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Smooth Cord Grass (Spartina Alterniflora) Response to Simulated Oil Spills in Sediment-Water Microcosms

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SMOOTH CORD GRASS (*SPARTINA ALTERNIFLORA)* RESPONSE TO SIMULATED OIL SPILLS IN SEDIMENT-WATER MICROCOSMS

by

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Graduate College
The University of Iowa
Iowa City, Iowa

CERTIFICATE OF APPROVAL

MASTER'S THESIS

This is to certify that the Master's thesis of

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has been approved by the Examining Committee
for the thesis requirement for the Master of Science degree
in Civil and Environmental Engineering
at the August 2013 graduation.

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Craig L. Just

To my family and friends for convincing me to
"be cool and stay in school."

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ABSTRACT

Simulated oil spills were created in *S. alterniflora* sediment-water microcosms to determine the effect of the applied crude oil on *S.alterniflora* during two 90-day studies. In the first experiment only oil dosage was varied at 0-250 mg crude oil/g wet soil to determine the lethal dosage level in a simulated oil slick. In the second experiment, oil type, dosage, and soil type were varied to determine the effects of oil under multiple scales of resolution. A light, medium, and heavy crude oil at dosages ranging from 0-150 mg crude oil/g wet soil was used in addition to soil which had been acclimated for four months with 0 or 5 mg crude oil/g wet soil. Following the completion of the 90-day experiment, several key findings were observed: (1) The lethal dosage limit was reached at 250 mg crude oil/g wet soil during the first experiment; (2) At the heaviest dosages applied as a simulated oil slick, concentrations of 150 mg crude oil/g wet soil, evapotranspiration rates were negatively affected by the oil (significant at p=0.05 in a one-tailed t-test); (3) Light, heavy, and then medium crude oil showed the lowest biomass growths, in that order, indicating that light crude oil was the most toxic in these microcosm experiments with *S. alterniflora*; (4) The 10 mg oil/g wet soil out-performed the 0 mg oil/g wet soil in transpiration and biomass growth.

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Introduction and Motivation

On the evening of April 20, 2010, the Deepwater Horizon oil-drilling platform operated by BP exploded, killing 11 workers, spilling 210 million US gallons of oil, and causing the largest marine oil spill in history (BP, 2013). Since then, BP has spent $25 billion for clean-up, restoration costs, and claim payments; and more than 1000 organizations contributed to the response of the oil spill (United States Coast Guard, 2011).

In spite of this unprecedented catastrophe, oil spills of a smaller nature are commonplace, and BP projects offshore drilling to increase from 7% to 10% of total global oil production by 2020 (BP, 2012).

It is evident that oil spills, especially offshore oil spills, are an increasing problem that is not expected to go away anytime soon. Thus, while it is important to reduce the rate at which oil spills occur, it is also imperative to better understand their impact to coastal and marine ecosystems to aid response and cleanup efforts. Wetlands and coastal saltmarshes, in particular, are frequently affected by oil spills and encounter some of the highest concentrations of oil due to their proximity to the ocean. During the 2010 Deepwater Horizon oil spill, concentrations up to 510 mg oil/g soil were measured in the saltmarshes (Lin & Mendelssohn , 2012). Furthermore, wetlands are some of the most biologically productive habitats in the world, acting as buffers against hurricanes, and supporting complex, profitable ecosystems (Nebel & Kormondy, 1981).

Funding provided by a summer research opportunity program (SROP) and Dr.

Jerald Schnoor allowed students Elliott Beenk and Aaron Gwinnup to travel to Louisiana shortly after the 2010 Deepwater Horizon oil spill and to begin a preliminary experiment. A picture of the author from this trip can be seen in Figure 1.

Figure 1: Elliott Beenk at Airplane Lake in Southern Louisiana During the 2010 Deepwater Horizon Oil Spill

Success from the initial experiment, referred to as experiment one, led to funding from BP to conduct a more comprehensive study on the effects of oil on the saltmarsh species *Spartina alterniflora.* The second experiment is the primary focus of this thesis and will be referred to as experiment two.

While a substantial body of literature exists on the effects of oil on saltmarsh plants, few studies account for multiple environmental and oil conditions simultaneously. Thus, additional research that considers multiple environmental variables simultaneously is needed to better inform best management practices. Accordingly, experiment two was developed to address this need.

Objectives

This research was performed in an attempt to understand the effect of oil on the saltmarsh species *S. alterniflora* under multiple conditions simultaneously. More specifically, experiment two studied the effect of oil type, dosage, and soil/sediment type on *S. alterniflora* biometrics. The specific objectives were as follows:

Primary Objective I

Determine the effect of crude oil type on *S. alterniflora* biometrics:

(a) Determine the effect of a light, medium, and heavy crude oil on *S. alterniflora* evapotranspiration rates

(b) Determine the effect of a light, medium, and heavy crude oil on the change in biomass for *S. alterniflora*

Primary Objective II

Determine the effect of oil vs. non-oiled soil/sediment on *S. alterniflora* biometrics:

(a) Determine the effect of oiled vs. non-oiled soil/sediment on *S. alterniflora* evapotranspiration rates

(b) Determine the effect of oiled vs. non-oiled soil/sediment on the change in biomass for *S. alterniflora*

Primary Objective III

Determine the effect of variable oil concentrations on *S. alterniflora* biometrics:

(a) Determine the effect of 0, 10, 50, and 150 mg crude oil/g wet soil on *S. alterniflora* evapotranspiration rates

(b) Determine the effect of 0, 10, 50, and 150 mg crude oil/g wet soil on the

change in biomass for *S. alterniflora*

(c) Determine the dose-response relationship for survival of *S. alterniflora* for Louisiana Sweet Crude oil

CHAPTER 2 LITERATURE REVIEW

Deepwater Horizon Oil Spill

On April 20, 2010 the Macondo MC252 wellhead exploded, killing 11 workers and starting the uncontrolled release of approximately 200 million gallons of crude oil into the Gulf of Mexico. Two days after the initial explosion the oil-drilling platform sank to the floor of the Gulf. This event is more commonly known as the Deepwater Horizon (DWH) oil spill and released oil for 87 days, totaling nearly 5 million barrels of oil spilled (National Commission on the BP Deepwater Horizon Oil Spill and Offshore Drilling, 2011). Since the spill, BP for has spent $25 billion on claim payments and restoration costs. (United States Coast Guard, 2011).

The DWH oil spill has been referred to as the worst environmental disaster in history and has caused significant environmental, economic, and human damages. A map of the total area affected by the spill can be seen in Figure 2 below.

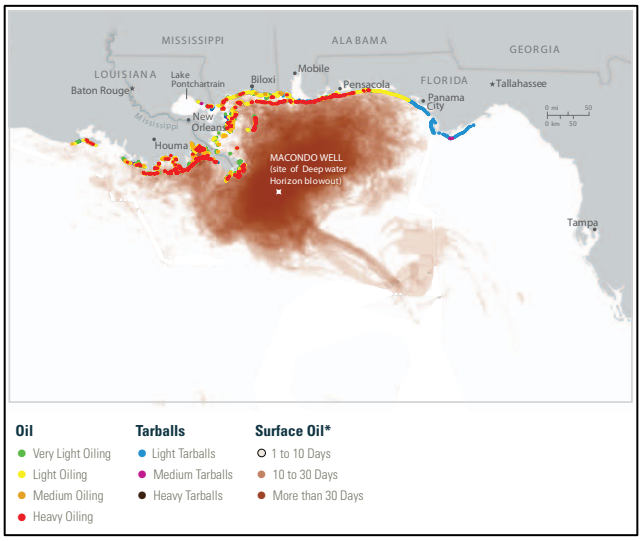

Figure 2: Total Area of Deepwater Horizon Oil Spill (National Commission on the BP Deepwater Horizon Oil Spill and Offshore Drilling, 2011)

As seen in Figure 2, the DHW oil spill heavily impacted a large area including the states of Louisiana, Mississippi, Alabama, and Florida. This complex environmental disaster requires a complete understanding of the full range of impacts to allow effective restoration and recovery efforts. However, during the oil spill, limited access to the response and heavily oiled zones inhibited the ability of independent researchers to study the impacts of the spill. Thus, additional research was needed to better understand the environmental effects of a large-scale oil spill (National Commission on the BP Deepwater Horizon Oil Spill and Offshore Drilling, 2011).

Saltmarshes

Coastal saltmarshes are the most heavily impacted non-marine environments due to their proximity to offshore drilling operations. Furthermore, saltmarshes are some of the most biologically productive habitats in the world and offer a host of economic and ecosystem services; Gulf of Mexico shoreline habitats generate more than $10 billion annually through tourism and fishing services they provide (Silliman, 2012).

Saltmarshes are present along a large percentage of the Gulf of Mexico shoreline and *S. alterniflora* is the most common saltmarsh grass. An example of a saltmarsh can be seen in Figure 3.

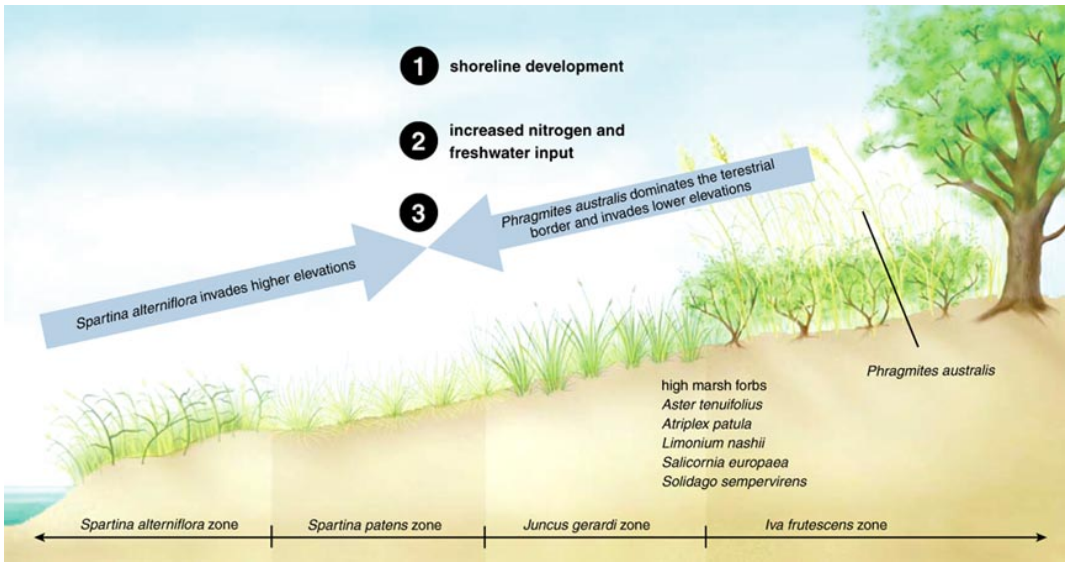

Figure 3: Example of the S. alterniflora Zone in a Coastal Salt Marsh (Miller, 2012)

Louisiana saltmarshes received the heaviest impact from the DWH spill and it was estimated that 75 linear km of saltmarshes in Louisiana experienced moderate to heavy oiling. It has been shown that saltmarsh ecosystems are quite resilient to oil spills by creating a natural buffer (Silliman, 2012). Figure 4 shows a comparison of an impacted and non-impacted saltmarsh in Louisiana following the DWH oil spill.

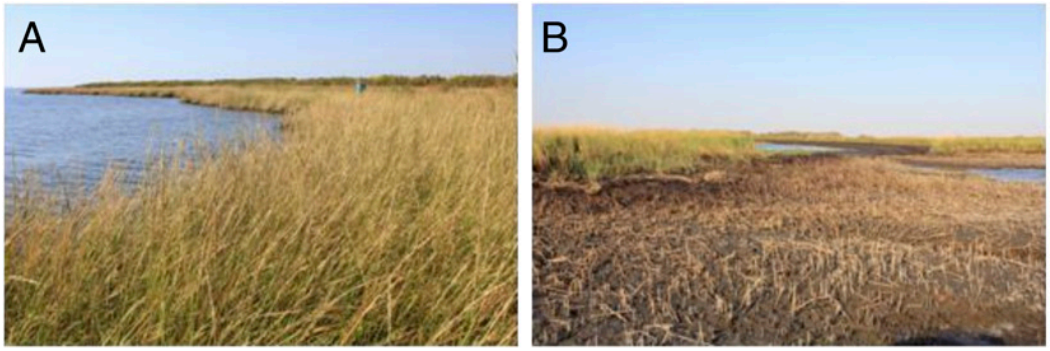

Figure 4: A Comparison of a Non-Impacted (C) and Impacted (D) Saltmarsh following the Deepwater Horizon Oil Spill (Silliman, 2012)

Observable in Figure 4, above ground die-off occurred in the saltmarsh impacted by the oil spill. The buffering nature of saltmarshes offers distinct advantages and disadvantages. On one hand, it confines and concentrates the oil to the edges of the saltmarsh causing relatively little oil to be detected at distances greater than 15m from the shoreline towards land; on the other hand, complete above ground mortality can occur along the shoreline due to the high concentrations of oil. Furthermore, this die-off is accelerated by anthropogenic activities like the channeling of the Mississippi. Shoreline erosion represents an additional environmental stressor on coastal ecosystems (Silliman, 2012). Thus, the full effects of the combined environmental impacts of anthropogenic activities plus oil spills to coastal saltmarshes are unknown.

Spartina alterniflora

Spartina alterniflora, also known as smooth cord grass, is the most abundant saltmarsh plant species. A variety of factors influence the impact of oil on saltmarsh grasses; this includes but is not limited to, oil type and concentration, method of exposure, soil type, and macrophyte species variation.

Oil Type and Characteristics

Petroleum hydrocarbons are organic compounds composed exclusively of carbon and hydrogen. Hydrocarbons and their associated refined products have come to be of great importance to industrial societies as sources of energy and as feedstocks for chemical products. Consequently, increased extraction and transportation of oil around the world has greatly increased the potential for oil spills (Harayama & Kishira, 1999).

Crude oil is a naturally occurring liquid consisting of a wide range of hydrocarbons, both aromatics and aliphatic hydrocarbons, typically categorized into a

gasoline and diesel range. The lighter hydrocarbons represent the gasoline range while the heavier hydrocarbons represent the diesel range of crude oil. Oil can be further categorized by relating its density to the density of water. The American Petroleum Institute (API) uses this relation, known as the API rating, to divide oil into five subcategories. These classifications can be found in Table 1 (Pezeshki & Hester, 2000).

Table 1: Classification of Oil Type by API

Type of oil	Examples	API Range [Degrees API]
Very light oils	Jet fuels and gasoline	>> 31.1
Light oils	Light crude oil, no. 2 fuel oil, and diesel	> 31.1
Medium oils	Most crude oils	23.3 to 31.1
Heavy oils	Heavy crude oils, No. 6 fuel oils, and Bunker C	< 23.3
Very heavy oils	Select No. 6 oils	< 10.0

Source: Pezeshki & Hester, The effects of oil spill and clean-up on dominant US Gulf coast marsh macrophytes: a review, 2000

Research has shown a variety of chemically induced affects depending on the type of oil. It is generally accepted that lighter weight oils are more immediately toxic to plants than heavier oils and medium oils are the least toxic (Pezeshki & Hester, 2000). That being said, when involving effects related to the inhibition of the gas-exchange surfaces of plants and into soil, heavier weight oils can be as detrimental as lighter weight oils (Pezeshki & Hester, 2000). To illustrate, the refined light oils are seemingly able to penetrate into plants and inhibit leaf and shoot regeneration where a variety of heavy oils showed little short-term impacts on *Spartina alterniflora* (Webb,

1994; Pezeshki S. D., 1995; Pezeshki & Hester, 2000).

Oil Lethal Dosage Limits to Saltmarsh Plants

Studies have shown a variety of responses by plants to oil ranging from being unaffected, to negative impacts, and in some cases, stimulating plant growth. Some studies report that acute dosages of crude oil pose no negative effects on plants or salt marsh ecosystems for dosages under 50mg/g (Delune et al., 1979). However, additional studies report that oil concentrations greater than 10.5 mg/g caused decreased plant biomass and stem density and led to long-term impacts (Alexander & Webb 1987). Furthermore, several studies have shown 100% mortality to plants at dosages greater than 400 mg oil/g dry soil (Pezeshki & Hester, 2000). Recent reports from studies of the DWH oil spill on saltwater marshes report that there is little toxicity to plants as long as the aerial portion of the grasses are not covered with oil.

Oil has been shown to decrease stem density and above and below ground biomass in *Spartina alterniflora*. Furthermore, a decrease in vegetation biomass led to "unconsolidated sediments, increased topographical variation and, ultimately, loss of salt marsh habitat." (Culbertson & Valiela, 2008).

Biomass metrics including stem density and biomass growth are negatively impacted by high total petroleum hydrocarbon (TPH) concentrations. Above-ground biomass has been shown to decrease with higher concentrations of TPH with effects observable at even relatively low TPH contents, 25 g/m^2. Root tissue has shown to be significantly more sensitive than rhizome tissue to oil (Culbertson & Valiela, 2008). To illustrate further, Lin and Mendelssohn observed that *Spartina alterniflora* was less sensitive than *Spartina patens* to South Louisiana crude but both species exhibited

complete mortality at oil dosages of 8 L of oil/m^2 and above (Pezeshki & Hester, 2000). To summarize, significant oiling can cause complete mortality in plants, but the dosage at which this can occur can significantly vary, and there is not yet an agreed upon dosage where effects occur.

Effects of Oil on an Environment

Significant research has been conducted analyzing the effects of oil on salt marshes; however, studies have only been able to focus on a limited number of variables at a time. The effect of oil on macrophytes can range from little to no effect to complete plant mortality depending on a number of factors. These impacts are due to physical effects and chemical toxicity, the extent of these effects can vary depending on mode of impact. Due to the frequency of oil pollution affecting salt marsh ecosystems, salt marsh plants have been of primary research interest; more specifically, the salt marsh genus *Spartina* and species *Spartina alterniflora*, *Spartina cynosuroides*, *Spartina townsendii*, and *Spartina patens* have been heavily studied. A more thorough list of plants that have exhibited the ability to tolerate petroleum hydrocarbons compiled from PhytoPet, a program developed by the University of Saskatchewan (Frick, Farrell, & Germida, 1999). This extensive list of 39 species further emphasizes the extent of research conducted regarding the effects of oil on plants.

Plant Biomass Metrics & Exposure Mechanisms

A number of different biomass metrics are used to assess the extent of the impact of oil to an ecosystem. In addition to mortality rates, several other plant biometrics are commonly measured, including but not limited to: evapotranspiration rates, plant stem density, above and below ground biomass, shoot regeneration, and

shoot height. The extent of oil impact depends upon a number of factors including the type and amount of oil, soil type, plant species, extent of oil coverage, and the weather conditions at the time of spillage (Burk, 1977; Hershner & Moore, 1977; Mendelssohn I. H., 1990; Alexander & Webb, 1985; Lin & Mendelssohn, 1996).

Plants can be affected by oil through physical and chemical means. The physical effects result from the coating of oil on the leaves, soil, and roots; effectively blocking transpiration, nutrient uptake, and gas-exchange pathways. This in turn can also increase the temperature of the leaves due to blocked transpiration pathways and reduce leaf photosynthesis because of blocked stomatal pores, reducing the entry of CO2 (Pezeshki & DeLaune, 1993; Pezeshki S. D., 1995). Extensive oil coverage can negatively impact the aforementioned biomass factors, with the extent of impact also depending on oil type and characteristics, hydrologic conditions, and dispersion of the oil. From these physical effects, biomass growth and regrowth can be inhibited, evapotranspiration rates reduced, and shoot generation limited (Pezeshki & DeLaune, 1993; Webb, 1994).

The physical effects of oil have also been found to be detrimental for plant to root oxygen diffusion. The transfer of atmospheric oxygen to plant roots is essential for plants growing in wetlands and flooded environments and is closely related to biomass growth. Thus, oil coating plant leaves reduces oxygen to root transfer and can negatively affect plant biomass growth (Pezeshki, DeLaune, & Patrick, 1989; Mendelssohn & McKee, 1988). This effect is amplified when oil also covers the sediment surface, limiting sediment root oxygen transfer and resulting in more anaerobic soils (Ranwell, 1968; Cowell, 1969).

In addition to physical effects, oil can cause chemically induced effects on plants and surrounding soils. Chemical fouling of leaves can penetrate plants and inhibit shoot and leaf regeneration (Pezeshki, DeLaune, Jugsujinda, Canevari, & Lessard, 1997; Webb, 1994). Furthermore, petroleum hydrocarbons can damage the root membranes of select salt-tolerant plants, negatively affecting the ionic balance of the plants and limiting their ability to grow in salt-water environments (Gilfillan, et al., 1989). The extent of chemically induced effects of oil has been found to be strongly dependent on the type of oil. Furthermore, heavy and medium crude oils exhibited few short-term effects on *Spartina alterniflora*, whereas refined light oils prevented plant biomass regeneration (Webb, 1994; Pezeshki S. D., 1995; Pezeshki, DeLaune, Jugsujinda, Canevari, & Lessard, 1997). Hydrologic patterns and movement of the oil also influence the extent of leaf and soil fouling. Salt marshes are frequently subject to changing tides due to tidal and storm variations in water levels, which influences the mode of impact of the oil. For example, with a low tide, the oil can directly impact the sediment and induce sediment fouling. Soil fouling is also associated with chronic exposure to petroleum hydrocarbons. In contrast, during a high tide the oil can directly coat the leaves and induce leaf fouling (Pezeshki & Hester, 2000).

Two soil/sediment characteristics that have been shown to have a noticeable impact are the concentration of organic matter and the particle size distribution. In one study, a clay soil slowed degradation rates and increases the time that plants were exposed to oil. However, when *Spartina alterniflora* was grown in multiple substratums, the plants grown in a more finely textured marsh substratum were less affected by oil than those grown in coarser sand. It is speculated that this is because the

coarser sand allowed oil to penetrate deeper more quickly than in the finely textured substratum (Head & Jones, 2006).

The direct impacts of oil on marsh macrophytes can inhibit plant transpiration and photosynthesis, are typically most detrimental on aboveground biomass, and can be directly toxic. In addition, if oil interacts with the soil, it can lead to increased oxygen stress in the rhizome, the area surrounding the roots, and reduce gas exchange between the soil and atmosphere. This can have a number of detrimental effects including disrupting root membranes and vegetative regrowth of new shoots (Pezeshki & Hester, 2000).

Oil fouling of leaves appears to induce more dramatic short-term effects to plants whereas fouling of soil tends to induce longer lasting effects. With a high enough dosage of oil, fouling of the leaves can cause complete mortality to above ground biomass. It is hypothesized that this is largely due to a breakdown of the photosynthetic apparatus in leaves directly affected by oil. Furthermore, following leaf fouling, *Spartina alterniflora* have been found to show no detectable photosynthetic activity and reduced stomatal conductance, the rate of carbon dioxide or water vapor entering or leaving a plant through the stomatal. (Pezeshki S. D., 1995).

In addition to fouling of the leaves, soil fouling can occur due to chronic exposure to oil and tidal variation, allowing for oil penetration and accumulation in the soil (Pezeshki & Hester, 2000). The extent of negative effects from soil fouling has been found to be primarily dependent on the properties of the plant followed by soil type and soil particle size. Soil organic matter (SOM) has been found to play an important role in the fate of oil in soil, and the effect of oil on plants and plant

regrowth. This is likely because SOM can replace oil as a substrate for hydrocarbon degrading bacteria and SOM also has the ability to sorb oil; thus, while higher SOM can decrease the toxicity to plants it may also limit rates of biodegradation of the oil (Pezeshki & Hester, 2000).

Due to a high concentration of oil drilling and refining operations, marsh ecosystems can be subject to oil spills of a variety of intensities. The impact of the oil on marsh ecosystems depends on how plants and ecosystems are exposed to oil, the species of macrophyte affected, the type and characteristics of oil spilled, current weather conditions, and concentration of oil exposure.

Macrophyte Species Variation

A variety of macrophytes have been studied and are typically classified by their aquatic environments; freshwater, saltwater, or brackish environments. While oil spills occur in both freshwater and saltwater mashes, the majority of research has been focused on the effect of oil on saltmarsh environments. In addition, saltwater and brackish marshes tend to support more commercially profitable ecosystems than freshwater marshes (Pezeshki & Hester, 2000).

The impact of oil on plants has been shown to be dependent on the type of environment and species affected. The following have been shown to have increasing sensitivity to crude oil in the following order: *Spartina lancifolia, Spartina alterniflora* and *Spartina patens.* The relative sensitivity of these plants is based on the effect of oil on live and dead biomass, photosynthetic rate, plant-stem density, and plant regrowth after a year. This specific study reports that the relative oil sensitivity was partially due to differences in soil organic matter among the marsh-types. In addition, soil organic

matter (SOM) played an integral role in accelerating penetration of oil into the soil compared to more mineral marshes. In addition, while SOM can lessen the toxicity of oil to plants, it can also reduce biodegradation rates (Pezeshki & Hester, 2000). However, the generally greater stress tolerance of salt marsh plants like *Spartina patens*, was not shown to extend to oil impacts. Rather, the fresh water plant *Spartina lancifolia* was shown to exhibit the greatest oil resistance up to 24 L/m^2 (Pezeshki & Hester, 2000).

Depending on the environment, oil has been shown to have long lasting effects on an ecosystem up to 40 years later. This includes instability and erosion of sediment related to location and content of residual petroleum (Culbertson & Valiela, 2008). In one study, very little living plant matter was found in areas with greater than 1000-2000 mg oil/g wet soil (Burns & Teal 1979).

Phytoremediation

The ability of plants, specifically *S. alterniflora*, to degrade oil has been well studied (Pezeshki & Hester, 2000). This method of degradation is known as phytoremediation and involves using plants to assist in the degradation of a contaminant. With this in mind, it is important to fully understand the impact of oil on plants to in turn understand the influence of plants on the degradation of oil.

CHAPTER 3 EXPERIMENTAL METHODS

Experiment One

Following the 2010 oil spill, a preliminary experiment was set up by graduate students Aaron Gwinnup and Elliott Beenk under the direction of Professor Jerald Schnoor to determine the survivability of the plants and the effect of variable oil concentrations on *S.alterniflora*. Oil concentration was the only experimental variable and four replicates of four oil concentrations at 0, 10, 50, and 250 mg crude oil/g wet soil were used to create the microcosms, totaling 16 samples. Furthermore, the microcosms were constructed by adding the soil, plants, water, and then oil. The plants were watered and photographed weekly. In addition, 5 ml water samples from the water phase were extracted at the start of the experiment and after 90 days at the end of the experiment and survival rates were determined by visual inspection.

Sediments

Salt marsh sediment was collected on June 23rd, 2010 from Airplane Lake in Louisiana, a *Spartina alterniflora* (cordgrass) dominated marsh. A soil core sample 5 inches in diameter and 90cm in length was used to collect the sample. The sediment was collected in the salt marsh in a stand of *Spartina alterniflora* and the shoots were pushed aside to access the sediment. Pictures from this procedure can be seen in Figure 5.

Figure 5: Soil Sampling Procedure Used in Experiment One (upper left photograph shows Elliott Beenk on the left and Aaron Gwinnup on the right; photographs were taken by collaborator R. Eugene Turner, Louisiana State University)

To collect the samples, *S. alterniflora* toppings were cut to expose the sediment. A master 90cm soil core was used and samples were cut at successive increments. The soil samples included five 30 cm core samples and five 10 cm core samples. Samples were extracted from a visibly homogenous salt marsh site and were presumably clean. The extracted soil after transport can be seen in Figure 6.

Figure 6: Sediment in a Cooler Transported from Airplane Lake Used for Experiment One

Following, the soil was mixed by hand until visibly homogenous and major stem and root structures were removed. This soil was used for all 16 samples.

Oil

Sweet Louisiana Crude oil was acquired from ONTA, a commercial oil supplier. This Sweet Louisiana Crude was chosen as a surrogate for the oil from the Deepwater Horizon Spill, also a type of Sweet Louisiana Crude. Four concentrations of 0, 10, 50, and 250 mg oil/ g wet soil were used to determine the effect of oil concentration on plant survivability. These concentrations were chosen based on literature that suggested the lethal dosage for *Spartina alterniflora* is around 250 mg oil/g wet soil

(Lin & Mendelssohn , 2002).

Total Extractable Hydrocarbons

The total extractable hydrocarbons (TEH) from the water phase of the microcosm were determined at 0 and 90 days. The water phase was sampled by penetrating the top oil layer using a glass pipet and extracting a 5ml sample. The sample was then delivered to the State Hygienic Laboratory at the University of Iowa and analyzed for TEH using the same methods as presented in Luke Smith's M.S. thesis and EPA 3510: Extraction of Total Extractable Hydrocarbons in Water (Smith, 2013).

Watering

The plants were watered weekly with a mixture of quarter strength Hoagland's solution at 10 parts per thousand (ppt) salinity to resemble the nutrient composition of saltmarsh water (Childers, McKellar, Dame, Sklar, & Blood, 1993). The composition of Hoagland's used can be found in Table 2.

Table 2: Quarter Strength Hoagland's Solution

Stock Solution	Volume of Solution per 1 L
1M $Ca(NO_3)*4H_2O$	1 mL
2M KNO_3	0.75 mL
2M $NH_4H_2PO_4$	0.5 mL
MICRONUTRIENTS	0.5 mL
20mM Fe-EDTA	0.5 mL
1M $MgSO_4*7H_2O$	0.25 mL
1M NaOH	Added until pH reaches 6.8

Source: Hoagland, D. (1920). Optimum Nutrient Solutions for Plants. *Science* , 562-564.

Deionized (DI) with UV disinfection was used to avoid organic contamination of the samples. An Instant Ocean® aquarium salt mixture was added to the Hoagland's solution to bring the salinity to 10 ppt. 10ppt was chosen to resemble typical salinity conditions in the saltmarshes and to be comparable to past literature. Furthermore, while the plants were initially watered with a 10ppt saline solution, salt accumulation problems became apparent around 45 days into the experiment as pure, freshwater was transpired by the plants, thus concentrating the salinity. After 45 days, a freshwater solution was used for the duration of the experiment. It is possible that this increased salt concentration could have stressed the plants in addition to the oil. To summarize, the salinity in the small microcosms of Experiment One was initially 10 ppt, climbed

throughout the experiment to ~30 ppt on day 45, and then was help roughly constant throughout the remainder of the experiment to 90 days.

Experiment Two

The first experiment showed encouraging results with the degradation of oil and plant survival rates; accordingly, the experimental setup of the second experiment was informed and modeled based on observations from the first experiment. In order to determine the effects of multiple variables simultaneously, several treatments and reference controls were imposed in the second experiment. Variable conditions were used for the soil type and oil type and oil concentration.

The second experiment was conducted in a climate-controlled greenhouse maintained at 68°F in Oakdale, Iowa and only natural lighting was used. The experiment was initially setup in a northwest facing room but was moved to a southwest facing room in the greenhouse due to an outbreak of spider mites in the previous location. One L beakers were used to create the microcosms and were selected because they were large enough to allow for root growth and their wide openings allowed for easy planting of the *S. alterniflora* shoots. Initial concerns of excessive odors from the oil in the first experiment proved to be unfounded; consequently, the flasks with cotton stoppers used in the first experiment proved unnecessary. Furthermore, sampling of the water phase in the first experiment required penetrating the oil layer with a syringe. In an effort to improve this process, the flasks were modified and sampling ports were included ¾ of the way up at the 750ml mark on the flasks used in the second experiment. An example of this experimental setup can be seen Figure 7.

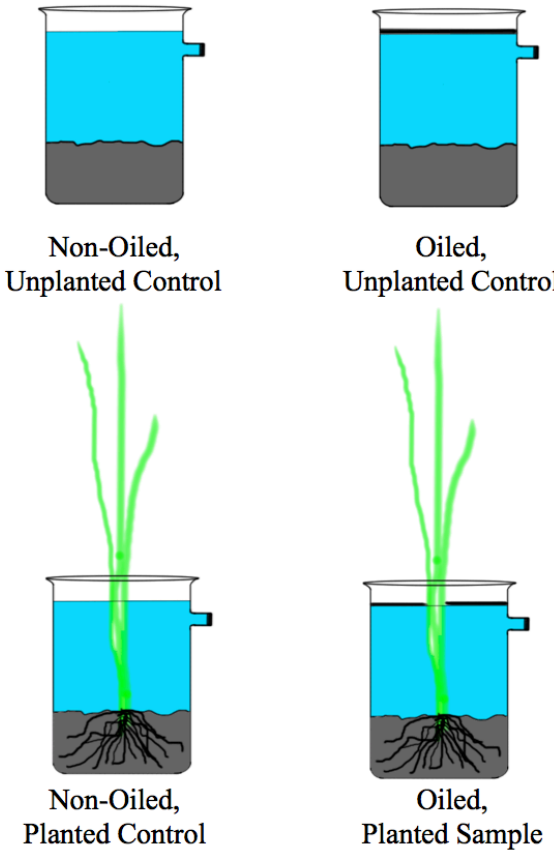

Figure 7: Beakers Used in Experiment Two

As seen in Figure 7, all samples consisted of soil and water with *S. alterniflora* and oil added for the various conditions. In addition to the samples displayed, the soil type was also varied with oiled and non-oiled soil. Henceforth, soil mixed with water will be referred to as sediment and "plants" will be in reference to the species *S. alterniflora*.

Soil was added to the 400 ml mark on the beaker and was not compacted. These soil conditions were chosen to resemble the headspace of water and non-load bearing nature of the soil that was observed in Louisiana saltmarshes during the 2010 trip. Subsequently, the mass of the beakers was measured and the microcosm was again

weighed individually after addition of the soil, plant, water, and oil. A Mettler Toledo® Delta Range scale was used for all measurements and was calibrated and leveled at the beginning of each day of measuring.

The equivalent of one plug, or three shoots, of *S.alterniflora* were added to each of the planted microcosms. Hundreds of *S.alterniflora* shoots were ordered from various nurseries in Louisiana during the period of acclimating the soil but only the 60 healthiest and most uniform plugs were used for the experiment. Furthermore, the plants were transported in freshwater and as suggested by the first experiment, conversion to saline conditions could shock the plants. Thus, saltwater was not used for the second experiment. Next, the beakers were filled to the 1000 ml mark with quarter strength Hoagland's and the appropriate microcosms were oiled from the top opening of the beaker. In summary, the microcosms were constructed by adding the components, when appropriate, in the following order: soil, plant, water, oil.

Following assembly of the microcosms, cardboard boxes were constructed to cover the samples to prevent algal growth, photolysis, and to reduce volatilization of the oil. Figure 8 shows an example of these boxes.

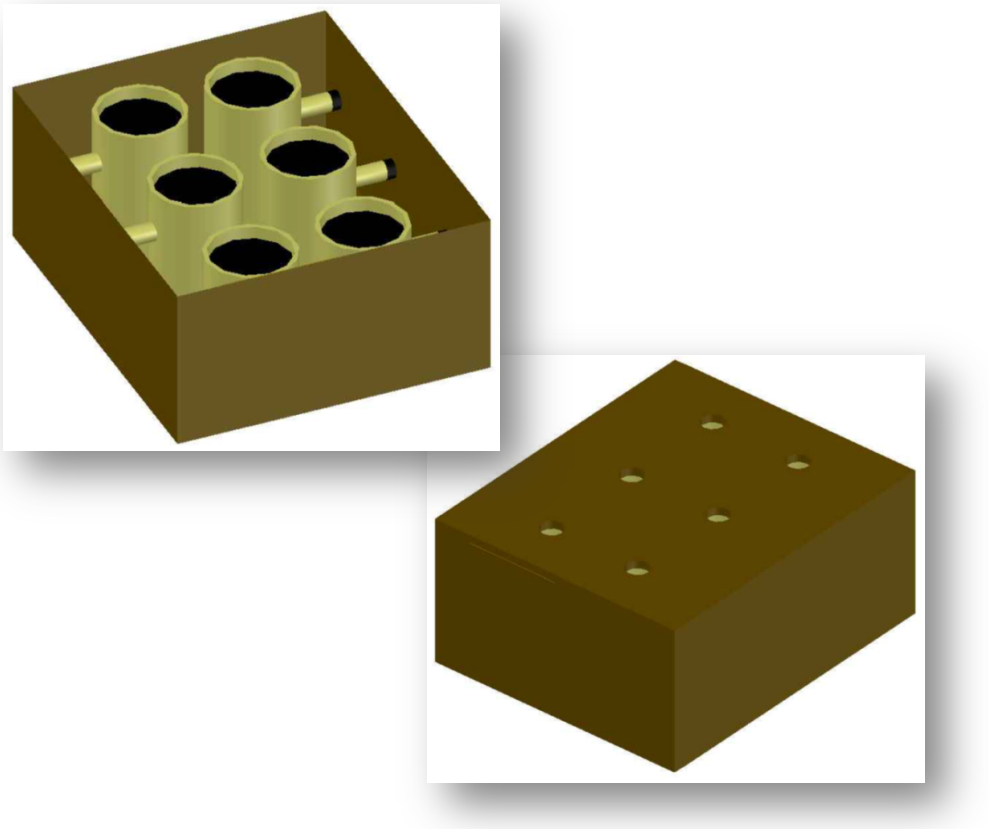

Figure 8: Boxes Used to Cover Samples in Experiment Two (Smith, 2013)

Observable in Figure 8, while the boxes covered the beakers, constructed openings allowed for penetration of *S. alterniflora* shoots.

Soil

To observe the effect of initial oil contamination in the soil, oiled and non-oiled soils were used. Sediments in saltmarshes are typically non-uniform, anaerobic, and have low organic content (Materne, 2009). Thus, with the aim of achieving homogeneity in the soil, an all-purpose organic potting soil was used for all samples. The soil was divided into two pots and one half was oiled with 5 mg Louisiana Sweet Crude oil/g soil and mixed thoroughly, while the other was left unoiled. This is a low

dosage of oil relative to the oil applied to the water phase. Both conditions were left exposed to the environment to be weatherized for 4 months, after which the experiment was started outdoors. In addition, soil samples were taken at the beginning and end of the four-month weatherization period and after the 90-day experiment and sent to the State Hygienic Laboratory at the University of Iowa to be tested for total extractable hydrocarbons (TEH). This was conducted to determine the concentration of oil in the soil that would potentially impact the plants. In addition, all samples were photographed weekly.

Oil Type & Concentration

A light, medium, and heavy crude oil was used at concentrations of 10, 50, and 150 mg crude oil/g wet soil to determine the effect of various oil types and concentrations on plant biomass metrics. The crude oils were obtained from BP and a sample of the light, medium, and heavy crude can be seen in Figure 9 smeared on the sides of the Erlenmeyer flasks.

Figure 9: Example of the Heavy, Medium, and Light (from left to right) Crude Oils Used in Experiment Two

Watering

The plants were watered weekly to the 1000ml mark on the beaker with quarter strength Hoagland's solution in an attempt to resemble the nutrient conditions of saltmarsh water (Childers, McKellar, Dame, Sklar, & Blood, 1993). The formula and makeup of Hoagland's solution is the same as that used in experiment 1 and can be found in Table 2**.** Deionized (DI) with UV disinfection was used to avoid organic contamination of the samples. After planting, the beakers were watered to the 1000 ml mark, submerging the sediment and creating conditions typical to a Louisiana saltmarsh.

TEH Sampling

Weekly 5ml water samples were taken through the side port of each sample at 0, 30, 60, and 90 days to test for TEH concentrations. While TEH degradation was not

the focus of this experiment, it was used to inform the concentrations of oil affecting the plants at various times. The sampling procedure and method for determination of TEH is the same as used in Luke Smith's thesis (Smith, 2013).

Plant Metrics

In order to study the effects of various conditions on plant health, the plant metrics of evapotranspiration rates, biomass growth rates, and plant survivability were calculated.

Evapotranspiration Rates were calculated using weekly plant watering data. Furthermore, unplanted controls were used to determine the extent of transpiration vs. evaporation.

Equation 1:

$$Weekly\ Change\ in\ Evapotranspiration\ Rates = V_n - V_{n-1}$$

Where V is the volume of water added weekly and n is the sampling week.

Biomass rates were calculated from weekly mass measurements of the entire microcosm after the plant was watered to the starting water height of 1 L. It is assumed that any change in the mass of the sediment and beaker is negligible. Accordingly, it is assumed the only weekly change in weight after the water level was retuned to 1 L was from the plant.

Equation 2:

$$Cumulative\ Change\ in\ Biomass = m_n - m_0$$

Where m is the mass of the microcosm and n is the sampling week.

The survival rate of the plants was determined by visual inspection. A one-tailed, p =0.05, type three significance was conducted using Microsoft Excel ®.

CHAPTER 4 RESULTS AND DISCUSSION

The survival rates were studied in both experiments one and two to determine the lethal dosage level of crude oil on *S. alterniflora.* In addition, weekly evapotranspiration rates and change in biomass were measured and calculated in experiment two to determine the effects of oil vs. non-oiled soil, oil type, and oil-concentration. While neither set of data for change in biomass or evapotranspiration is fully conclusive or consistent, both provide insight into trends under variable conditions. Furthermore, the biomass data seems to be more indicative of trends than the evapotranspiration data for experiment two. Conclusions are able to be drawn from all three environmental treatments of soil type, oil type and oil concentration.

Survival Rates

The survival rates for experiment one and two were measured at the end of both 90-day experiments and the results from experiment one can be found in Figure 10 below.

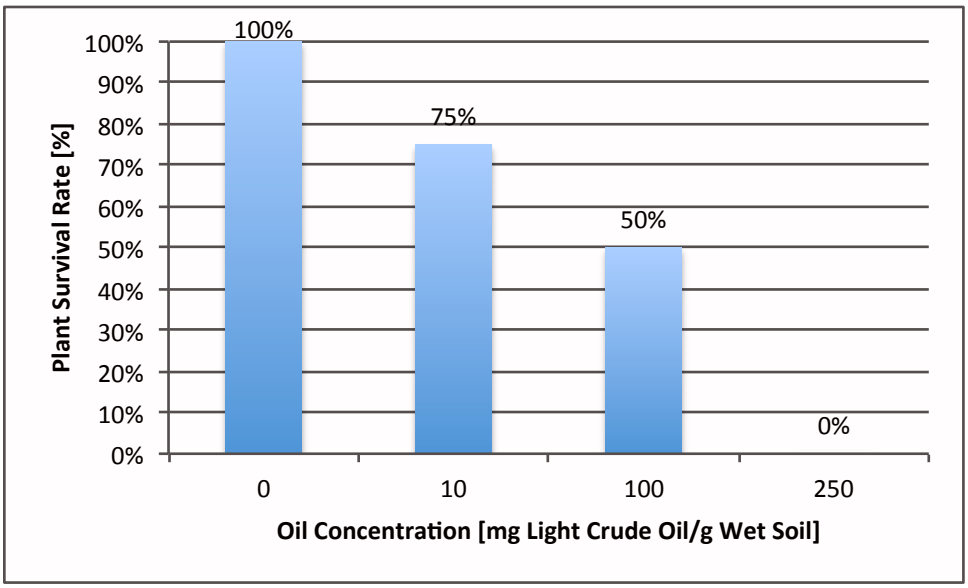

Figure 10: Plant Survival Rate vs. Oil Concentration for Experiment One with Louisiana Sweet Crude Oil (n =4)

As expected, the plant survival rates from experiment one decreased with oil concentration of the Sweet Louisiana Light Crude. Furthermore, the concentration of 250 mg crude oil/g wet soil caused complete plant mortality. To be noted, it is also suspected that increasing salinity concentrations during experiment one could have contributed to additional plant stress in addition to increasing oil concentrations.

The lethal dosage for *S. alterniflora* was not reached during experiment two by design; thus, all of the plants survived the 90-day experiment. This was expected since the highest concentration used in experiment two was 150 mg crude oil/g wet soil, 100 mg crude oil/g wet soil less than the concentration that exhibited complete mortality in experiment one. Experiment two was designed such that all the plants (or nearly all) would survive.

Cumulative Change in Biomass

The weekly change in biomass was measured and calculated for all 72 samples in experiment two. The same set of data is presented in three different ways to visually compare the effects of oiled vs. non-oiled soil, oil concentration, and oil type, respectively. In addition, the results of a 95% confidence, one-tail, t-test are presented at the end of each sub-section to highlight the statistical significance of the data.

Effect of Oiled vs. Non-Oiled Soil on Cumulative Change in Biomass

The cumulative change in biomass for all planted samples is presented in Figure 11through Figure 20; each data point represents a triplicate. The following figures illustrate the effect of oiled vs. non-oiled soil on biomass where each figure represents one oil type and concentration.

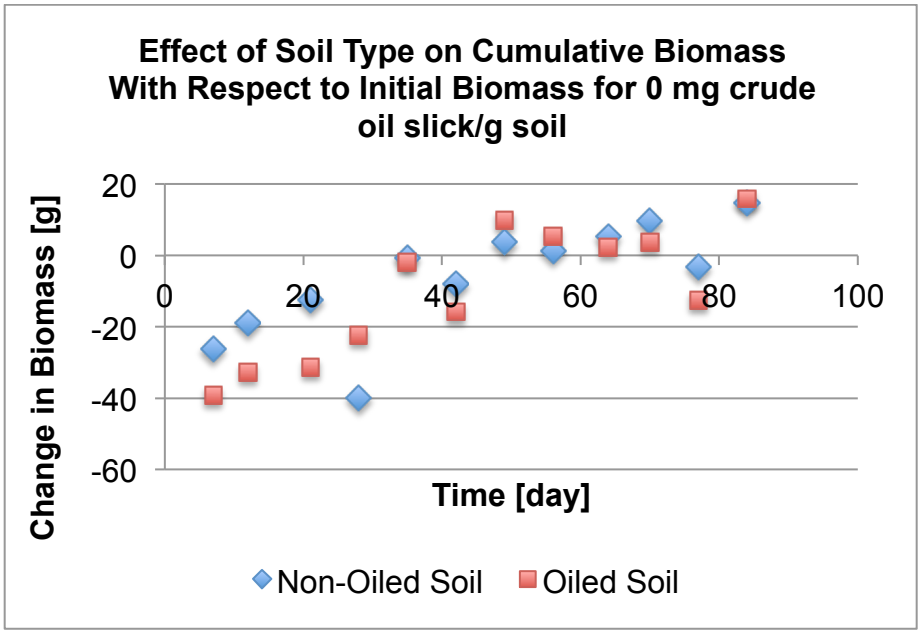

Figure 11: Effect of Soil Type on Cumulative Biomass With Respect to Initial Biomass for 0 mg crude oil slick /g soil (not statistically different)

As seen in Figure 11, all samples showed negative cumulative change in biomass until around day 40. This is possibly due to transplant shock of moving the plants and changing the environments. Furthermore, Figure 11 is significant because it represents the non-oiled controls on which all future Figures are based on.

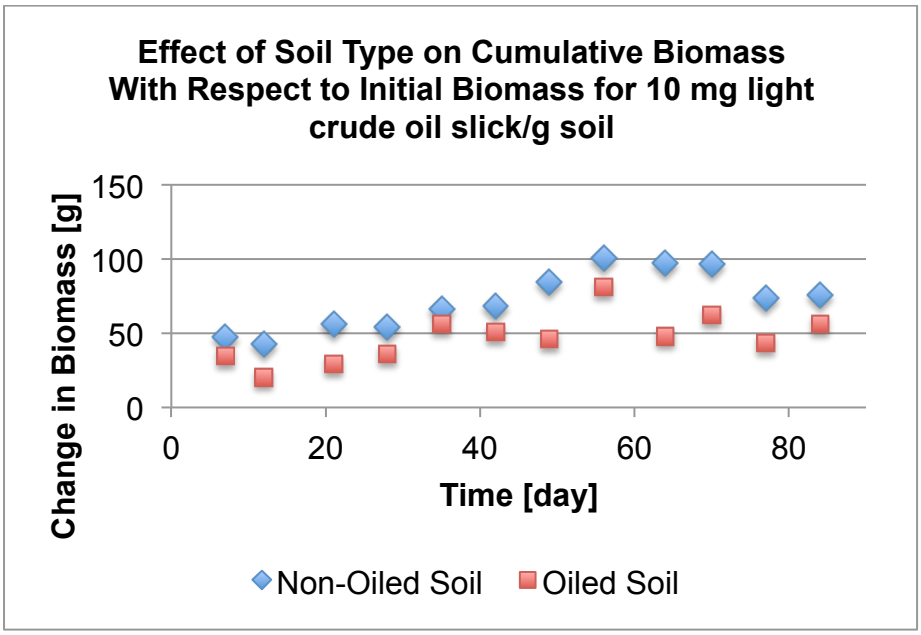

Figure 12: Effect of Soil Type on Cumulative Biomass With Respect to Initial Biomass for 10 mg light crude oil slick/g soil (significantly different at p=0.05)

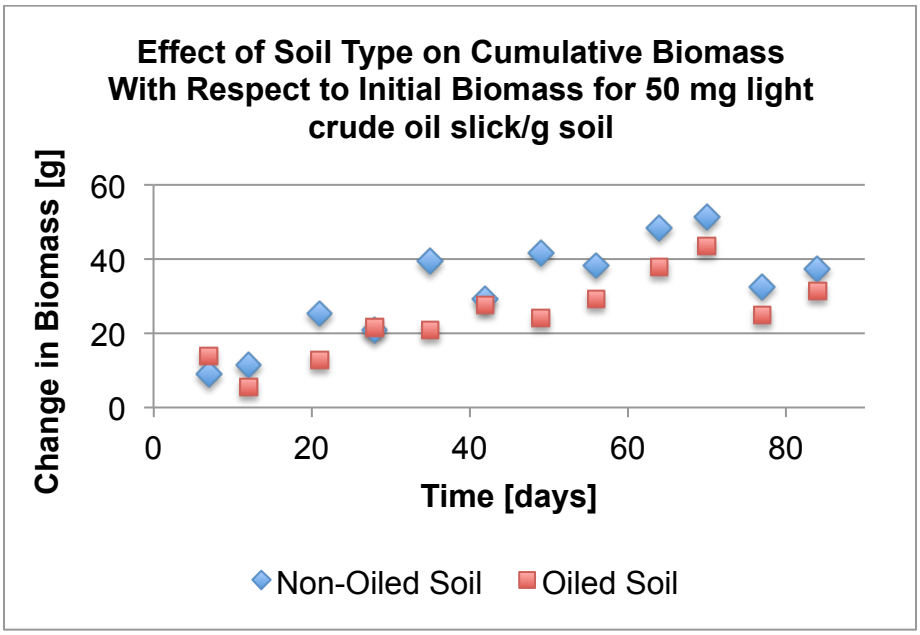

Figure 13: Effect of Soil Type on Cumulative Biomass With Respect to Initial Biomass for 50 mg light crude oil slick/g soil (significantly different at p= 0.05)

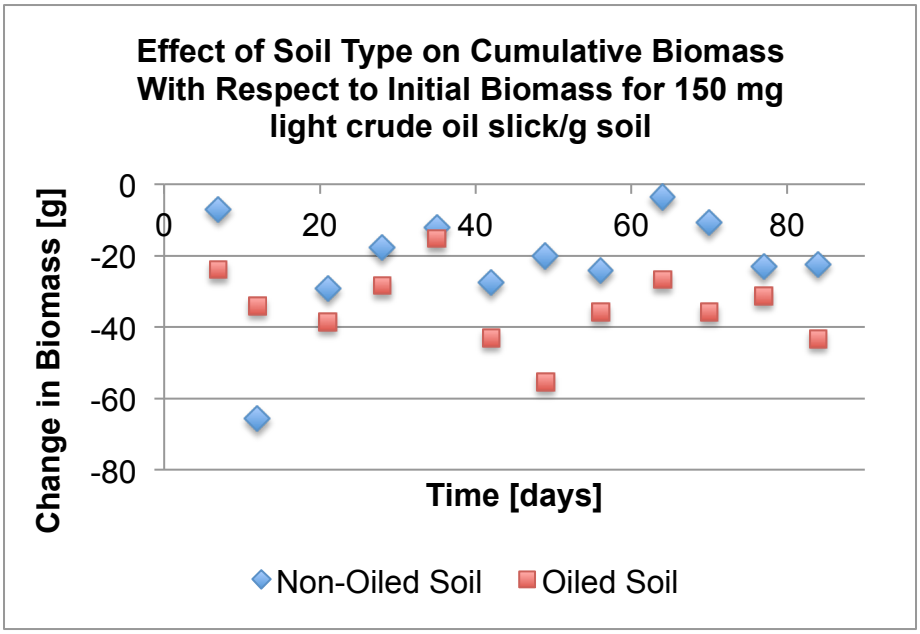

Figure 14: Effect of Soil Type on Cumulative Biomass With Respect to Initial Biomass for 150 mg light crude oil slick/g soil (not statistically different)

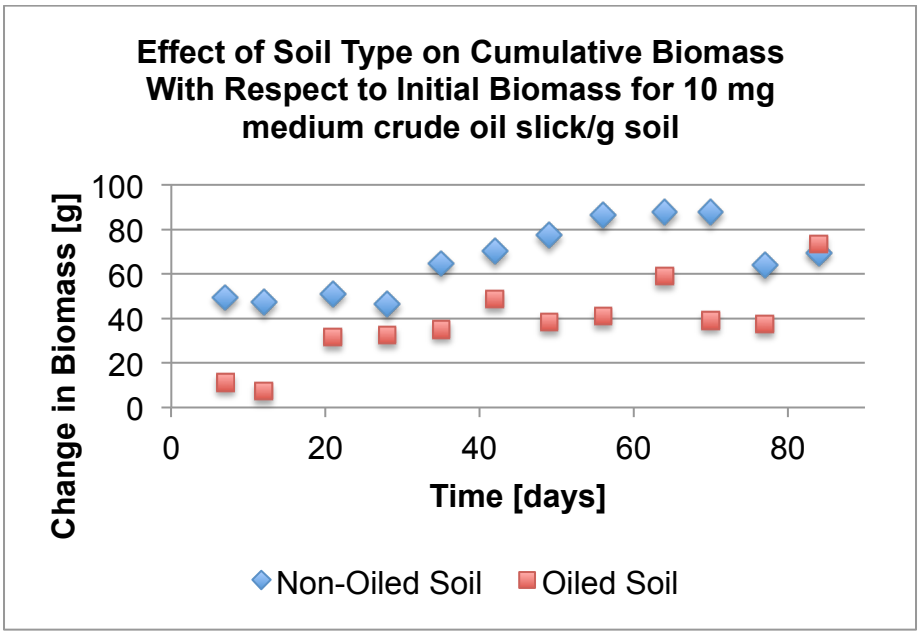

Figure 15: Effect of Soil Type on Cumulative Biomass With Respect to Initial Biomass for 10 mg medium crude oil slick/g soil (statistically different, p=0.05)

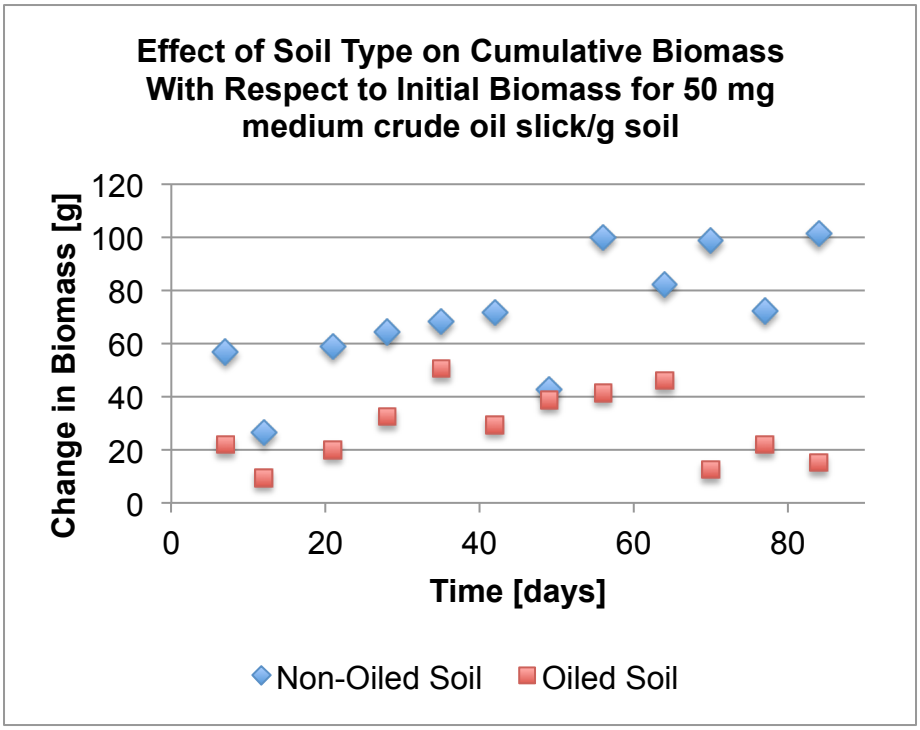

Figure 16: Effect of Soil Type on Cumulative Biomass With Respect to Initial Biomass for 50 mg medium crude oil slick/g soil (not significantly different at p= 0.05)

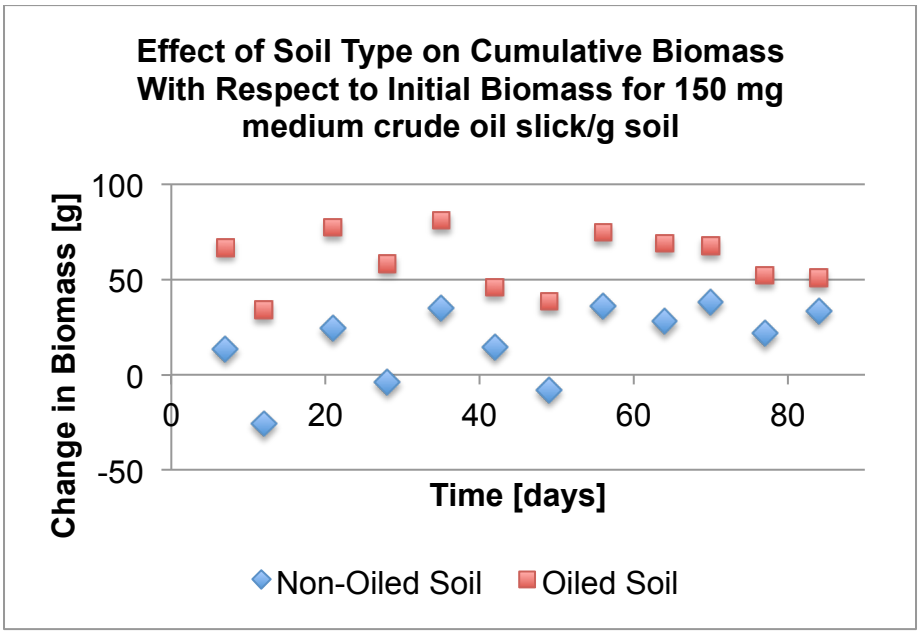

Figure 17: Effect of Soil Type on Cumulative Biomass With Respect to Initial Biomass for 150 mg medium crude oil slick/g soil (significantly different at p= 0.05)

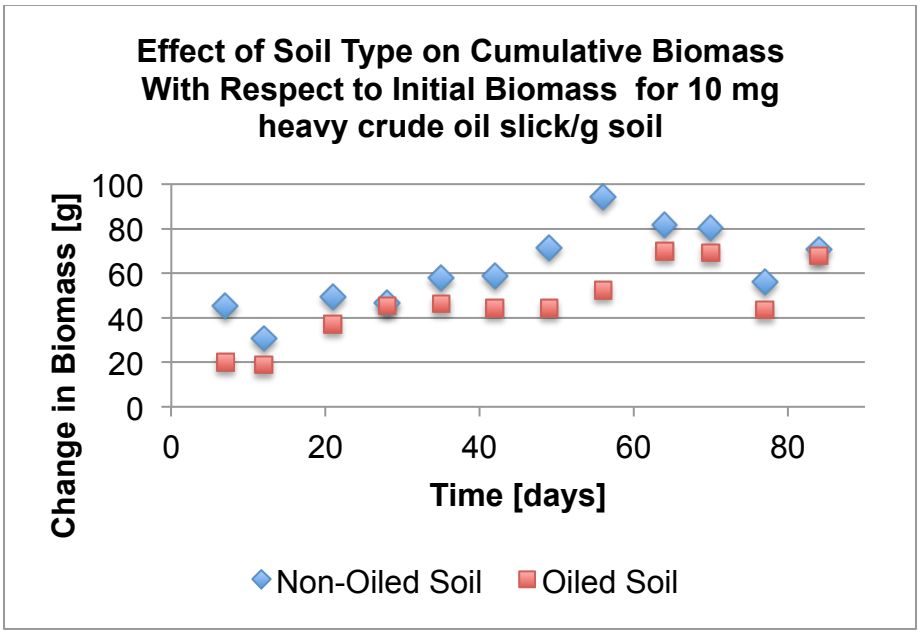

Figure 18: Effect of Soil Type on Cumulative Biomass With Respect to Initial Biomass for 10 mg heavy crude oil slick/g soil (significantly different at p= 0.05)

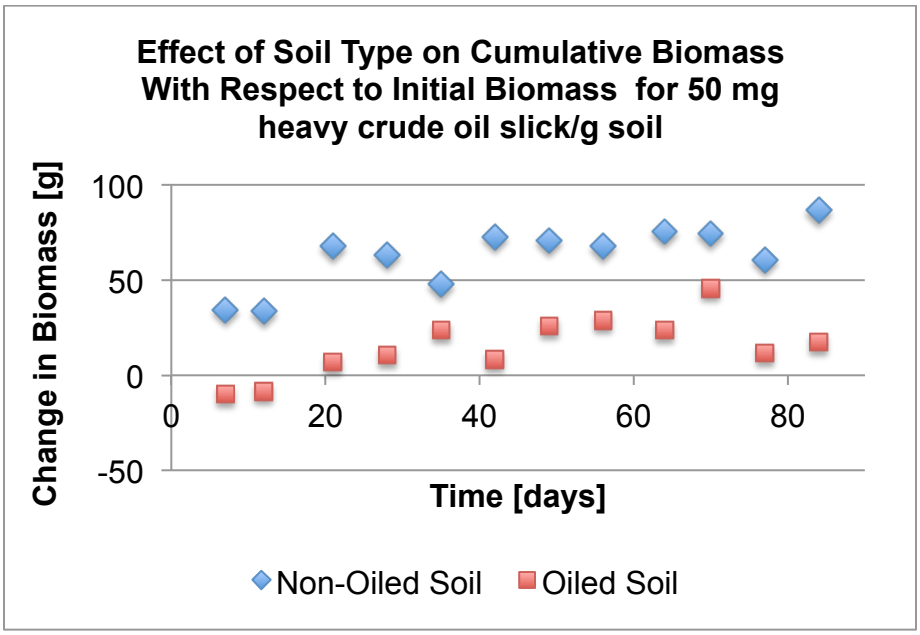

Figure 19: Effect of Soil Type on Cumulative Biomass With Respect to Initial Biomass for 50 mg heavy crude oil slick/g soil (significantly different at p= 0.05)

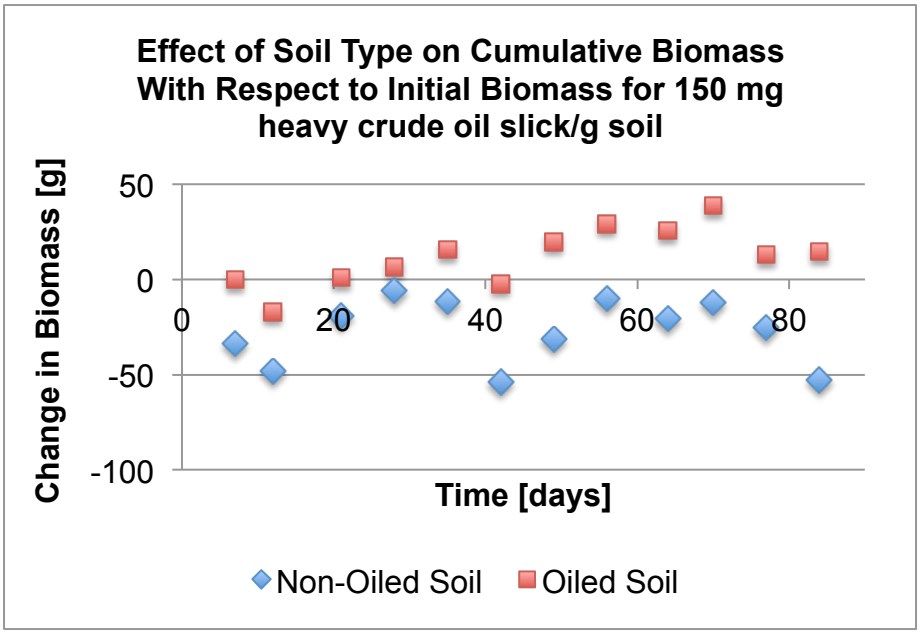

Figure 20: Effect of Soil Type on Cumulative Biomass With Respect to Initial Biomass for 150 mg heavy crude oil slick/g soil (significantly different at p= 0.05)

The statistical significance of the effect of oiled vs. non-oiled soil at a 95%

confidence interval can be found in Table 3.

Table 3: Statistical Significance of Difference in Oil Types on Biomass Based on T-test With 95% Confidence

Oiled vs. Non-Oiled Soil		
Oil Type/Concentration	**p**	**Significant Difference?**
0 mg crude oil/g wet soil	0.3028366	No
Light [10 mg crude oil/g wet soil]	0.0014889	Yes
Medium [10 mg crude oil/g wet soil]	0.0001910	Yes
Heavy [10 mg crude oil/g wet soil]	0.0216415	Yes
Light [50 mg crude oil/g wet soil]	0.0661487	No
Medium [50 mg crude oil/g wet soil]	0.0000186	Yes
Heavy [50 mg crude oil/g wet soil]	0.0000001	Yes
Light [150 mg crude oil/g wet soil]	0.0186658	Yes
Medium [150 mg crude oil/g wet soil]	0.0000055	Yes
Heavy [150 mg crude oil/g wet soil]	0.0000033	Yes

From Figures 11-20, it is clear that the light crude is the most toxic oil to the plants. For example, Figure 14 at the highest oil dosage in the simulated oil slick (150 mg/g) very clearly illustrates that there was no cumulative growth of biomass by either the microcosms with oiled soil (acclimated microorganisms) or the ones without oiled soil. The plants in microcosms with soils that were not oiled appeared to do slightly better (they lost less biomass during the experiment) than those with oiled soils. This may be attributed to the high level of toxicity in the light crude oil treatments at the highest dosage (150 mg/g). At the highest dosage, even a small amount of "extra" oil added to the soil is an additional stress that the plants could not afford.

From Figure 11-Figure 20 and Table 3, it is also evident that the soil type (oiled soil vs. non-oiled soil) caused a significant effect on cumulative biomass for all

treatments (except for the light crude oil sample at a concentration of 50 mg crude oil/g wet soil). At concentrations of 10 and 50 mg crude oil/g wet soil, the cumulative change (growth, increase) in biomass was lower for the oiled soil in all six conditions. However, at a concentration of 150 mg crude oil/g wet soil, this trend appeared to flip and the oiled soil presented a higher cumulative change (increase) in biomass than the non-oiled soil microcosms. For example, in Figure 20 for the heavy crude oil at the highest dosage, it is apparent that the plants with oiled soil had cumulative biomass growth during the course of the experiment while those without oiled soil did not. It is likely that the microcosms with oiled soil had developed microorganisms during the 4-month prior acclimation period that were helpful in degrading the toxic constituents in the oil, and which allowed these plant systems to be healthier than those without acclimated microorganisms. Because the "heavy crude" in Figure 20 was not so toxic as the "light crude" in Figure 14, it allowed the plants in the oiled soil (with acclimated microorganisms) to grow. However, the (more toxic) light crude oil at 150 mg crude oil/g wet soil in Figure 14 does not clearly follow this trend. It should be noted that the data in Figure 14 is relatively non-uniform with R^2 values around 0.1, and it is possible that no actual trend is observable from this data.

These results suggest that at concentrations of 50 mg crude oil/g wet soil and lower, the concentration of oil in the soil adversely affects the change in biomass more than the above-ground oil. However, at a concentration of 150 mg crude oil/g wet soil, the concentration of aboveground oil is great enough that this trend flips and the acclimated organisms associated with the oiled soil actually helps the plants to cope with the heavy dosage of oil.

Effect of Oil Concentration on Change in Biomass

The cumulative change in biomass for all planted samples is presented below with each Figure representing one oil and one soil type to illustrate the effect of different oil concentrations. The results can be seen in Figures Figure 21Figure 26; each data point represents a triplicate.

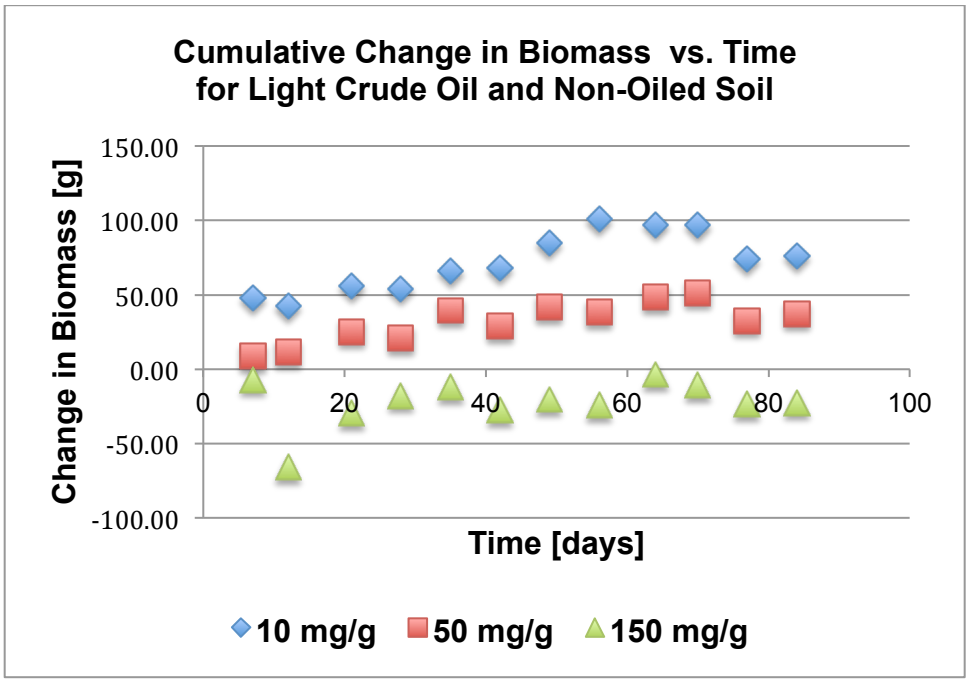

Figure 21: Cumulative Change in Biomass vs. Time for Light Crude Oil and Non-Oiled Soil

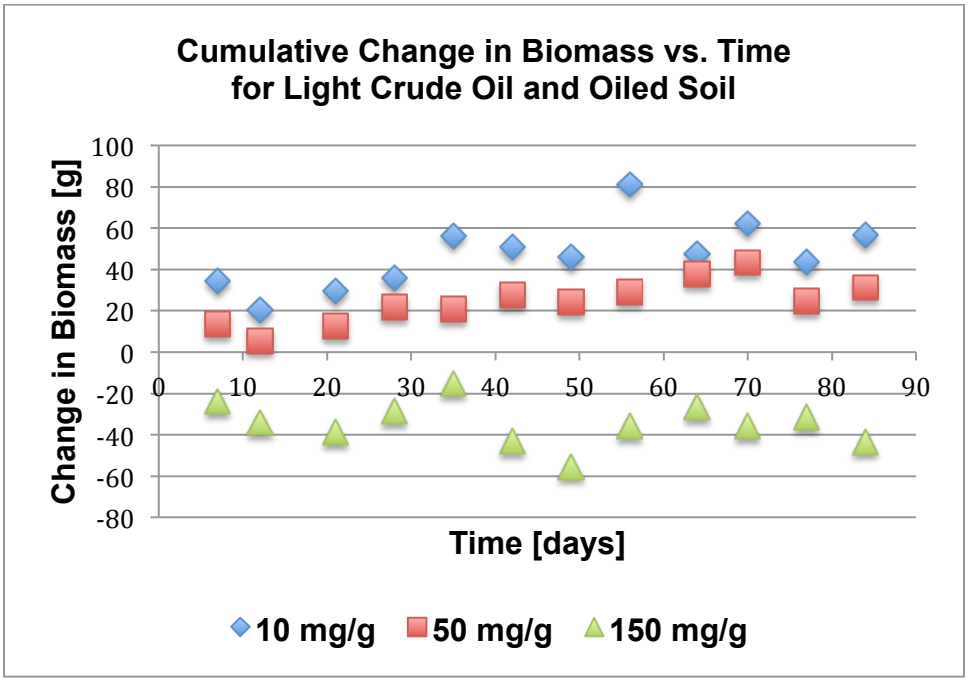

Figure 22: Cumulative Change in Biomass vs. Time for Light Crude Oil and Oiled Soil

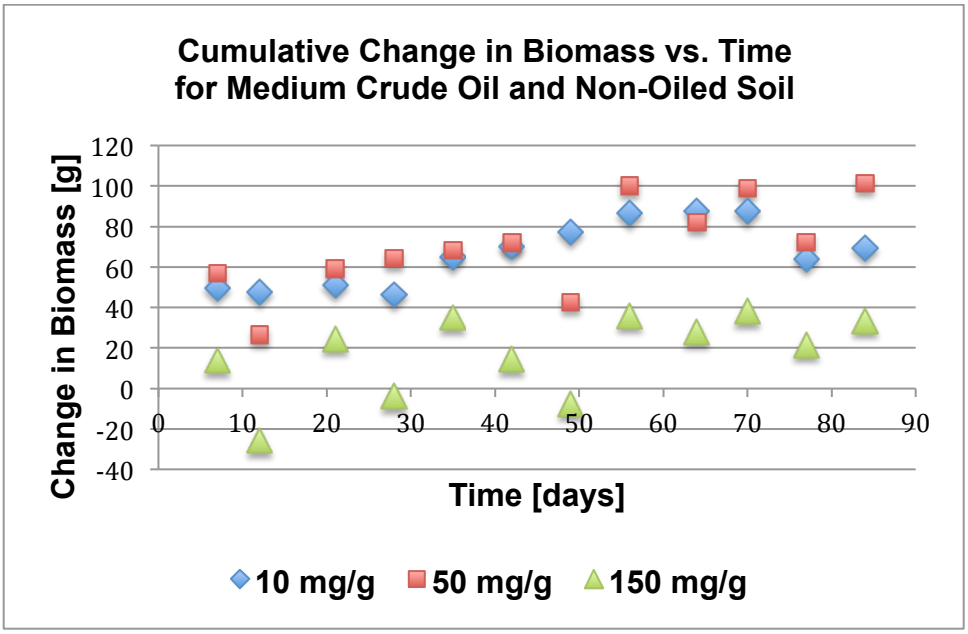

Figure 23: Cumulative Change in Biomass vs. Time for Medium Crude Oil and Non-Oiled Soil

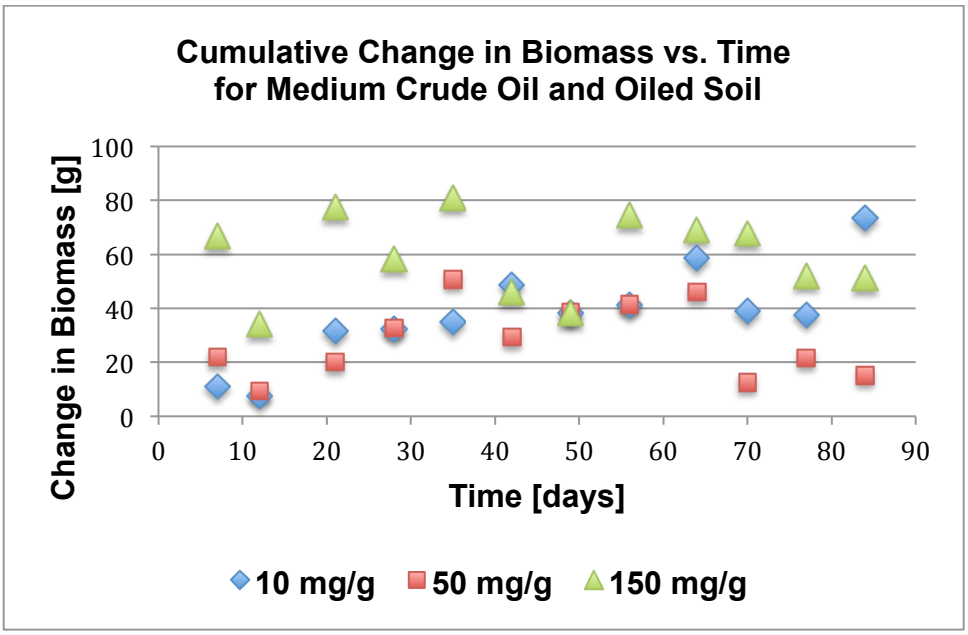

Figure 24: Cumulative Change in Biomass vs. Time for Medium Crude Oil and Oiled Soil

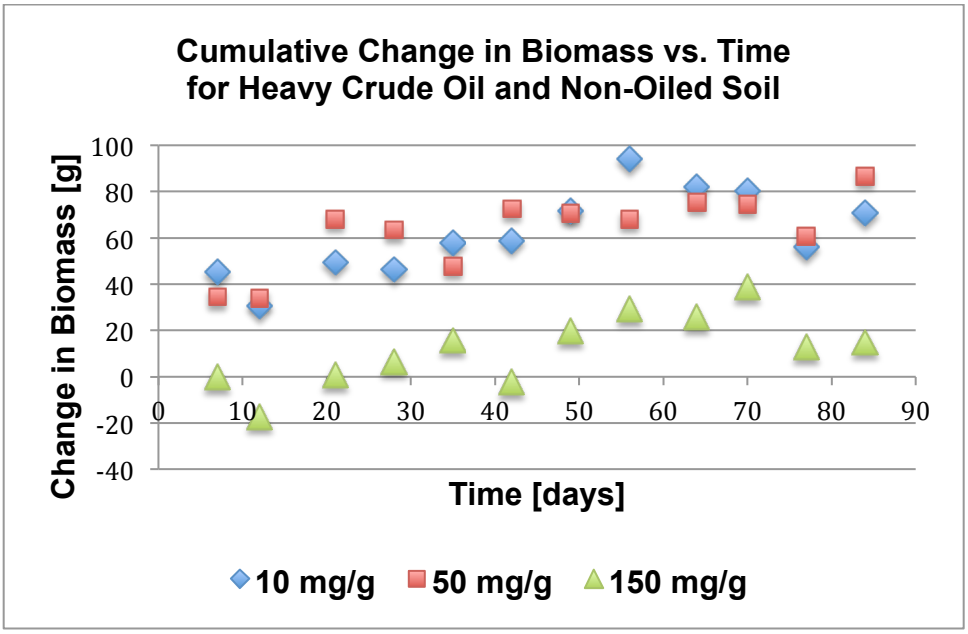

Figure 25: Cumulative Change in Biomass vs. Time for Heavy Crude Oil and Non-Oiled Soil

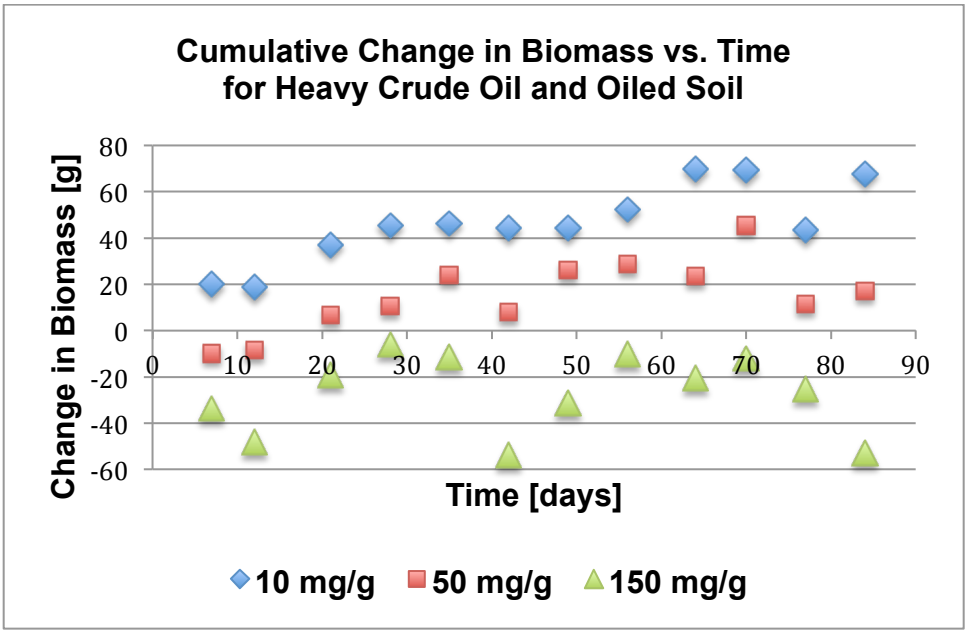

Figure 26: Cumulative Change in Biomass vs. Time for Heavy Crude Oil and Oiled Soil

The statistical significance of the effect of oil concentration at a 95% confidence interval can be found in Table 4.

Table 4: Statistical Significant Difference for Oil Concentration on Biomass Based on a Paired, Two-tailed, T-test (p=0.05)

Light Crude Oil, Non-Oiled Soil				
Concentration [mg crude oil/g wet soil]	**0**	**10**	**50**	**150**
0		Yes	Yes	Yes
10	Yes		Yes	Yes
50	Yes	Yes		Yes
150	Yes	Yes	Yes	
Light Crude Oil, Oiled Soil				
Concentration [mg crude oil/g wet soil]	**0**	**10**	**50**	**150**
0		Yes	Yes	Yes
10	Yes		Yes	Yes
50	Yes	Yes		Yes
150	Yes	Yes	Yes	
Medium Crude Oil, Non-Oiled Soil				
Concentration [mg crude oil/g wet soil]	**0**	**10**	**50**	**150**
0		Yes	Yes	Yes
10	Yes		No	Yes
50	Yes	No		Yes
150	Yes	Yes	Yes	
Medium Crude Oil, Oiled Soil				
Concentration [mg crude oil/g wet soil]	**0**	**10**	**50**	**150**
0		Yes	Yes	Yes
10	Yes		No	Yes
50	Yes	No		Yes
150	Yes	Yes	Yes	
Heavy Crude Oil, Non-Oiled Soil				
Concentration [mg crude oil/g wet soil]	**0**	**10**	**50**	**150**
0		Yes	Yes	Yes
10	Yes		No	Yes
50	Yes	No		Yes
150	Yes	Yes	Yes	
Heavy Crude Oil, Oiled Soil				
Concentration [mg crude oil/g wet soil]	**0**	**10**	**50**	**150**
0		Yes	Yes	Yes
10	Yes		Yes	Yes
50	Yes	Yes		Yes
150	Yes	Yes	Yes	

From Figure 21-Figure 26 and Table 4, oil concentration creates a significant difference in the cumulative change of biomass with the exception of the medium and heavy oil at concentrations of 50 mg crude oil/g wet soil.

In all six conditions, the concentrations of 10 and 50 mg crude oil/ g wet soil and outperformed the non-oiled samples. In the condition of medium crude oil and oiled soil, the concentration of 150 mg crude oil/ mg wet soil led to the greatest change in biomass. This is suggestive that the oil created biomass stimulation in the plants, however, this level of stimulation was unexpected. In fact, the non-oiled controls performed the worst in terms of cumulative change of biomass. This was also definitely not expected.

Effect of Oil Type on Cumulative Change in Biomass

To demonstrate the effect of oil type on cumulative change in biomass, the change in biomass is presented in the figures below with each figure representing one oil concentration and one soil type. The results can be seen in Figures Figure 27Figure 32; each data point represents a triplicate.

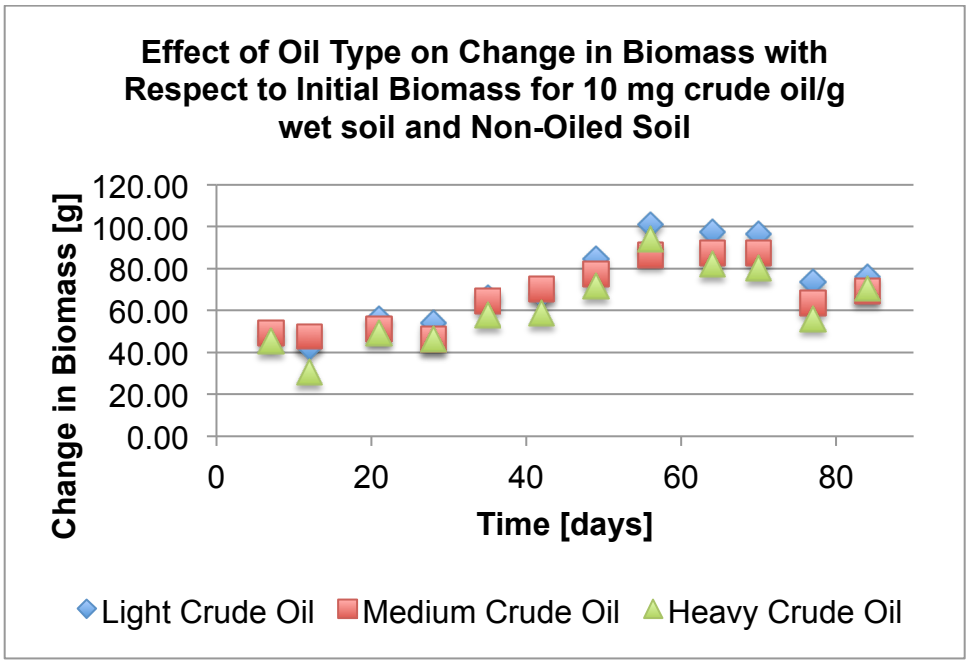

Figure 27: Effect of Oil Type on Cumulative Change in Biomass with Respect to Initial Biomass for 10 mg crude oil/g wet soil and Non-Oiled Soil

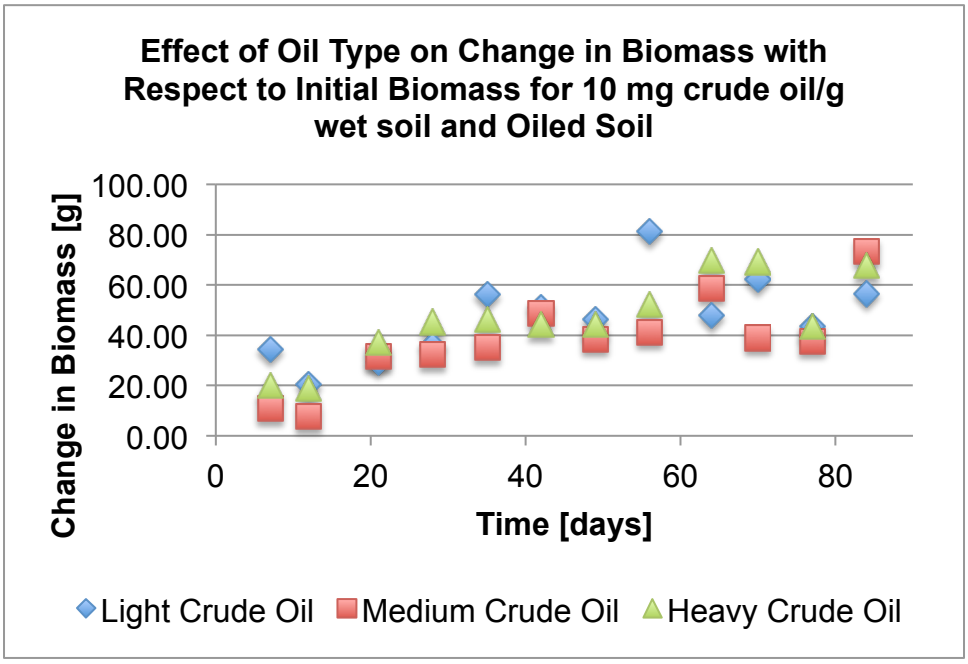

Figure 28: Effect of Oil Type on Cumulative Change in Biomass with Respect to Initial Biomass for 10 mg crude oil/g wet soil and Oiled Soil

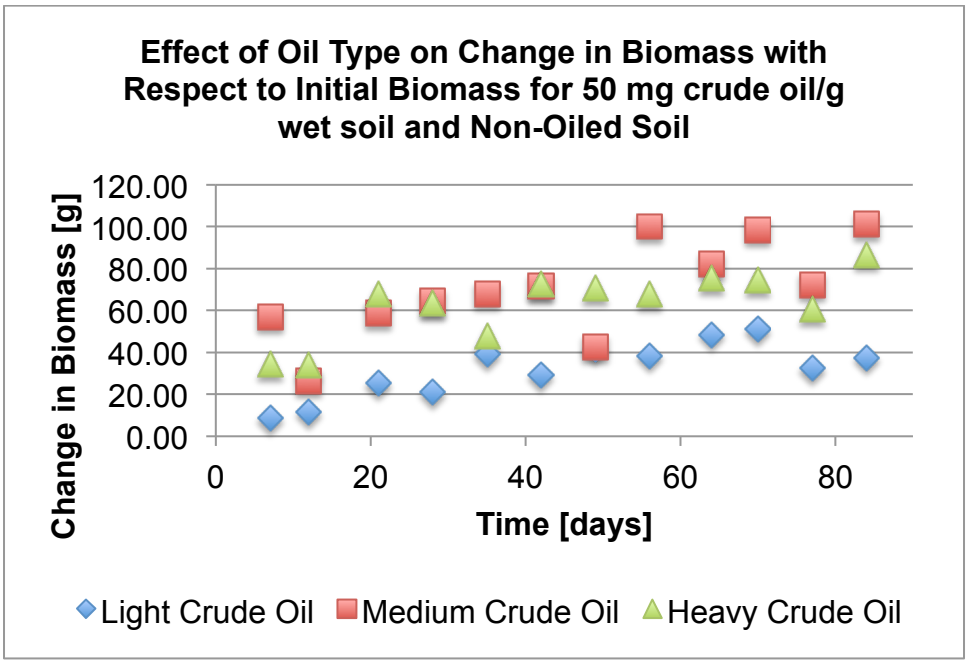

Figure 29: Effect of Oil Type on Cumulative Change in Biomass with Respect to Initial Biomass for 50 mg crude oil/g wet soil and Non-Oiled Soil

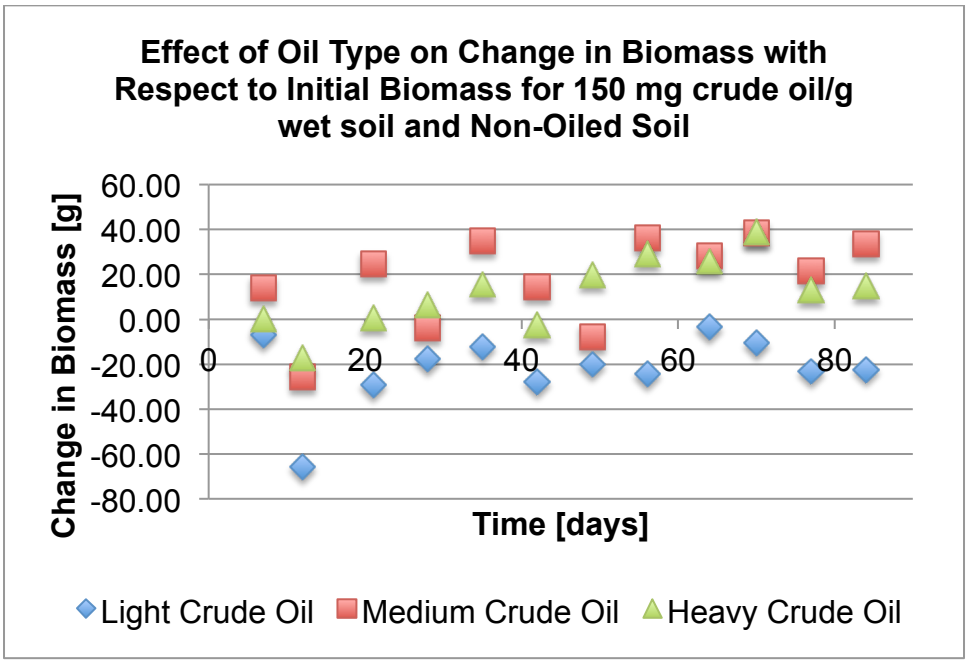

Figure 30: Effect of Oil Type on Cumulative Change in Biomass with Respect to Initial Biomass for 150 mg crude oil/g wet soil and Non-Oiled Soil

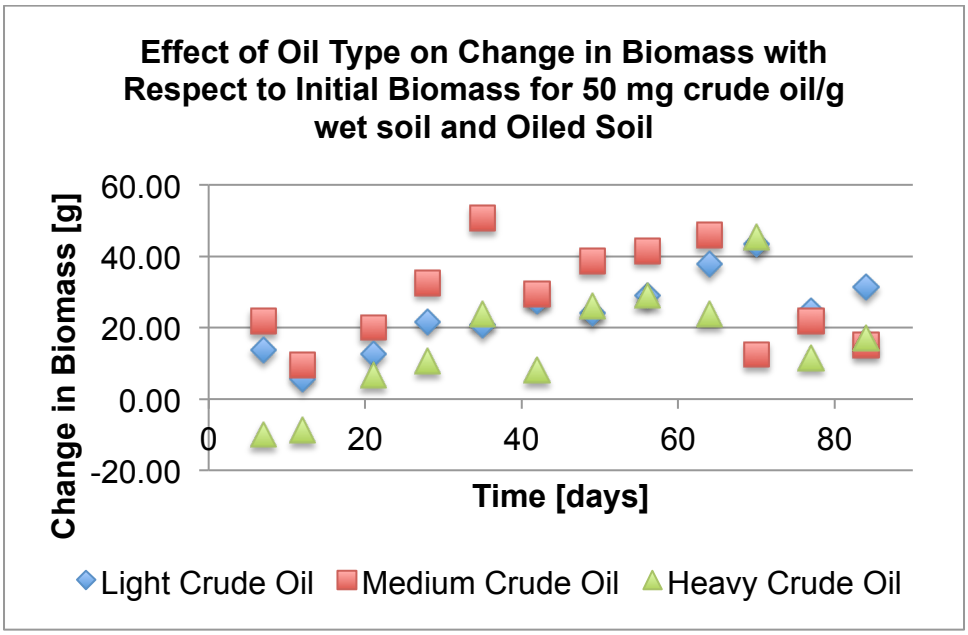

Figure 31: Effect of Oil Type on Cumulative Change in Biomass with Respect to Initial Biomass for 50 mg crude oil/g wet soil and Oiled Soil

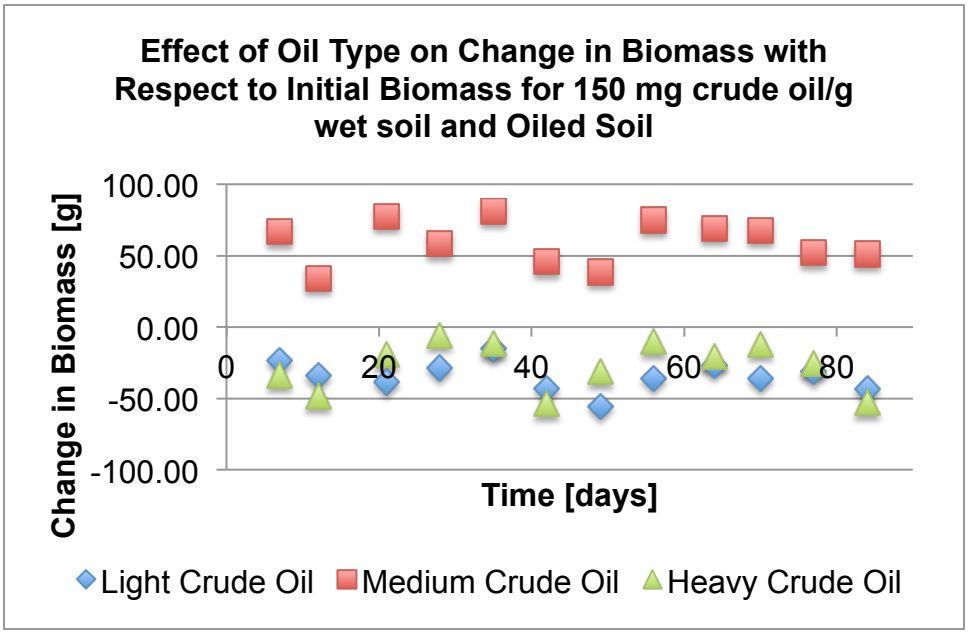

Figure 32: Effect of Oil Type on Cumulative Change in Biomass with Respect to Initial Biomass for 150 mg crude oil/g wet soil and Oiled Soil

The statistical significance of the effect of oil type at a 95% confidence interval can be found in Table 5.

Table 5: Statistical Significance of Difference in Oil Types on Biomass Based on T-test With 95% Confidence

Concentration	p	Significant Difference?
Non-Oiled Soil		
Light vs. Medium (10mg/g)	0.24702	No
Light vs. Heavy (10mg/g)	0.10711	No
Medium vs. Heavy (10mg/g)	0.24569	No
Light vs. Medium (50mg/g)	5.27E-05	Yes
Light vs. Heavy (50mg/g)	0.00003	Yes
Medium vs. Heavy (50mg/g)	0.18983	No
Light vs. Medium (150mg/g)	1.60E-05	Yes
Light vs. Heavy (150mg/g)	1.30E-05	Yes
Medium vs. Heavy (150mg/g)	0.24337	No
Oiled Soil		
Light vs. Medium (10mg/g)	0.10126	No
Light vs. Heavy (10mg/g)	0.47178	No
Medium vs. Heavy (10mg/g)	0.11674	No
Light vs. Medium (50mg/g)	0.21979	No
Light vs. Heavy (50mg/g)	0.05814	No
Medium vs. Heavy (50mg/g)	0.02123	Yes
Light vs. Medium (150mg/g)	1.45E-13	Yes
Light vs. Heavy (150mg/g)	0.11288	No
Medium vs. Heavy (150mg/g)	4.42E-12	Yes

Table 5 shows that only approximately half of the conditions show a statistical difference for an oil type. Furthermore, none of the 10 mg crude oil/g wet soil concentrations show a significant difference for oil type. This is not surprising since it is hypothesized that oil type and concentration has a lesser effect than the oiled soil for low concentrations. In addition, medium crude oil in Figure 29 through Figure 32 is the

oil type of crude oil that exhibited positive biomass growth in all four conditions. This is to be expected since medium crude oil is typically least toxic of crude oils followed by heavy crude oil with light crude being the most toxic. Furthermore, in Figure 29 and Figure 30, the only two conditions that showed a significant difference, the light crude oil was more detrimental to the growth in biomass. This is less conclusive than the low toxicity of the medium crude oil, however, results suggest that the light crude is more toxic than both medium and heavy crude oil. The medium crude is the least toxic.

Evapotranspiration Rates

Evapotranspiration rates were measured and calculated weekly. Similar to the biomass data, the same set of data is presented in three different ways to visually compare the effects of oiled vs. non-oiled soil, oil concentration, and oil type, respectively. In addition, the results of a 95% confidence t-test are presented at the end of each sub-section to highlight the statistical significance of the data. While less statistical differences were observed for the evapotranspiration data than the biomass data, indicative trends are still observable for the effect of the 150 mg crude oil/g wet soil concentration.

Effect of Oiled vs. Non-Oiled Soil on Evapotranspiration Rates

The evapotranspiration rates for all planted samples are presented in Figure 33Figure 42; each data point represents a triplicate. The following figures illustrate the effect of oiled vs. non-oiled soil on evapotranspiration rates where each figure represents one oil type and concentration.

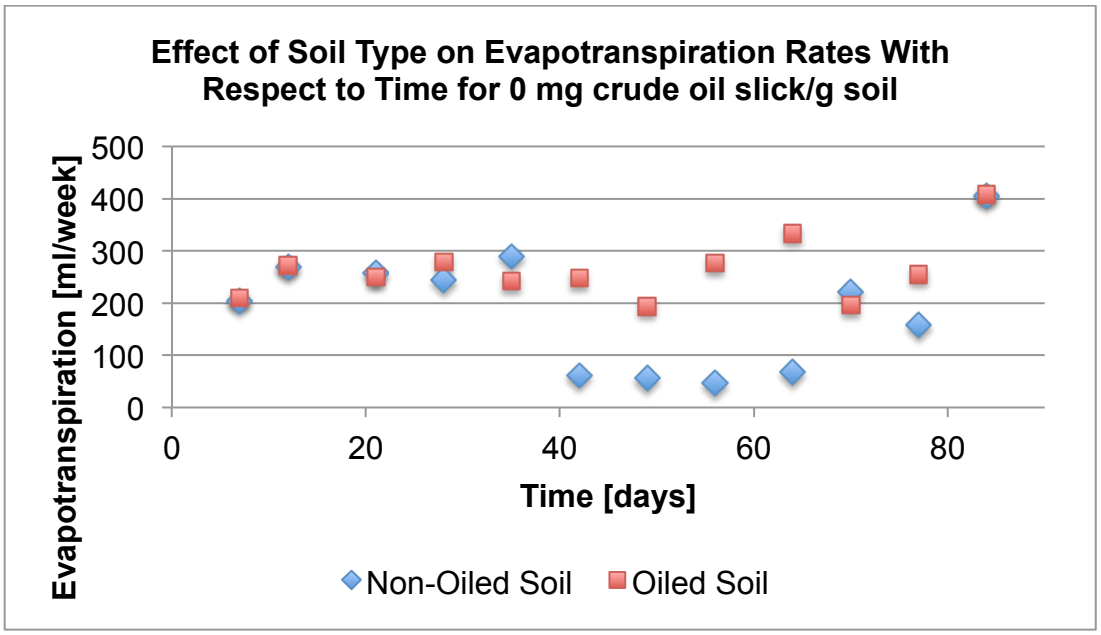

Figure 33: Effect of Soil Type on Evapotranspiration Rates With Respect to Time for 0 mg crude oil slick/g soil

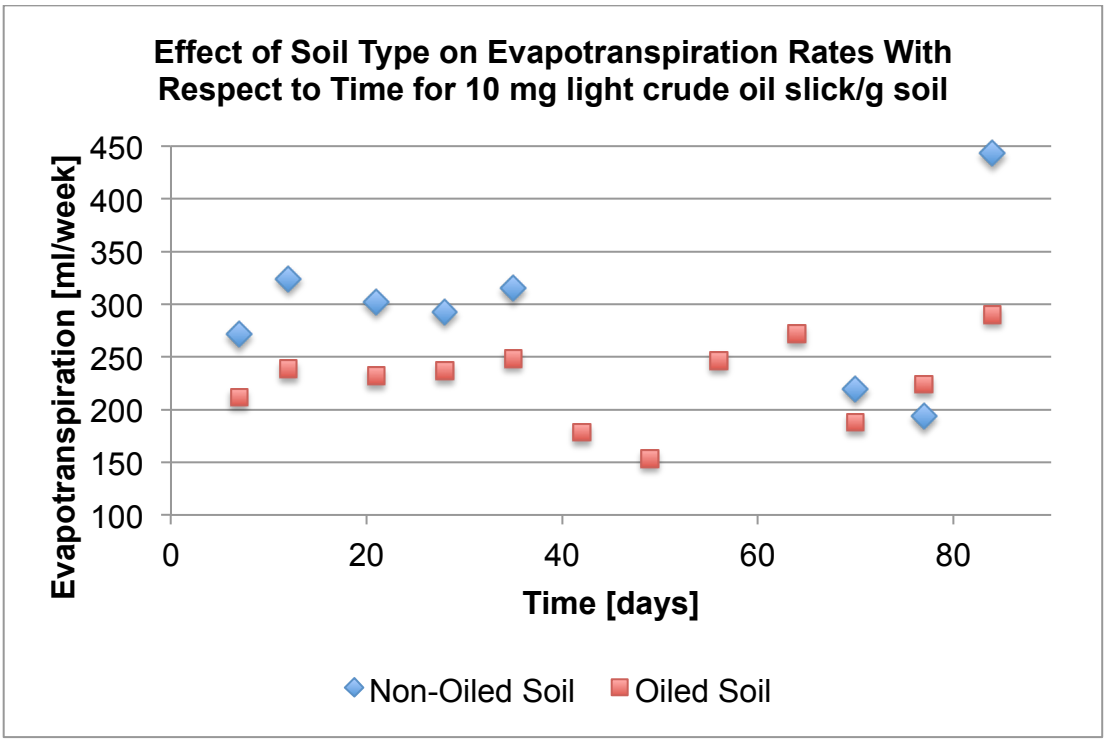

Figure 34: Effect of Soil Type on Evapotranspiration Rates With Respect to Time for 10 mg light crude oil slick/g soil

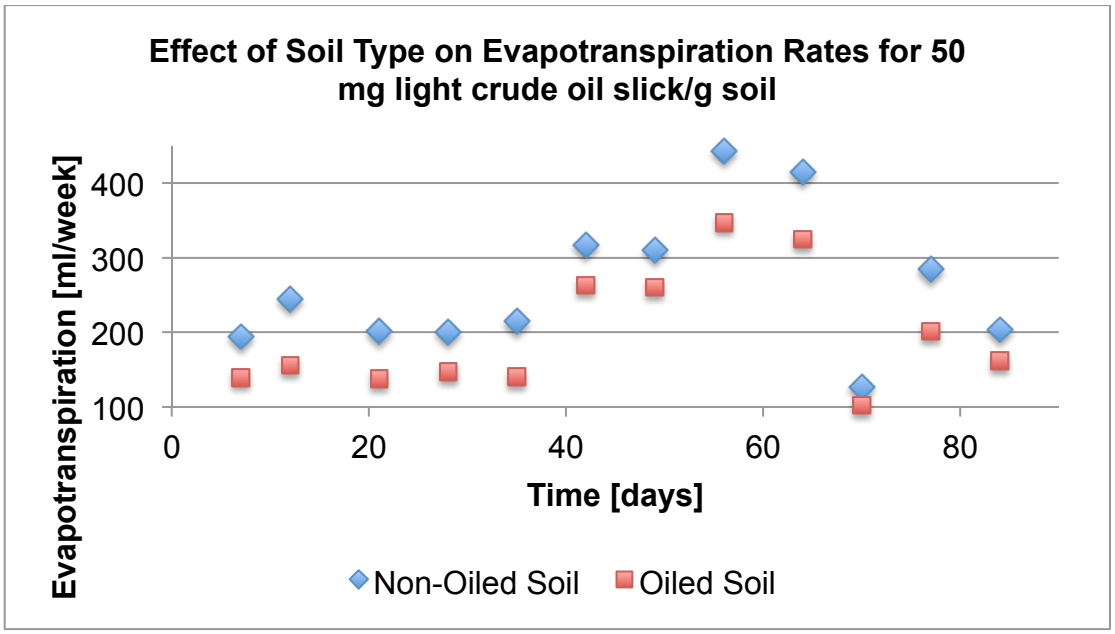

Figure 35: Effect of Soil Type on Evapotranspiration Rates for 50 mg light crude oil slick/g soil

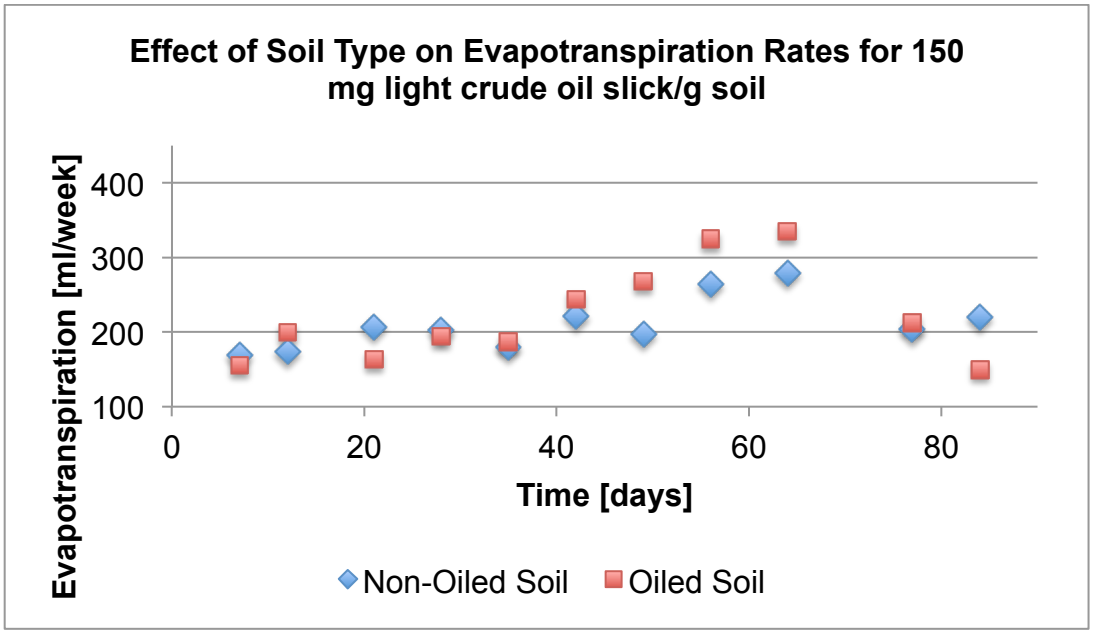

Figure 36: Effect of Soil Type on Evapotranspiration Rates for 150 mg light crude oil slick/g soil

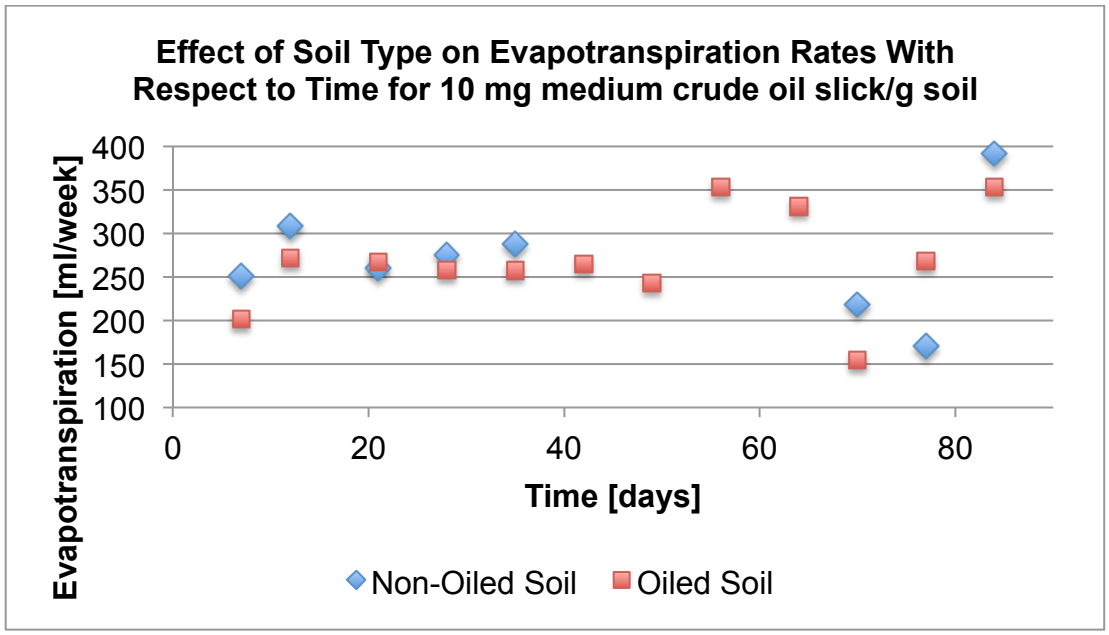

Figure 37: Effect of Soil Type on Evapotranspiration Rates With Respect to Time for 10 mg medium crude oil slick/g soil

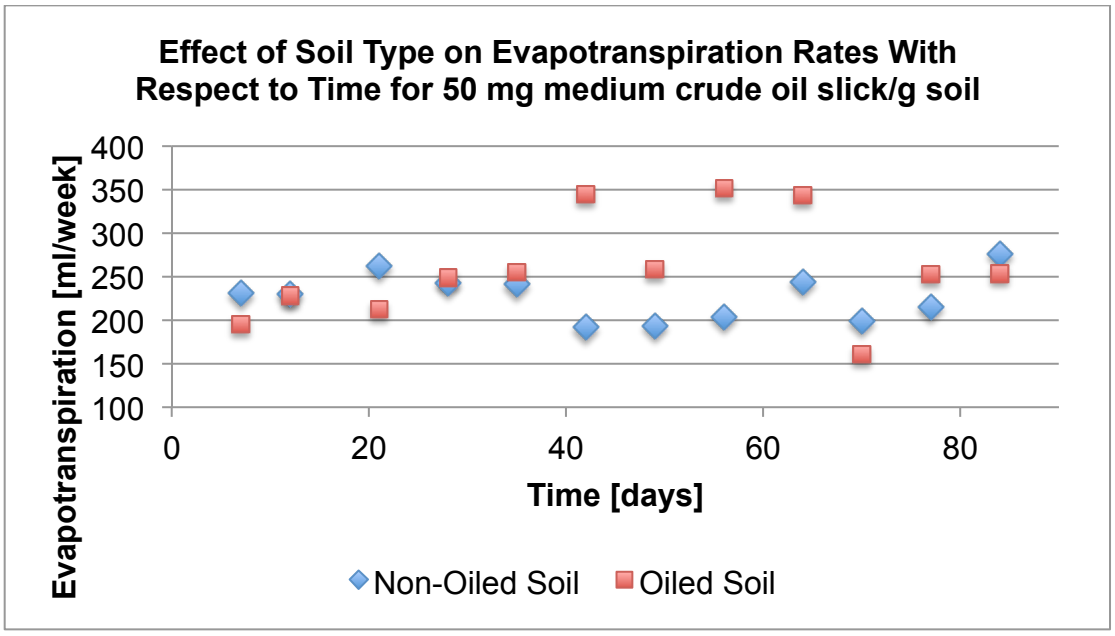

Figure 38: Effect of Soil Type on Evapotranspiration Rates With Respect to Time for 50 mg medium crude oil slick/g soil

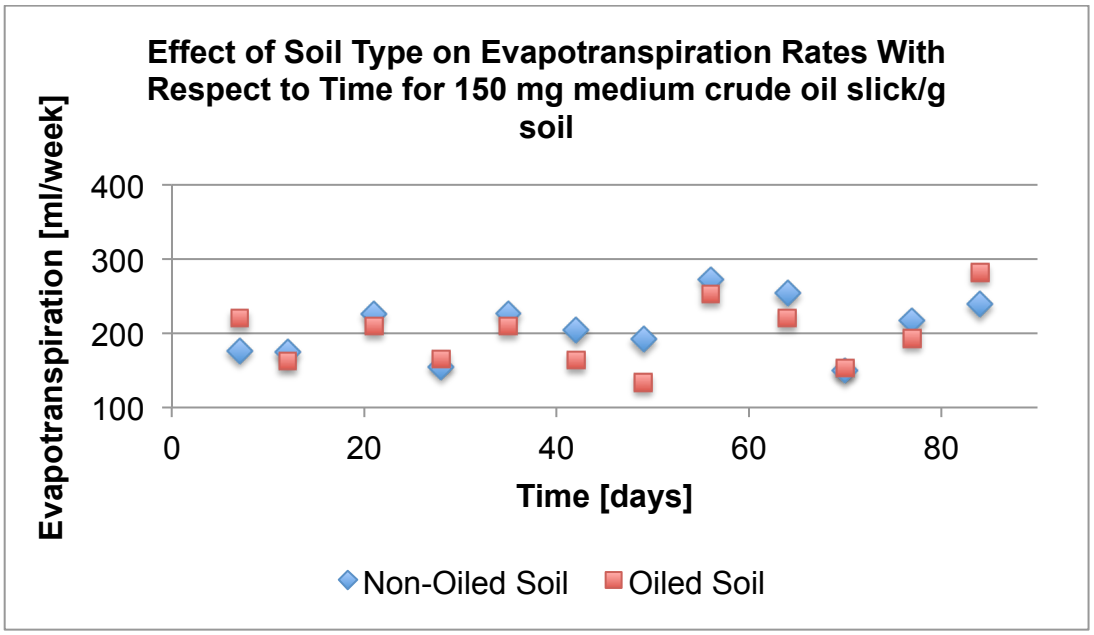

Figure 39: Effect of Soil Type on Evapotranspiration Rates With Respect to Time for 150 mg medium crude oil slick/g soil

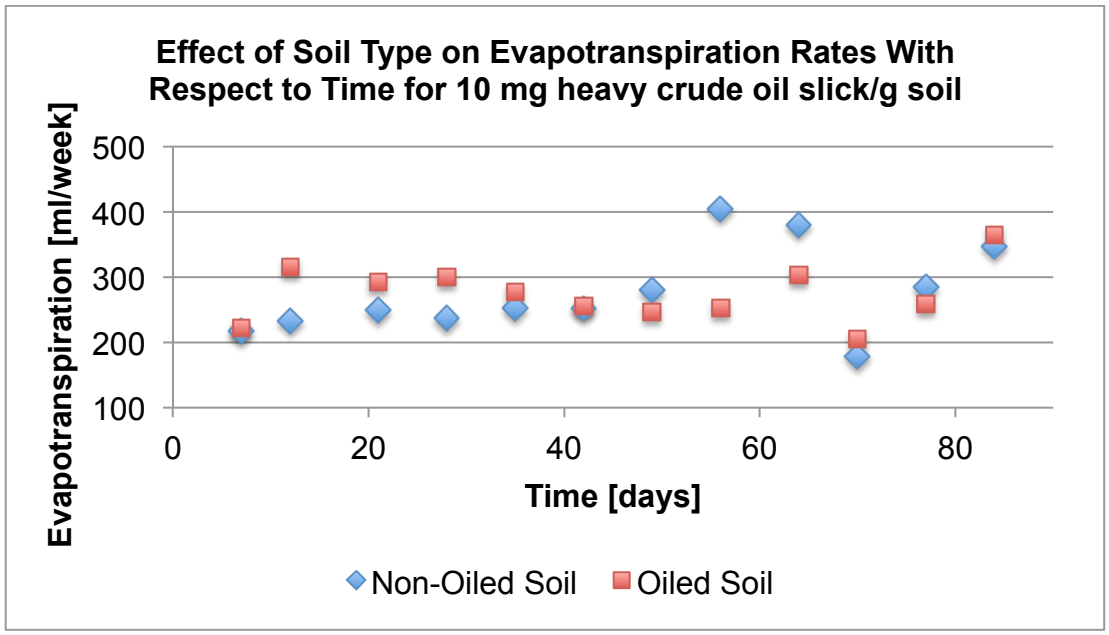

Figure 40: Effect of Soil Type on Evapotranspiration Rates With Respect to Time for 10 mg heavy crude oil slick/g soil

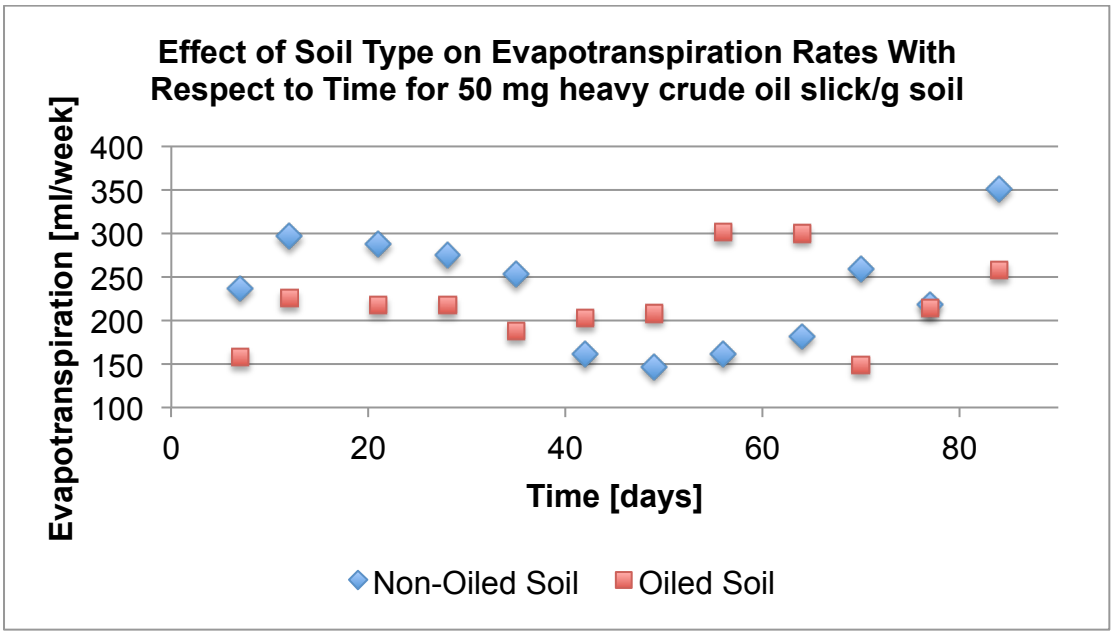

Figure 41: Effect of Soil Type on Evapotranspiration Rates With Respect to Time for 50 mg heavy crude oil slick/g soil

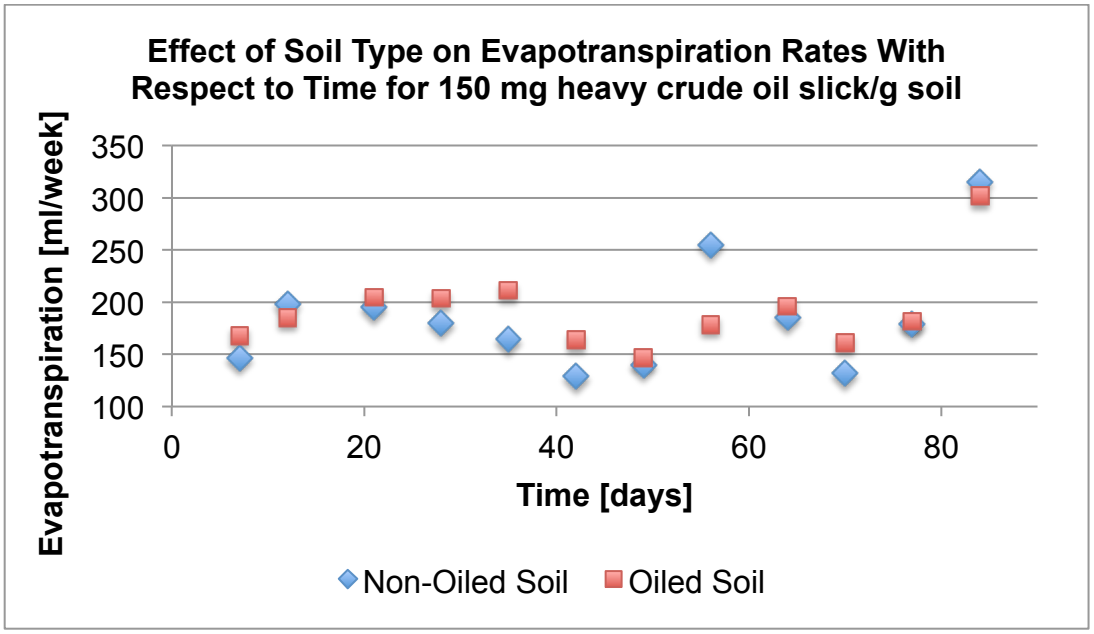

Figure 42: Effect of Soil Type on Evapotranspiration Rates With Respect to Time for 150 mg heavy crude oil slick/g soil

The statistical significance of the effect of oil type at a 95% confidence interval can be found in Table 6.

Table 6: Statistical Significance of Difference in Soil Type on Evapotranspiration Rates Based on a Paired, Two-tailed, T-test (p=0.05)

Oiled vs. Non-Oiled Soil		
Oil Type/Concentration	**p**	**Significant Difference?**
0mg/g	1.82E-02	Yes
Light (10mg/g)	4.07E-01	No
Medium (10mg/g)	5.26E-02	No
Heavy (10mg/g)	4.59E-01	No
Light (50mg/g)	3.56E-07	Yes
Medium (50mg/g)	7.72E-02	No
Heavy (50mg/g)	2.65E-01	No
Light(150mg/g)	2.82E-01	No
Medium (150mg/g)	1.36E-01	No
Heavy (150mg/g)	2.33E-01	No

Figure 33 and Figure 35 are the only two that show a significant difference of soil type on evapotranspiration rates. From Table 6, it is evident that there is little statistical significant difference in soil type on evapotranspiration rates, and it would appear that evapotranspiration was not as sensitive of an indicator of plant health compared to cumulative biomass. This set of data showed more variability than desired. Accordingly, it is difficult to draw conclusions with much degree of certainty. To be noted, Figure 35 does show a statistically significant difference and agrees with the biomass data by showing that the non-oiled soil out- performed the oiled soil with respect to evapotranspiration.

Effect of Oil Concentration on Evapotranspiration Rates

The evapotranspiration rates for all planted samples are presented below with each Figure representing one oil and one soil type to illustrate the effect of different oil concentrations. The results can be seen in Figure 43Figure 48; each data point represents a triplicate.

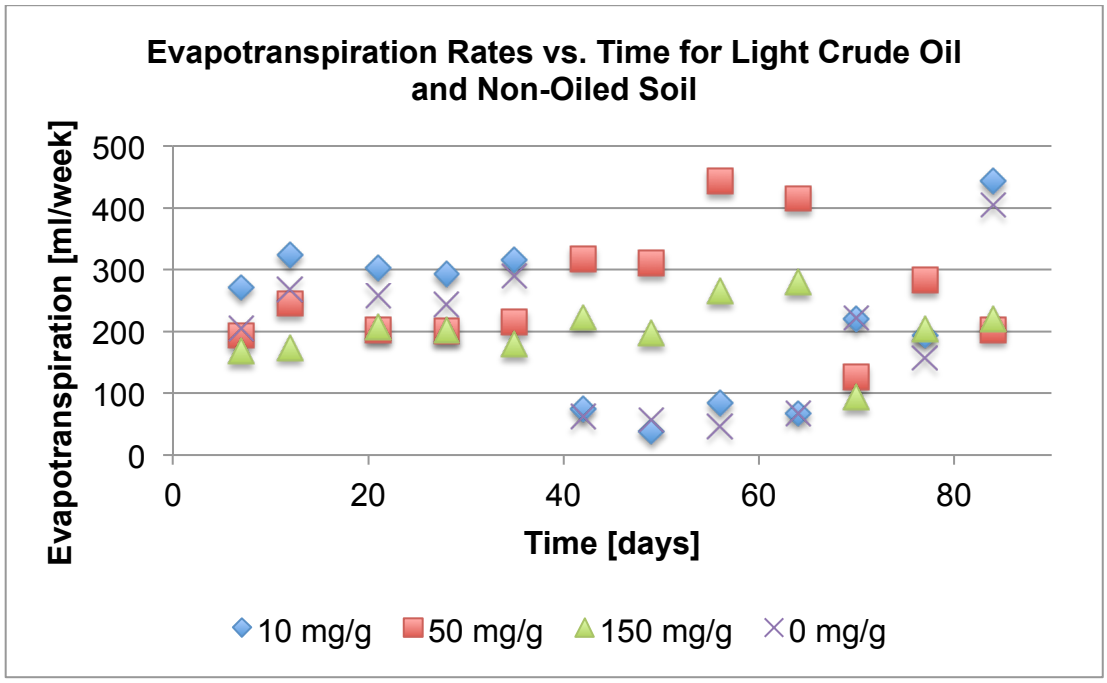

Figure 43: Evapotranspiration Rates vs. Time for Light Crude Oil and Non-Oiled Soil

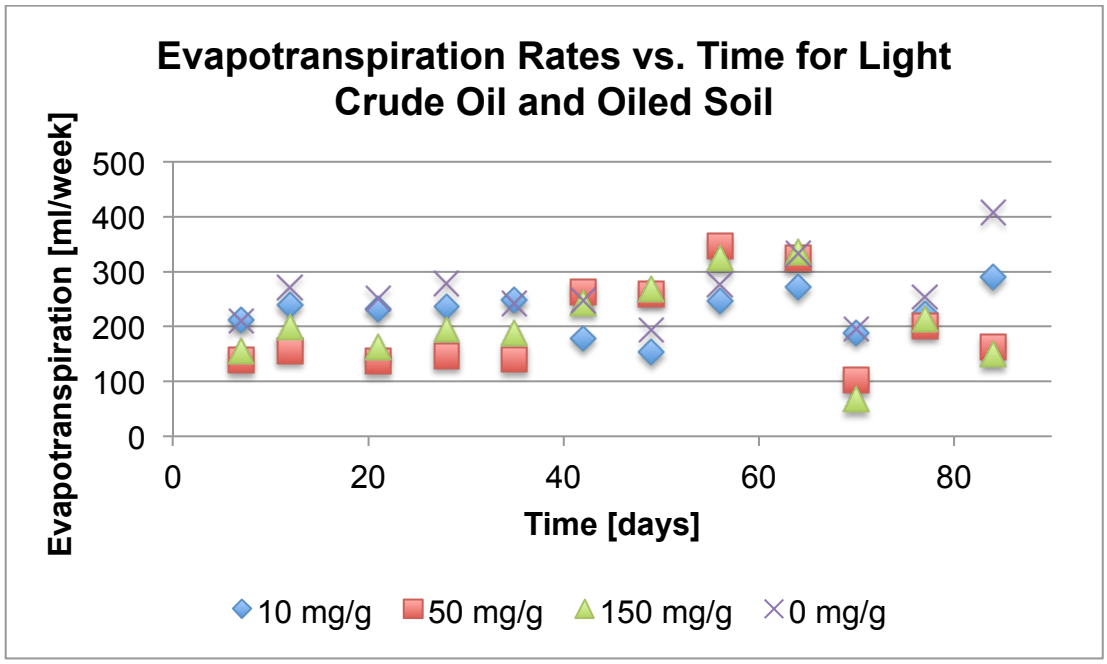

Figure 44: Evapotranspiration Rates vs. Time for Light Crude Oil and Oiled Soil

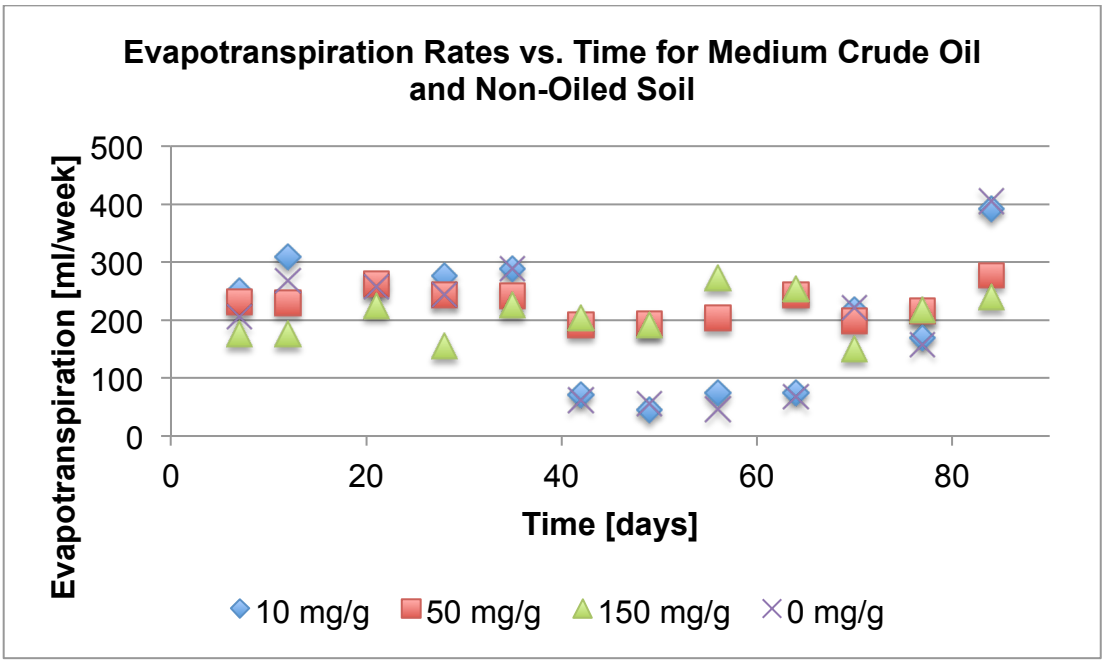

Figure 45: Evapotranspiration Rates vs. Time for Medium Crude Oil and Non-Oiled Soil

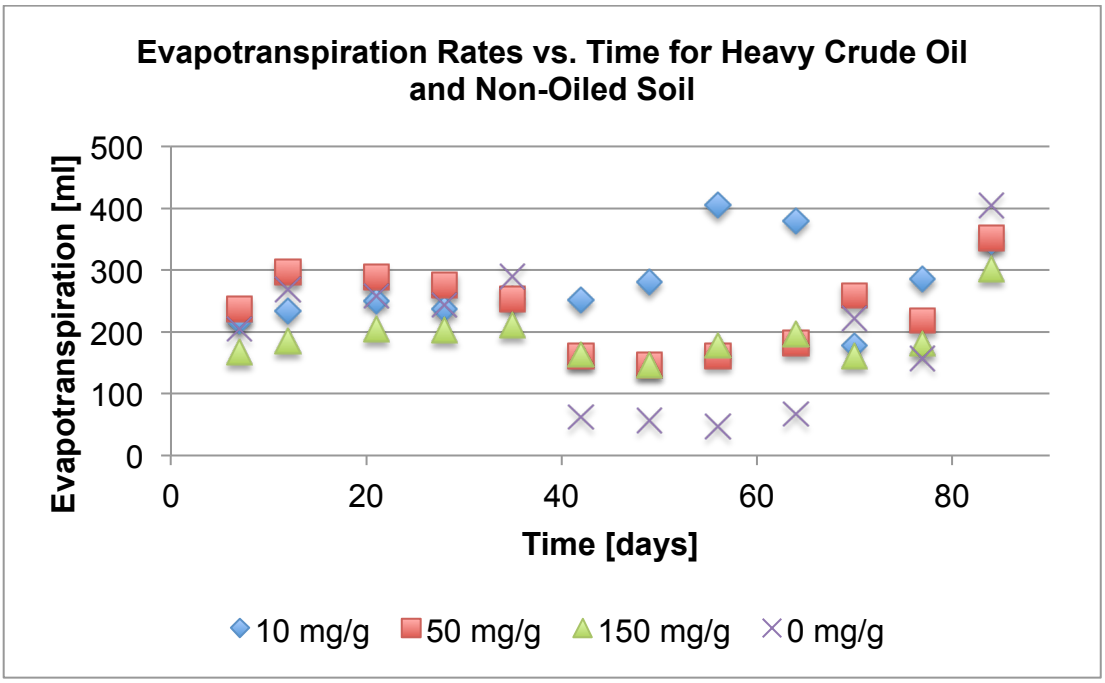

Figure 46: Evapotranspiration Rates vs. Time for Heavy Crude Oil and Non-Oiled Soil

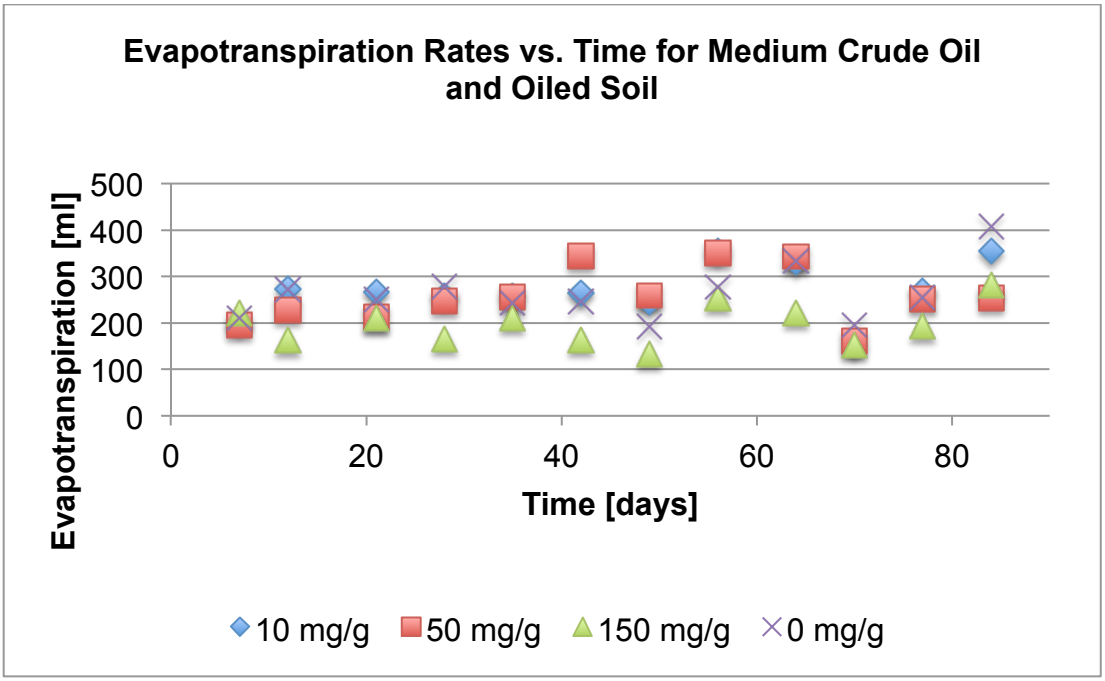

Figure 47: Evapotranspiration Rates vs. Time for Medium Crude Oil and Oiled Soil

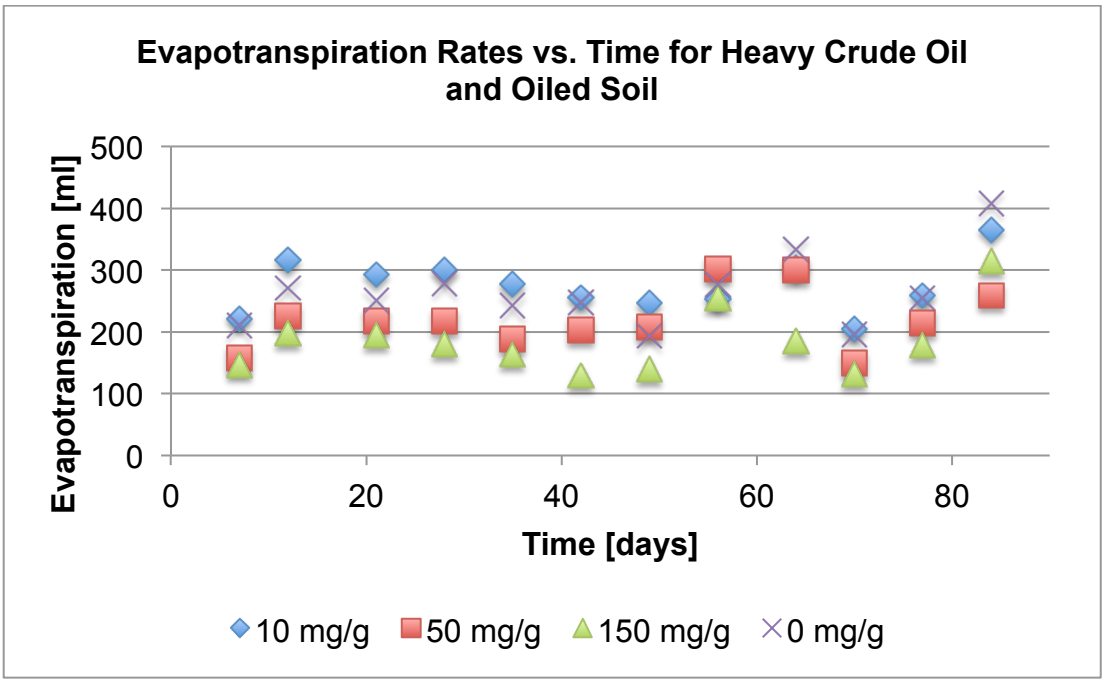

Figure 48: Evapotranspiration Rates vs. Time for Heavy Crude Oil and Oiled Soil

The statistical significance of the effect of oil concentration on evapotranspiration rates at a 95% confidence interval can be found in Table 7.

Table 7: Statistical Significance of Difference in Oil Concentration on Evapotranspiration Rates Based on T-test With 95% Confidence (p = 0.05)

Light Crude Oil, Non-Oiled Soil				
Concentration [mg crude oil/g wet soil]	**0**	**10**	**50**	**150**
0		No	No	No
10	No		No	Yes
50	No	No		Yes
150	No	Yes	Yes	
Light Crude Oil, Oiled Soil				
Concentration [mg crude oil/g wet soil]	**0**	**10**	**50**	**150**
0		Yes	Yes	Yes
10	Yes		No	No
50	Yes	No		No
150	Yes	No	No	
Medium Crude Oil, Non-Oiled Soil				
Concentration [mg crude oil/g wet soil]	**0**	**10**	**50**	**150**
0		No	No	No
10	No		No	No
50	No	No		No
150	No	No	No	
Medium Crude Oil, Oiled Soil				
Concentration [mg crude oil/g wet soil]	**0**	**10**	**50**	**150**
0		No	No	Yes
10	No		No	Yes
50	No	No		Yes
150	Yes	Yes	Yes	
Heavy Crude Oil, Non-Oiled Soil				
Concentration [mg crude oil/g wet soil]	**0**	**10**	**50**	**150**
0		Yes	No	No
10	Yes		No	Yes
50	No	No		Yes
150	No	Yes	Yes	
Heavy Crude Oil, Oiled Soil				
Concentration [mg crude oil/g wet soil]	**0**	**10**	**50**	**150**
0		No	Yes	Yes
10	No		Yes	Yes
50	Yes	Yes		No
150	Yes	Yes	No	

Table 7 shows once again that most of the conditions exhibit no significant difference for evapotranspiration rates based on oil concentration. However, the one consistent trend is that for all concentrations of 150 mg crude oil/g wet soil that show a statistical significant difference, i.e., the evapotranspiration rates are lower than all the other concentrations including the non-oiled samples. Fifty percent of the 150 mg crude oil/g wet soil concentrations showed a significant difference for evapotranspiration rates compared to the other dosages applied, whereas the other showed no statistically significant difference. This agrees with the biomass data (Figures 11-20) that 150 mg crude oil/g wet soil is a high enough concentration of oil that its effects dominate the evapotranspiration rates of the microcosm and indicate toxicity. At lower concentrations this conclusion is less certain.

Effect of Oil Type on Evapotranspiration Rates

To demonstrate the effect of oil type on evapotranspiration rates, evapotranspiration rates are presented in the figures below with each figure representing only one oil concentration and one soil type. The results can be seen in Figure 49-Figure 54; each data point represents a triplicate.

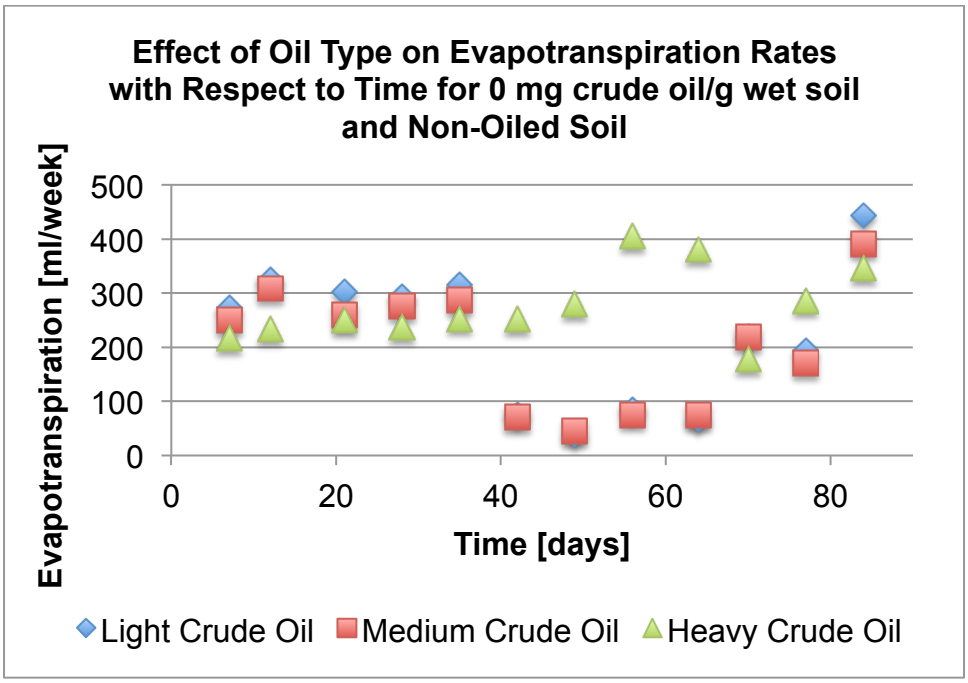

Figure 49: Effect of Oil Type on Evapotranspiration Rates with Respect to Time for 0 mg crude oil/g wet soil and Non-Oiled Soil

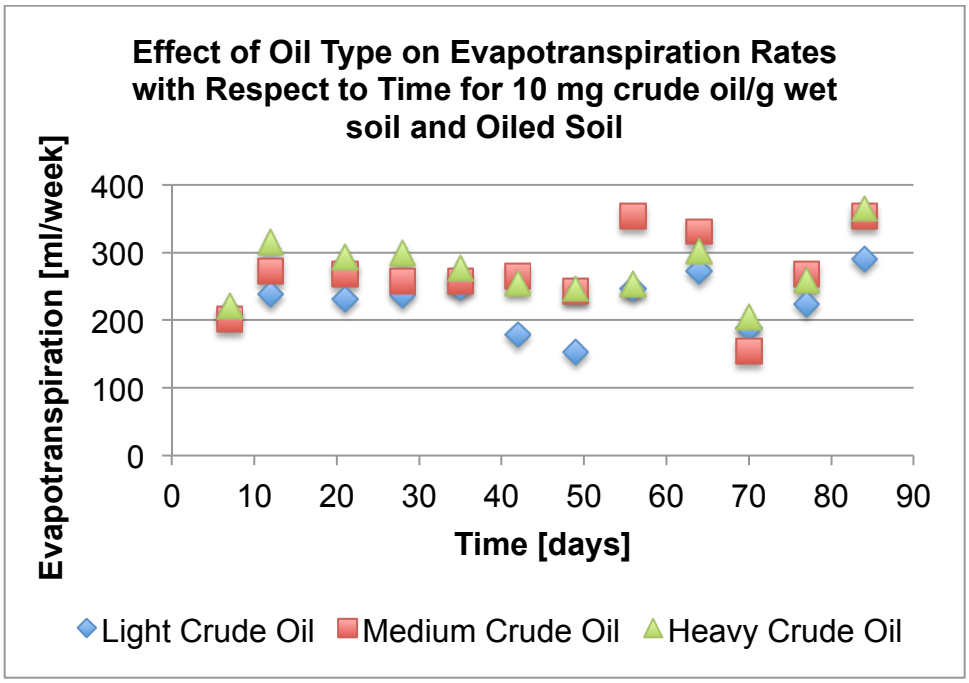

Figure 50: Effect of Oil Type on Evapotranspiration Rates with Respect to Time for 10 mg crude oil/g wet soil and Oiled Soil

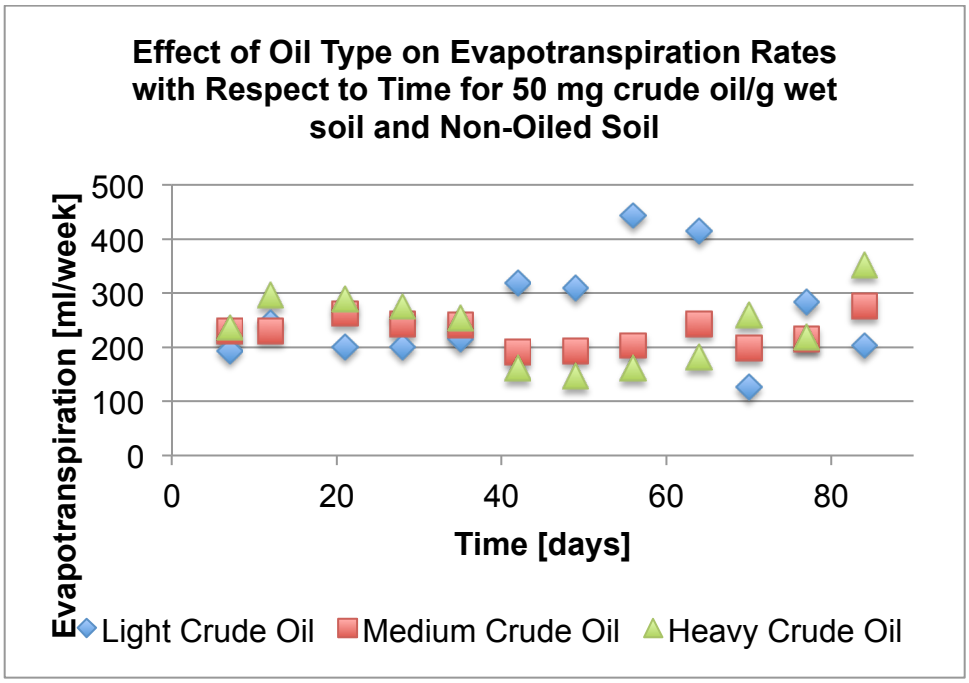

Figure 51: Effect of Oil Type on Evapotranspiration Rates with Respect to Time for 50 mg crude oil/g wet soil and Non-Oiled Soil

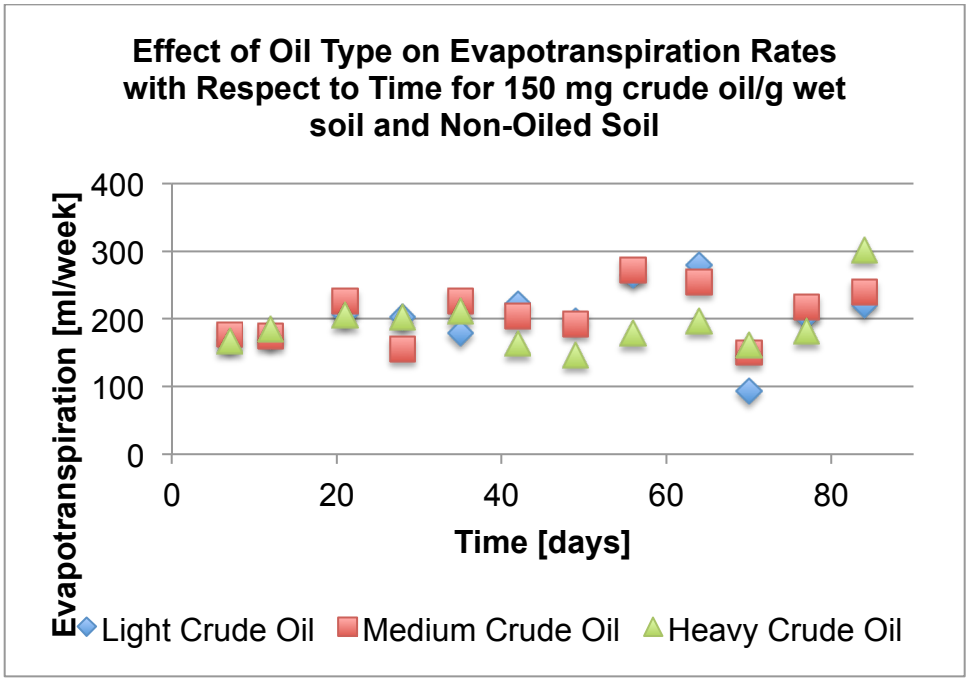

Figure 52: Effect of Oil Type on Evapotranspiration Rates with Respect to Time for 150 mg crude oil/g wet soil and Non-Oiled Soil

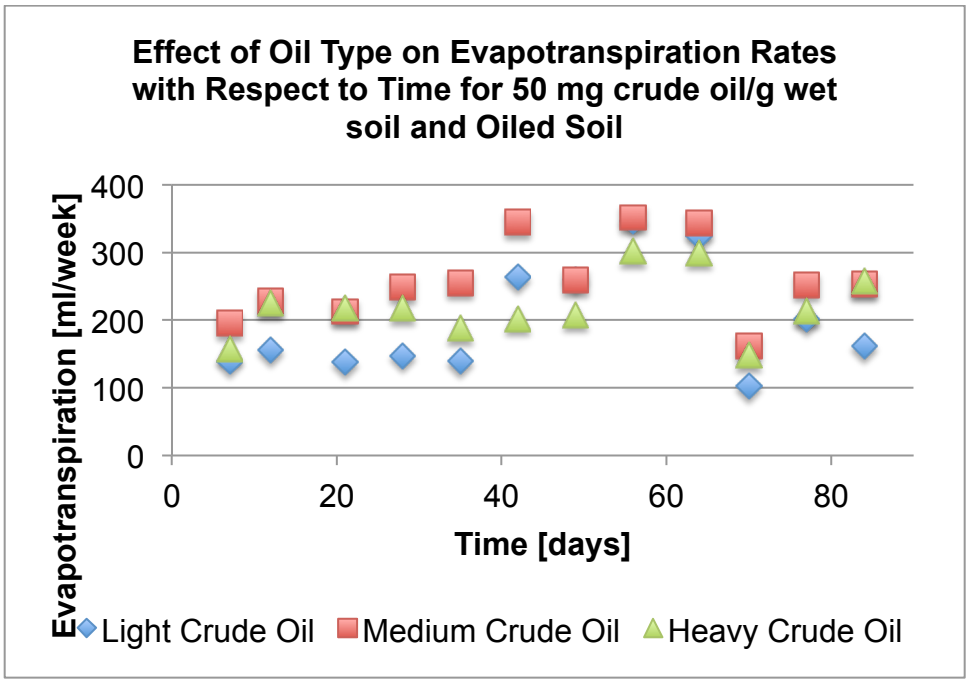

Figure 53: Effect of Oil Type on Evapotranspiration Rates with Respect to Time for 50 mg crude oil/g wet soil and Oiled Soil

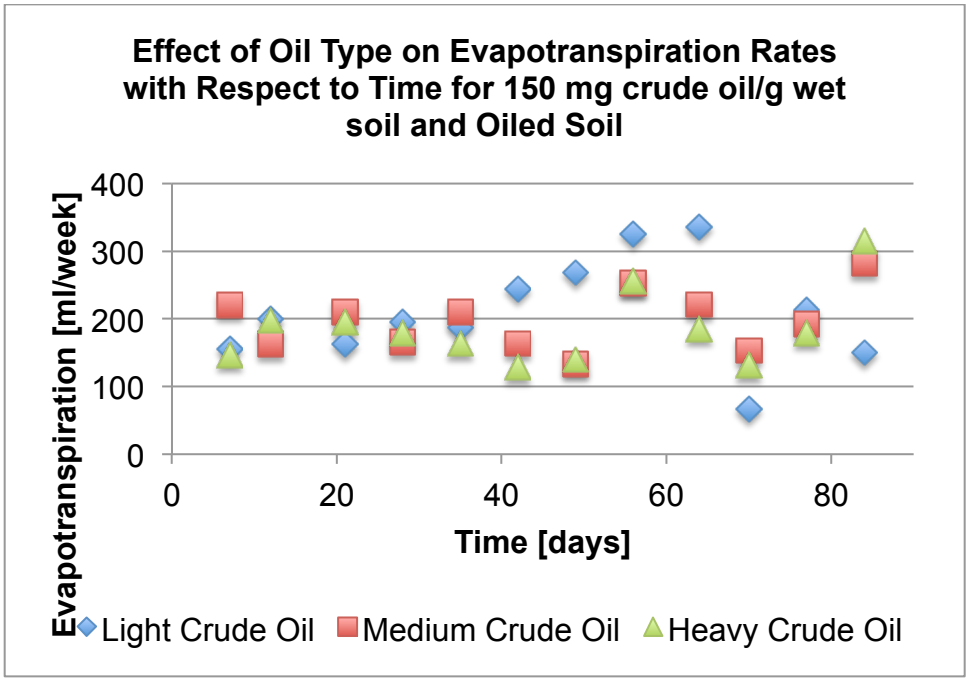

Figure 54: Effect of Oil Type on Evapotranspiration Rates with Respect to Time for 150 mg crude oil/g wet soil and Oiled Soil

The statistical significance of the effect of oil type on evapotranspiration rates at a 95% confidence interval can be found in Table 8.

Table 8: Statistical Significance of Difference in Oil Type on Evapotranspiration Rates Based on a Paired, Two-tailed, T-test (p=0.05)

Concentration	p	Significant Difference?
Non-Oiled Soil		
Light vs. Medium (10mg/g)	3.71E-01	No
Light vs. Heavy (10mg/g)	9.37E-02	No
Medium vs. Heavy (10mg/g)	3.39E-02	Yes
Light vs. Medium (50mg/g)	1.18E-01	No
Light vs. Heavy (50mg/g)	2.11E-01	No
Medium vs. Heavy (50mg/g)	3.39E-01	No
Light vs. Medium (150mg/g)	3.61E-01	No
Light vs. Heavy (150mg/g)	3.05E-01	No
Medium vs. Heavy (150mg/g)	1.70E-01	No
Oiled Soil		
Light vs. Medium (10mg/g)	2.46E-02	Yes
Light vs. Heavy (10mg/g)	4.92E-03	Yes
Medium vs. Heavy (10mg/g)	3.94E-01	No
Light vs. Medium (50mg/g)	2.51E-02	Yes
Light vs. Heavy (50mg/g)	2.17E-01	No
Medium vs. Heavy (50mg/g)	4.72E-02	Yes
Light vs. Medium (150mg/g)	3.31E-01	No
Light vs. Heavy (150mg/g)	1.95E-01	No
Medium vs. Heavy (150mg/g)	2.72E-01	No

Figure 49 through Figure 54 and Table 8 show that oil type played a more important role in the oiled soil than non-oiled soil. However, the data shows that oil type did not play a significant role for any of the conditions with 150 mg crude oil/g wet soil.

In comparison to the biomass data (Figures 11-20), the evapotranspiration data is significantly less conclusive. There is greater variability in the data than desired. In

the future, a more precise evapotranspiration measurement device would be better to ensure less error and greater precision. Furthermore, it is possible that a spider mite infestation and change of room location influenced the evapotranspiration rates in addition to the intended variables around day 40. Also, while evapotranspiration (ET) and biomass rates are linked, ET rates are expected to be more sensitive than change in biomass when environmental factors are variable. Which potentially caused the ET data to have too much noise to provide conclusive results. These occurrences introduced additional unintended variables into the experiment that likely caused the evapotranspiration data to be less conclusive than the biomass data.

CHAPTER 5 CONCLUSIONS AND RECOMENDATIONS

Experiment two was the largest laboratory study on the effects of oil on saltmarsh plants that was uncovered in the literature search. It represents nearly 3000 points of biometric data. While there was more variability in the data than desired, significant trends can still be observed, especially from the biomass data. Furthermore, the evapotranspiration data was less definitive than the biomass data, but when a significant difference was observed, the evapotranspiration data supported the conclusions drawn from the biomass data. The following specific conclusions were reached:

(1) The lethal dosage for *S. alterniflora* was 250 mg light crude oil/g wet soil for Sweet Louisiana Crude in experiment one, which caused death of the plants (0 % survival). This dosage can be considered a lethal dose for light crude oil spills with smooth cord grass in saltwater marshes. It is also the concentration of a simulated oil slick (at approximately an oil thickness of 3 mm).

(2) Two types of oil exposures were used in this research: a) oil applied as a simulated spill which floated on the surface of the water in the microcosms; and b) oil which was added to soil prior to preparing the microcosms and which was acclimated for four months. At initial oil slick concentrations (dosages) of 10 and 50 mg crude oil/g wet soil, the oiled soil (pre-acclimated for 4 months) was more influential in decreasing cumulative biomass growth rates compared to oil applied at the oil-water interface, for the light crude used (Experiment #2).

(3) At the heaviest dosages applied as a simulated oil slick, concentrations of 150 mg crude oil/g wet soil, biomass and evapotranspiration rates were negatively

affected by the oil (significant at p=0.05 in a one-tailed t-test).

(4) Light, heavy, and then medium crude oil showed the lowest cumulative biomass growths, in that order, indicating that light crude oil was the most toxic in these microcosm experiments with *S. alterniflora*.

(5) The 10 mg oil/g wet soil out-performed the 0 mg oil/g wet soil in transpiration and biomass growth

These conclusions could potentially be used to guide best management practices in restoring coastal marsh ecosystems impacted by oil. Tidal hydraulic modeling would need to be incorporated to determine the proper headspace height but results suggest that substratum contamination of oil adversely affects *S. alterniflora* at significantly lower concentrations than oil slick-water interface contamination.

APPENDIX A MICROBIAL CONSIDERATIONS

A supplementary microbial study was conducted to qualitatively determine the effect of planted and oiled samples on microbial community populations. This experiment was conducted as part of an Environmental Microbiology class under the supervision of Dr. Tim Mattes. 120 spread plates were created with four types of samples, three types of spread plates, and eight dilution levels with the objective of quantifying the growth of microbial colonies.

Experimental Setup

Aqueous samples were taken from the water phase of four microcosms and streak plated. The four microcosm types sampled included an oiled-planted sample, a non-oiled-planted sample, an oiled-unplanted sample, and a non-oiled-unplanted sample. All microcosms contained approximately 300 g of organic potting soil in addition to DI water and all planted samples used *S.alterniflora* as the only plant species. The four sample conditions were selected to isolate the effect of oil and *S.alterniflora* on microbe populations. In addition, eight dilutions of the four sample types were used to attempt to achieve a dilution level that allowed visibly countable colony numbers. The dilutions of 10^{0}, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} were used to provide a wide range of growth densities.

Three types of plate medium were used for the growth plates. The three plate types used included one trypticase soy agar (TSA) plate, TSA with oil applied to the surface, and a mineral salts medium (MSM) plate with oil applied to the surface. For the plates with applied oil, three drops of light crude oil were spread uniformly over the plate to provide oil as a carbon source to potential microorganisms. The TSA medium was

used because it is known to support a wide range of microbe types and the oiled MSM plate was used to determine if microorganisms could growth with oil as the sole carbon source (Madigan M, 2005). Following, all plates were sterilized using an autoclave and the samples were streak plated. After the streak plating, the 120 plates were observed daily for a week. Temporal restrictions were responsible for the limited observation window. A picture from this process can be seen in Figure A1.

Figure A1: Lab Partner Andy Awad Preparing Streak Plates

Results and Discussion

Microbial growth was observed on all plate types during the weeklong observation period. Due to resource and time limitations, colony counts were based on visual estimation rather than a computer based graphical method. From this, several trends were observed.

The planted and oiled sample and the unplanted oiled sample exhibited comparable growth rates on the oiled-MSM plates but the planted and oiled samples exhibited slightly higher growth rates at dilutions of 10^{-1} and 10^{-2}. These results can be found in Table A1.

Table A1: Estimated Colonies of Three Sample Conditions for Oiled-MSM Plates

Sample	Dilution	Estimated Colonies
Oiled Plant	10^{0}	130
	10^{-1}	60
	10^{-2}	35
Oiled Soil	10^{0}	130
	10^{-1}	25
	10^{-2}	30
Plant	10^{0}	1
	10^{-1}	0
	10^{-2}	2

As seen in Table A1, the non-oiled-plated sample exhibited essentially no growth in comparison to the oiled samples. This was to be expected and suggests that the oiled samples had oil degrading microbial populations that were able to propagate with oil as the sole carbon source. In addition, no observable growth was detected in

the oiled-MSM plates at dilutions greater than 10^{-2}. Furthermore, a visual comparison can be observed in Figures A2 and A3.

Figure A2: Oiled and Planted Sample at No Dilution on an Oiled-MSM Plate

Figure A3: Oiled and Unplanted Sample at No Dilution on an oiled-MSM Plate

Figure A2 and Figure A3 show a visual comparison of a non-planted and planted sample at zero dilution on an oiled-MSM plate. Both showed similar colony counts but the planted sample showed the colonies less uniformly distributed and more clumped together. In addition, it appears there was only one microbe type of a white microbe colony.

All of the TSA plates exhibited growth at all dilution levels. This was to be expected since the TSA substrate supports the widest range of microorganisms. In addition, a wider range of microorganisms were observed on the TSA growth plates including orange, white, and red colonies, whereas, the MSM plates showed primarily white colonies during the week observation period. One of the TSA growth plates can be seen in Figure A4 below.

Figure A4: Non-Oiled and Planted Sample at 10^{-7} Dilution on a TSA Plate

Figure A4 shows extensive microbial growth of a non-oiled and plated sample on a TSA plate at a dilution of 10^{-7}. This shows growth at greater dilution rates than

with the MSM plates, which indicates that the TSA plates were able to support greater growth.

While the results suggest that the oiled planted samples exhibited a more robust microbial population than the unplanted oiled samples, there are several key limitations to this experiment. Only one plate was used for each of the conditions and consequently there exists a high statistical variability; this could be remedied in the future by using triplicates for all the samples. In addition, none of the microbe colonies were sequenced or characterized. Thus, while the plates could be showing oil-degrading microbes, it is impossible to conclude this with any degree of certainty. Based on these results, the decrease in Total extractable hydrocarbons during the course of the experiments shown in Chapter 5 is likely due to biodegradation, as seen in these streak plates. While not definitive, results suggest that planted microcosms performed better in degrading TEH due to the microbial population of oil-degrading microorganisms that was supported in the root zone of the plants.

APPENDIX B RAW DATA

Table B1: Initial Conditions for Samples in Experiment Two

Sample #	Plants	Oil type	Soil Type	Concentration (mg/g)	Beaker Mass (g)	Beaker + Soil Mass (g)	Beaker+Soil+ Plant Mass	Initial Soil and Biomass Mass (g)	Mass of Oil Added (g)
1	NO	n/a	Non-Oiled	0	409.40	704.15	n/a	294.75	-
2	NO	n/a	Non-Oiled	0	404.63	692.47	n/a	287.84	-
3	NO	n/a	Non-Oiled	0	407.23	708.86	n/a	301.63	-
4	NO	n/a	Oiled	0	406.77	708.62	n/a	301.85	-
5	NO	n/a	Oiled	0	412.44	698.67	n/a	286.23	-
6	NO	n/a	Oiled	0	401.62	703.61	n/a	301.99	-
7	NO	Medium	Non-Oiled	50	410.15	695.45	n/a	285.30	14.27
8	NO	Medium	Non-Oiled	50	410.43	714.76	n/a	304.33	15.22
9	NO	Medium	Non-Oiled	50	410.23	704.71	n/a	294.48	14.72
10	NO	Medium	Oiled	50	409.36	695.77	n/a	286.41	14.32
11	NO	Medium	Oiled	50	407.34	707.52	n/a	300.18	15.01
12	NO	Medium	Oiled	50	409.55	702.63	n/a	293.08	14.65
13	YES	n/a	Non-Oiled	0	410.12	704.67	846.40	436.28	-
14	YES	n/a	Non-Oiled	0	408.72	710.30	848.68	439.96	-
15	YES	n/a	Non-Oiled	0	409.39	709.91	861.25	451.86	-
16	YES	n/a	Oiled	0	407.01	704.82	804.65	397.64	-
17	YES	n/a	Oiled	0	405.30	696.61	806.76	401.46	-
18	YES	n/a	Oiled	0	411.25	704.02	836.52	425.27	-
19	YES	Light	Non-Oiled	10	409.27	707.93	817.95	408.68	4.09
20	YES	Light	Non-Oiled	10	409.80	702.10	912.11	502.31	5.02
21	YES	Light	Non-Oiled	10	409.68	704.73	862.85	453.17	4.53
22	YES	Light	Non-Oiled	50	407.75	708.64	869.37	461.62	23.08
23	YES	Light	Non-Oiled	50	408.03	703.09	840.06	432.03	21.60
24	YES	Light	Non-Oiled	50	410.73	701.95	887.51	476.78	23.84
25	YES	Light	Non-Oiled	250	408.95	702.69	850.75	441.80	110.45
26	YES	Light	Non-Oiled	150	405.34	706.40	841.06	435.72	65.36
27	YES	Light	Non-Oiled	150	407.77	701.07	827.63	419.86	62.98
28	YES	Light	Oiled	10	407.69	707.24	847.09	439.40	4.39
29	YES	Light	Oiled	10	407.78	703.60	812.20	404.42	4.04
30	YES	Light	Oiled	10	408.72	709.72	851.04	442.32	4.42
31	YES	Light	Oiled	50	409.86	705.22	776.16	366.30	18.32
32	YES	Light	Oiled	50	409.60	708.31	773.66	364.06	18.20
33	YES	Light	Oiled	50	407.03	710.23	834.39	427.36	21.37
34	YES	Light	Oiled	150	408.77	701.12	892.89	484.12	72.62
35	YES	Light	Oiled	150	406.44	704.41	805.87	399.43	59.91
36	YES	Light	Oiled	150	411.95	706.11	845.06	433.11	64.97
37	YES	Medium	Non-Oiled	10	410.53	709.22	822.36	411.83	4.12
38	YES	Medium	Non-Oiled	10	409.60	709.64	821.40	411.80	4.12
39	YES	Medium	Non-Oiled	10	411.59	707.38	836.39	424.80	4.25
40	YES	Medium	Non-Oiled	50	409.65	704.09	841.25	431.60	21.58
41	YES	Medium	Non-Oiled	50	408.41	706.68	822.19	413.78	20.69
42	YES	Medium	Non-Oiled	50	410.42	706.21	809.82	399.40	19.97
43	YES	Medium	Non-Oiled	250	408.61	703.16	817.62	409.01	102.25
44	YES	Medium	Non-Oiled	150	412.10	701.97	793.28	381.18	57.18
45	YES	Medium	Non-Oiled	150	408.81	705.75	878.42	469.61	70.44
46	YES	Medium	Oiled	10	409.43	715.16	789.07	379.64	3.80
47	YES	Medium	Oiled	10	407.17	708.81	853.88	446.71	4.47
48	YES	Medium	Oiled	10	410.89	701.64	811.68	400.79	4.01
49	YES	Medium	Oiled	50	409.65	703.64	806.76	397.11	19.86
50	YES	Medium	Oiled	50	408.14	705.20	789.91	381.77	19.09
51	YES	Medium	Oiled	50	407.69	713.11	781.24	373.55	18.68
52	YES	Medium	Oiled	150	402.85	702.73	781.86	379.01	56.85
53	YES	Medium	Oiled	150	408.31	700.82	803.45	395.14	59.27
54	YES	Medium	Oiled	150	408.64	704.03	799.95	391.31	58.70
55	YES	Heavy	Non-Oiled	10	403.60	705.61	805.64	402.04	4.02
56	YES	Heavy	Non-Oiled	10	404.10	705.36	864.90	460.80	4.61
57	YES	Heavy	Non-Oiled	10	404.22	707.02	770.51	366.29	3.66
58	YES	Heavy	Non-Oiled	50	407.50	705.40	840.53	433.03	21.65
59	YES	Heavy	Non-Oiled	50	409.47	703.99	788.12	378.65	18.93
60	YES	Heavy	Non-Oiled	50	409.32	721.56	832.31	422.99	21.15
61	YES	Heavy	Non-Oiled	150	406.07	705.01	805.36	399.29	59.89
62	YES	Heavy	Non-Oiled	150	409.99	746.58	860.12	450.13	67.52
63	YES	Heavy	Non-Oiled	150	407.14	705.69	892.97	485.83	72.87
64	YES	Heavy	Oiled	10	404.28	713.57	923.32	519.04	5.19
65	YES	Heavy	Oiled	10	407.24	705.99	828.17	420.93	4.21
66	YES	Heavy	Oiled	10	410.89	720.59	890.18	479.29	4.79
67	YES	Heavy	Oiled	50	408.38	703.44	855.15	446.77	22.34
68	YES	Heavy	Oiled	50	408.20	730.29	896.55	488.35	24.42
69	YES	Heavy	Oiled	50	406.98	709.91	841.21	434.23	21.71
70	YES	Heavy	Oiled	150	407.51	705.93	825.69	418.18	62.73
71	YES	Heavy	Oiled	150	411.13	691.20	778.45	367.32	55.10
72	YES	Heavy	Oiled	50	406.21	705.02	853.75	447.54	22.38

Table B2: Averaged Triplicate Cumulative Change in Biomass Data for Experiment Two

Plants	Oil type	Soil Type	Concentration (mg/g)	Day 7 (g)	Day 12 (g)	Day 21 (g)	Day 28 (g)	Day 35 (g)	Day 42 (g)	Day 49 (g)	Day 56 (g)	Day 64 (g)	Day 70 (g)	Day 77 (g)	Day 84 (g)
YES	Light	Non-Oiled	10	47.52	42.53	55.80	54.07	66.33	68.00	84.47	101.00	97.30	96.47	73.67	76.03
YES	Light	Oiled	10	34.45	20.40	29.33	35.97	56.30	50.77	46.20	81.17	47.80	62.17	43.57	56.60
YES	Light	Non-Oiled	50	8.96	11.57	25.37	20.90	39.40	29.20	41.80	38.33	48.43	51.33	32.66	37.43
YES	Light	Oiled	50	13.71	5.43	12.67	21.60	20.77	27.63	24.13	29.07	37.70	43.40	24.76	31.40
YES	Light	Non-Oiled	150	-6.97	-65.65	-29.15	-17.80	-12.15	-27.65	-20.10	-24.25	-3.65	-10.75	-23.15	-22.35
YES	Light	Oiled	150	-23.80	-34.00	-38.60	-28.40	-15.20	-43.10	-55.43	-35.87	-26.77	-35.83	-31.34	-43.30
YES	Medium	Non-Oiled	10	49.47	47.50	51.23	46.40	64.67	70.20	77.40	86.57	87.70	87.50	63.93	69.43
YES	Medium	Oiled	10	11.04	7.53	31.57	32.37	35.10	48.67	38.43	41.13	58.80	38.93	37.58	73.37
YES	Medium	Non-Oiled	50	56.95	26.47	58.97	64.43	68.07	71.87	42.63	100.00	82.23	98.73	72.32	101.47
YES	Medium	Oiled	50	21.89	9.47	20.07	32.53	50.70	29.43	38.67	41.50	45.90	12.43	21.79	15.13
YES	Medium	Non-Oiled	150	13.79	-25.80	24.55	-3.95	34.95	14.50	-7.80	36.00	28.05	38.30	21.58	33.50
YES	Medium	Oiled	150	66.73	34.00	77.53	58.17	81.13	45.90	38.40	74.70	69.17	67.70	52.14	51.10
YES	Heavy	Non-Oiled	10	45.34	30.63	49.37	46.47	57.73	58.83	71.67	94.30	82.03	80.33	56.15	70.63
YES	Heavy	Oiled	10	20.00	18.93	37.10	45.40	46.37	44.37	44.20	52.30	69.73	69.20	43.58	67.73
YES	Heavy	Non-Oiled	50	34.49	33.87	67.83	63.53	47.77	72.73	70.73	67.80	75.33	74.60	60.70	86.73
YES	Heavy	Oiled	50	-9.94	-8.60	6.80	10.70	23.83	8.15	26.18	28.93	23.73	45.43	11.54	17.23
YES	Heavy	Non-Oiled	150	0.04	-17.27	0.87	6.50	15.63	-2.40	19.80	29.27	25.87	38.97	12.92	14.83
YES	Heavy	Oiled	150	-33.67	-48.15	-19.15	-5.90	-11.40	-53.70	-31.45	-10.25	-20.70	-12.10	-25.40	-52.95
YES	n/a	Non-Oiled	0	-26.23	-18.97	-12.50	-39.93	-0.67	-8.00	3.73	1.40	5.27	9.60	-3.36	14.83
YES	n/a	Oiled	0	-39.13	-32.63	-31.30	-22.33	-1.90	-15.90	9.80	5.33	2.20	3.67	-12.58	15.93

Table B3: Averaged Triplicate Weekly Evapotranspiration Data for Experiment Two

Plants	Oil type	Soil Type	Concentration (mg/g)	Day 7 (ml)	Day 12 (ml)	Day 21 (ml)	Day 28 (ml)	Day 35 (ml)	Day 42 (ml)	Day 49 (ml)	Day 56 (ml)	Day 64 (ml)	Day 70 (ml)	Day 77 (ml)	Day 84 (ml)
YES	Light	Non-Oiled	10	271.67	324.00	302.67	292.67	315.33	74.00	38.33	83.33	67.33	220.00	193.41	443.33
YES	Light	Oiled	10	212.00	238.67	231.67	237.00	248.33	178.33	153.33	246.67	272.00	188.33	224.23	290.00
YES	Light	Non-Oiled	50	194.00	245.33	201.33	200.33	215.00	317.33	310.00	443.33	415.00	126.67	284.08	203.33
YES	Light	Oiled	50	139.00	155.67	137.67	147.00	140.00	263.33	260.00	346.67	324.67	102.67	201.18	161.67
YES	Light	Non-Oiled	150	169.00	173.50	207.00	202.50	180.00	222.00	197.50	265.00	279.00	94.00	203.83	220.00
YES	Light	Oiled	150	155.50	199.33	163.00	194.67	187.00	244.00	268.33	325.00	335.33	67.00	212.69	150.00
YES	Medium	Non-Oiled	10	250.33	308.67	260.33	276.00	287.67	70.33	45.00	75.00	74.67	218.33	170.34	391.67
YES	Medium	Oiled	10	201.67	272.33	267.67	258.33	257.67	265.00	243.33	353.33	330.67	154.33	268.71	353.33
YES	Medium	Non-Oiled	50	231.00	230.00	262.33	242.67	241.33	191.67	193.33	203.33	244.00	199.00	215.12	276.67
YES	Medium	Oiled	50	195.67	228.00	212.67	248.33	255.00	344.67	259.00	351.67	343.33	161.33	252.56	253.33
YES	Medium	Non-Oiled	150	177.00	175.00	225.50	155.50	226.50	204.00	192.50	272.50	254.00	150.00	217.36	240.00
YES	Medium	Oiled	150	220.33	162.67	210.00	165.33	210.00	163.33	133.33	253.33	221.00	153.33	192.73	281.67
YES	Heavy	Non-Oiled	10	217.33	233.67	250.00	237.33	253.00	251.67	280.00	405.00	380.00	178.00	285.48	346.67
YES	Heavy	Oiled	10	221.33	315.33	293.00	300.00	277.33	255.67	246.67	253.33	303.00	205.33	258.60	365.00
YES	Heavy	Non-Oiled	50	236.67	297.00	288.33	275.00	254.00	161.67	146.67	161.67	181.67	259.00	218.49	351.67
YES	Heavy	Oiled	50	157.67	225.67	217.33	217.33	188.00	202.67	208.33	301.67	299.67	148.67	214.07	258.33
YES	Heavy	Oiled	150	146.50	198.00	195.00	180.00	164.00	129.00	140.00	255.00	185.00	131.50	179.29	315.00
YES	Heavy	Non-Oiled	150	167.67	185.33	205.00	203.33	211.33	163.67	146.67	178.33	196.33	161.33	181.37	301.67
YES	n/a	Non-Oiled	0	205.00	268.00	257.33	243.67	289.33	61.67	56.67	46.67	67.00	221.33	157.58	405.00
YES	n/a	Oiled	0	210.00	271.67	250.00	278.33	242.33	247.00	193.33	276.67	333.67	195.67	254.35	408.33

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