed a maximum PAH concentration of 7,000 \( \mu \)g/kg for Port Fourchon Beach samples, and 100,000 \( \mu \)g/kg for Bay Jimmy samples. This was higher (especially in Bay Jimmy samples) than in the chamber experiment reported here. This may have resulted from differences in the exposure time and differences in the air concentrations of PAHs at the field locations. The chamber experiment was performed over a 25 - day period, while exposure in field locations was considerably longer. Higher concentrations also suggest a higher air concentration of PAHs in the Bay Jimmy marsh environment, which includes exposure to oil emulsion and oiled marsh sediment.
Figure 3-2: Average PAH concentrations (µg/kg) versus time in the Spartina plant cuticle (A = Port Fourchon Spartina alterniflora, B = Spartina alterniflora cv Vermillion)
After 10-15 days, the concentration of PAHs began to decrease, likely due to the depletion of PAHs in the system as PAH volatilization from the oil surface declined. The behavior of *Spartina* from Port Fourchon Beach was slightly different then that of the Vermillion *Spartina*. C3-naphthalene was measured but not detected in the cuticle of the Vermillion *Spartina* plants. This was not the case for *Spartina* from Port Fourchon Beach; C3-naphthalene was quantified in the cuticle of the samples. The same overall trend of an increase, followed by maximum, and a decrease in concentrations was observed in both types of *Spartina*.

Both types of *Spartina* were exposed to the same air PAH concentrations (within the same chamber). The measured time-weighted average air concentrations of PAHs (Table 3-1) followed the same general trend as the PAH concentration in the *Spartina* leaves. Air concentrations reported in the table determined for 5-15 day intervals represent time-weighted averages.

Utilizing the concentration of PAHs in the plant cuticle and the concentration of PAHs in the air phase, plant – air partition coefficients (Figure 3-3) were calculated. These coefficients quantify the relative distribution of PAHs in the cuticle of the leaf as compared to the concentration of PAHs in the air. The plant – air partition coefficients increased, reached a maximum value, and subsequently decreased, tracking the relative changes observed in the plant. The partition coefficients increased until Day 10-15 then decreased. Increased values for the plant – air partition coefficients would signify a higher uptake fraction of PAHs from the air into the cuticle of the leaves. The values of the plant – air partition coefficients were high, reaching 320,000 (L/kg).
Air sampling was initiated on Day 1 of the experiment. The pump was left to sample for 5 days. Subsequent air samples were taken on day 5 of the experiment, then at the time of experiment termination. Air samples for each day of the experiment were found based on integration with the air samples of days 1, 5, and 20, and 25.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Days 0-5</th>
<th>Days 5-25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>0.020</td>
<td>0.189</td>
</tr>
<tr>
<td>C1-naphthalenes</td>
<td>0.022</td>
<td>0.221</td>
</tr>
<tr>
<td>C2-naphthalenes</td>
<td>0.018</td>
<td>0.119</td>
</tr>
<tr>
<td>C3-naphthalenes</td>
<td>0.027</td>
<td>0.061</td>
</tr>
<tr>
<td>C4-naphthalenes</td>
<td>0.164</td>
<td>0.054</td>
</tr>
<tr>
<td>C1-phenanthrene</td>
<td>0.073</td>
<td>0.044</td>
</tr>
<tr>
<td>C2-phenanthrene</td>
<td>0.038</td>
<td>0.167</td>
</tr>
</tbody>
</table>

Plant – air partition coefficients were calculated for both Port Fourchon Spartina and the Vermillion Spartina. The Port Fourchon Spartina leaves show less variability in the partition coefficients, ranging from 430 - 51,000 (L/kg), while the partition coefficients for the Vermillion Spartina variety plant leaves ranged from 350 – 320,000 (L/kg). The leaves from the Spartina of Fourchon Beach were still high, however they were not as high as those of the Vermillion variety. These data are not explained by the mass of cuticle observed in each variety. There were no differences in the cuticle weights of the 2 Spartina sources. The octanol – air partition coefficients, $K_{oa}$, were also calculated using the equation 2 (Table 3-2). $K_{oa}$ is a ratio of the octanol-water coefficient, $K_{ow}$, and the dimensionless Henry’s Law constant, $K_{aw}$ [70, 75].
Figure 3-3: Plant - air partition coefficient, $K_{pa}$ (L/kg) vs. time (A = Port Fourchon *Spartina alterniflora*, B = *Spartina alterniflora cv Vermillion*). The gas sample intervals were calculated based on sorbent tubes 1, 2, and 3.
Values for $K_{ow}$ and $K_{aw}$ were taken from literature. $K_{oa}$ can be used to predict the distribution of PAHs between the air and *Spartina* plant leaves. Log $K_{oa}$ values ranged from 5.1 – 8. To the extent that the plant cuticle can be represented as octanol, the degree to which PAHs accumulate on the leaf is dependent on the $K_{oa}$ value. PAHs with a large $K_{oa}$ value are more likely to accumulate in the plant cuticle [77]. C2-phenanthrene held the highest log $K_{oa}$ value indicating that PAH should have the highest partition coefficient. C2-phenanthrene had the highest concentration of PAHs in the cuticle of the leaves (for both the Port Fourchon and the Vermillion *Spartina*) and the highest observed partition coefficient. Calculations of the concentration of PAHs in the leaves and the log $K_{oa}$ values are independent.

C2-phenanthrene behaved as expected in the chamber experiment.

Table 3-2: Octonal-water Partition coefficient ($K_{ow}$) [78], Octonal- air partition coefficient ($K_{oa}$), Dimensionless Henry's Law Constant ($K_{aw}$) [79] of select petrogenic PAHs. $K_{oa}$ is a ratio of the octanol-water coefficient, $K_{ow}$, and the dimensionless Henry's Law constant, $K_{aw}$ [70, 75]. Values for $K_{ow}$ and $K_{aw}$ were taken from literature. $K_{oa}$ can be used to predict the distribution of PAHs between the air and *Spartina* plant leaves.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Log $K_{ow}$</th>
<th>$K_{aw}$</th>
<th>Log $K_{oa}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>3.4</td>
<td>0.020</td>
<td>5.1</td>
</tr>
<tr>
<td>C1- Naphthalene</td>
<td>3.9</td>
<td>0.018</td>
<td>5.6</td>
</tr>
<tr>
<td>C2- Naphthalene</td>
<td>4.4</td>
<td>0.007</td>
<td>6.5</td>
</tr>
<tr>
<td>C3- Naphthalene</td>
<td>5.0</td>
<td>0.006</td>
<td>7.2</td>
</tr>
<tr>
<td>C4- Naphthalene</td>
<td>5.6</td>
<td>0.006</td>
<td>7.8</td>
</tr>
<tr>
<td>C1- Phenanthrene</td>
<td>5.1</td>
<td>0.003</td>
<td>7.7</td>
</tr>
<tr>
<td>C2- Phenanthrene</td>
<td>5.5</td>
<td>0.003</td>
<td>8.0</td>
</tr>
</tbody>
</table>
In the naphthalene family, C4-naphthalene had the highest log $K_{oa}$ value. Therefore, of the naphthalene family, C4-naphthalene should hold the highest concentration in the cuticle of the leaf. This was the case for the *Spartina* leaves from Port Fourchon; however, the leaves from the Vermillion *Spartina* did not behave similarly. C4-naphthalene was present in the cuticle of the Vermillion *Spartina* leaves, yet the naphthalene with the highest concentration of PAHs was C1-naphthalenes.

3.3.1 Discussion and Conclusions

Considering all values quantified, the chamber study revealed that *Spartina* leaves are susceptible to the uptake of PAHs in a controlled environment. The PAHs found in the leaves were consistent with the PAHs found on field sites (Port Fourchon and Bay Jimmy). Analysis of air concentrations revealed similar PAHs in the experimental chamber as those in the leaves. The PAHs quantified in this study, alkylated naphthalenes and phenanthrenes are present in MC252 crude oil. However, as discovered in the results of Paper1, alkylated dibenzothiophenes and chrysenes were not detected in any of the samples.
References


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Vita

Yasmin Mohammad, daughter of Louay and Eman Mohammad, was born (February 14, 1989) and raised in Baton Rouge, Louisiana. She is the youngest and only girl of three siblings. Yasmin graduated from the University Laboratory School in May 2007 and continued her education at Louisiana State University (LSU). In December of 2011, she received her Bachelor's of Science in Biological Engineering. Upon graduation, Yasmin was honored as a Distinguished Communicator. She then continued her education at LSU. With Dr. John Pardue as her advisor, Yasmin studied the Uptake and Deposition of PAHS on Spartina Leaves. In May 2015, Yasmin expects to earn her Master of Science in Civil and Environmental Engineering. She is now working with Chevron as an HES Engineer.