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Microcosm phytoremediation of crude oil using Spartina alterniflora and simulated via a mathematica model

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MICROCOSM PHYTOREMEDIATION OF CRUDE OIL USING SPARTINA ALTERNIFLORA AND SIMULATED VIA A MATHEMATICA MODEL

by

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CERTIFICATE OF APPROVAL

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MASTER'S THESIS

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This is to certify that the Master's thesis of

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has been approved by the Examining Committee
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To my family for all their endless support and to the love of my life Adrianna

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ABSTRACT

Light, medium and heavy crude oils were studied at three concentrations and with two different sediments in experimental microcosm settings to determine the ability of *Spartina alterniflora* and associated microbes to breakdown total extractable hydrocarbons (TEH) in the water. It was a baseline experiment designed to quantify the rates of biodegradation under relatively quiescent conditions from different crude oils at moderate doses ranging from 0-150 mg/g soil. Upon the completion of the experiment there were several key findings: (1) The lethal dosage for *Spartina alterniflora* was not reached within the 90 day experiment at these dosages, and all plants survived; (2) More than 97% of the total extractable hydrocarbons (TEH) were shown to be degraded by plants and rhizosphere microorganisms within the 90- day experiment; (3) The dose of oil introduced as a slick (simulated spill) on day zero did not significantly affect the results for TEH degradation within the range of dosages from 50-150 mg/g -- these dosages could be degraded by the marsh cord grass system; (4) A sediment type which was acclimated to oil for several months and one which was non-acclimated did not show significantly different results for TEH degradation in the microcosms -- both sediment systems resulted in TEH degradation over the 90-day experiment; and (5) A mathematical model was developed which simulated experiment results quite closely including TEH diffusion from the crude oil slick into the water and subsequent biodegradation.

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M_t = Total Mass of TEH in the system (g)
M_S = Mass of sediment in the system (g)
C_o = Concentration of TEH in the oil (g/L)
C_w = Concentration of TEH in the water at time t (g/L)
C_a = Concentration of TEH in the air at time t (g/L)
C_s = Concentration of TEH in the sediment at time t (g/L)
C_{s1} = Initial concentration of TEH in the sediment at time t = 0days (mg/g)
C_{wend} = integration of Cw with respect to time
C_{send} = integration of Cs with respect to time
V_s = Volume of the sediment (L)
V_o = Volume of the oil (L)
V_{o1} = Initial Volume of the oil (L)
V_w = Volume of the water (L)
V_a = Volume of the air (L)
k_L = Rate constant of TEH dissolving from water to sediment (1/day)
k_o = Rate constant of TEH dissolving from oil to water (1/day)
k_a = Rate constant of TEH dissolving from oil to air (1/day)
k_{bio} = Rate constant for biodegradation of TEH in water (1/day)
k_{bios} = Rate constant for biodegradation of TEH in sediment (1/day)
p = Vapor pressure for naphthalene (atm)
M_s = Mass of TEH in the sediment phase (g)
M_o = Mass of TEH ni the oil phase (g)
M_a = Mass of TEH in the air phase (g)
M_w = Mass of TEH in the water phase (g)
MW_{oil} = Average molecular weight of oil (g/mol)

CHAPTER 1 - INTRODUCTION

Background

Crude oil consumption is continuing to grow world-wide with an increase of 0.7 million barrels per day in 2011 (BP, 2011). Even though the Deepwater Horizon catastrophe occurred in 2010 it did not curtail oil production by BP: "2012 will be a busy year for us in the deep water regions of Angola, Brazil and the Gulf of Mexico" (BP, 2011). While this attitude of not letting a catastrophe overshadow continued exploration and drilling for oil is on one hand commendable, blindly continuing without determining what action can be taken to minimize the impact of another Deepwater Horizon catastrophe would be foolish. To continue in coming centuries as a viable and productive society, we must be stewards of our environment. For a company like BP – one of the largest producers of hydrocarbons in the Gulf of Mexico region (BP, 2011) – it is imperative to ensure that they not only understand the impact of another spill, but also that they have the resources and emergency measures to appropriately handle another disaster if it were to occur.

Is offshore drilling really that bad though? Humberto Fontova would argue that the production of oil is usually relatively clean and that it is its transportation that usually results in problems. Examples of transportation errors include the Exxon Valdez, Amoco Cadiz, and the Argo Merchant (Haerens, 2010). But even if offshore drilling was foolproof, which it is not, the issue of oil spills in the oceans would persist and its effects on our ecosystems would still be a problem with which to contend.

When an oil spill occurs in the ocean, it can affect entire ecosystems and possibly eliminate certain species. One of the most biologically productive habitats in the world is wetlands (Nebel & Kormondy, 1981). Wetlands can be broken into six basic classifications: swamps, marshes, bogs, fens, wet meadows, and shallow water. Swamps are ecosystems where trees become rooted in hydric soil, such as the mangroves in the

floodplains of the Mississippi (Keddy, 2010). Hydric soil refers to soil flooded for long enough periods of time to create anaerobic conditions in the upper portions of the soil (Hydric Soils - Introduction). Marshes, like swamps, have hydric soil but are dominated by herbaceous plants instead of trees, as for example the Louisiana salt marshes. A bog is a moss-based ecosystem that has a pH<5. Fen wetlands contain a predominately sedge- and grass-based ecosystem grounded in peat. The fifth type of wetland, the wet meadow, occurs along rivers in the floodplains and usually has herbaceous plants. The final type of wetland is the shallow water wetland, which refers to regions where plants are covered by at least 25cm of water (Keddy, 2010).

Of all wetlands, ocean oil spills are most likely to affect coastal saltmarshes, which serve as the first coastal buffer before oil moves inland. As a consequence, salt marshes are more likely to be exposed to higher or even lethal concentrations of oil compared to inland plants. For this reason, it is important to understand the impact of varying concentrations of oil on salt marshes. Salt marshes could also be used to 'clean up' oil, as they are known to be extremely effective filters of the water that passes through them. They can reduce the energy of tropical storms; and they can also remove a variety of waterborne toxins. Their ability to clean-up oil raises the question as to whether we need to act at all if an oil spill occurs which could potentially be cleaned-up by the native wetland plants. The plants are capable of cleaning up the contaminants in a process called phytoremediation demonstrated in Figure 1.

Even though we know that the coastal marshes will be the first to be impacted when a spill occurs, we do not know how oil moves throughout the saltmarsh. The research conducted here attempts to determine at what concentration hydrocarbons move throughout different physical phases (oil slick, water, soil, and plants) of a microcosm, intended to represent crudely the saltmarsh ecosystem at microcosm scale. This movement of hydrocarbons was also simulated using a mathematical program called Mathematica.

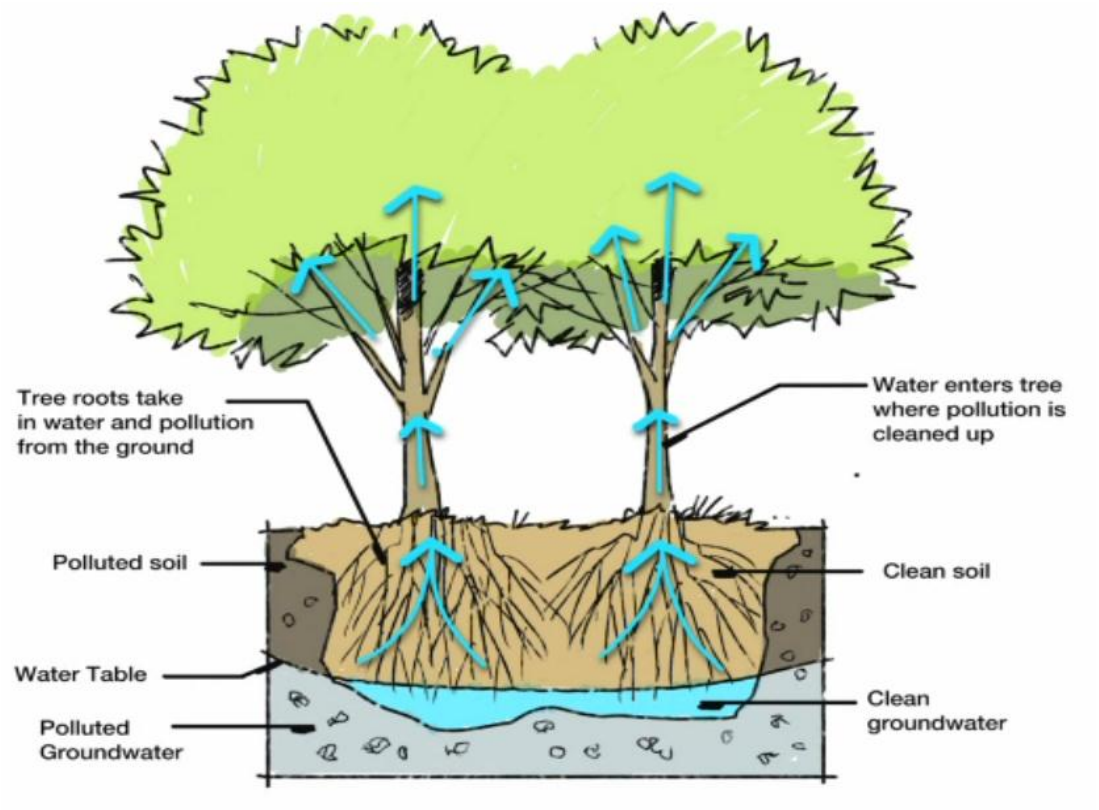


Figure 1-1: How phytoremediation works (Rice, 2012).

After the model was generated and compared to the research results, a decision tree was created that takes into account both the experimental results and the Mathematica model simulations to create a plan-of-action based on important decision-variables of an oil spill.

Objectives

The thesis has three main objectives: (1) To determine the extent of biodegradation and disappearance of Total Extractable Hydrocarbons (TEH) in small microcosms over three months of exposure; (2) To create a mathematical model to determine the movement and concentration of hydrocarbons throughout different phases

in the experimental environment (microcosm/beaker); (3) To create a decision tree for BP to use if an oil spill occurs.

The model created does not consider degradation due to photolysis, physical/chemical weathering processes at sea, or possible increases in volatilization caused by wind and wave mixing. These processes could not be simulated in the quiescent beakers and greenhouse environment available for experimentation. The "microcosms" were covered in cardboard boxes, so photolysis in the oil layer, water and sediment was precluded. The microcosms were open to the atmosphere in a greenhouse so gases could easily exchange, but there were no wind, tides, or wave action. For this reason, it is hypothesized that the rate at which oil degrades in the microcosm will be slower than its rate of degradation in nature and therefore would represent an engineering "safety factor". The Mathematica model was created to follow the movement of hydrocarbon concentrations when a spill occurs if the spill concentration is "mimicked" by one of the experimental concentrations tested.

The decision tree that has been created for BP is meant to be used with caution as there are many environmental and health factors that are hard to take into account based on a simple microcosm study. It is recommended that more experimental and field research be conducted to improve the reliability of the decision tree for actual field cases.

CHAPTER 2 - LITERATURE REVIEW

BP

The world record depth for ocean drilling is 35,055 feet – over a mile deeper than Mount Everest is high – set in 2009 by Deepwater Horizon drilling in the Keathley Canyon in more than 4,000 feet of water (Contreras, 2011). Deepwater Horizon was tapping into 4-6 billion barrels of oil (Contreras, 2011). Six months later, in a fireball that could be seen 35 miles away, Deepwater Horizon exploded in a blowout that killed 11 people. Perhaps as part of that tragedy, BP is now committed to studying how oil spills can be cleaned by plants, a process known as phytoremediation.

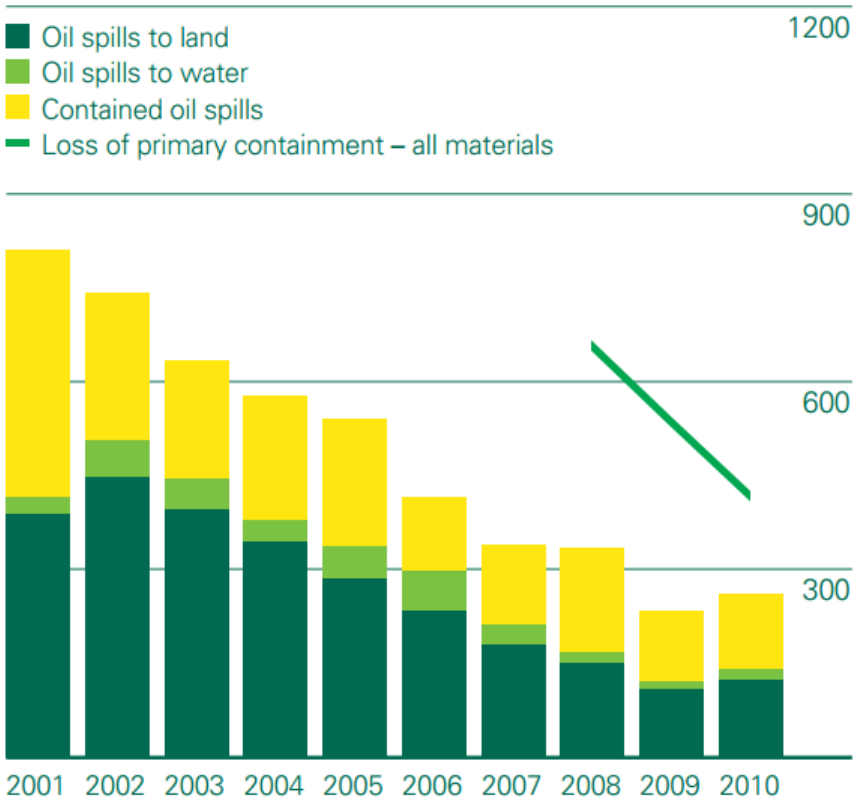


Figure 2-1: Shows the number of BP oil spills between 2001 - 2010 (BP, 2011).

Table 2-1: This table was created and presented to BP in an effort to receive funding for the research conducted in this thesis.

Location	**Oil**	**API**	**Plant**	**Climate**	**Salinity** (ppt)
Angola	Dalia	23.2	*Spartina maritima*	Semiarid	36.4
Australia[a]	Cossack Pioneer	47.7	*Spartina anglica*	Temperate	35
Libya[b]	Azeri	34.8	*Sarcocornia fruticosa*	Mediterranean	38
Gulf of Mexico	Southern Green Canyon	28.2	*Spartina alterniflora*	Temperate/Tropical	30
North Sea	Alba	19.7	*Spartina anglica*	Temperate	34

The table provides the location, type of oil, API of the oil presented the type of salt marsh plants closest to the drilling location, the climate of that region, and the ocean salinity in that region. Only one type of crude oil was selected from each region, due to the overall abundant variety of crude oils. The oil selected was based on the lowest API value.

Locations were taken from BP website (BP Crude Assays, 2013).

**Climate information was taken from* (Climate Zone, 2013).

**All information relating to oil was taken from the BP website.*

[a] *Deepwater exploration in the Ceduna Sub Basin is taking place; however, no oil is currently being extracted from there. Cossack Pioneer is taken from the North West Shelf Venture field in Australia.*

[b] *Deepwater exploration is currently being conducted so crude oil is not from Libya, but still is being extracted from the Mediterranean.*

Crude Oil

Crude oil (petroleum) is a mixture of hundreds of organic compounds and trace amounts of inorganic compounds. Although each organic compound has unique physical and chemical properties, collectively they are often divided into the paraffins, naphthenes and aromatic hydrocarbons (Screening-Level Hazard Characterization, 2011). Amongst the inorganic compounds are trace amounts of metals and sulfides (Chaudhuri, 2011).

Some of the most problematic organic components in crude oil are the polycyclic aromatic hydrocarbons (PAHs). Their bioaccumulation, airborne transportation and persistence in the environment are of concern since PAHs have been identified as carcinogenic, mutagenic and teratogenic (Kalf, Crommentuijn, & van de Plassche, 1997). In addressing PAHs and other components of crude oil, an effective petroleum remediation strategy (such as phytoremediation) must demonstrate treatment of bulk oil, measured as Total Extractable Hydrocarbons (TEH), and treatment of special constituents like PAHs, in the context of restoring or re-establishing the complex marsh grass ecosystem affected by offshore spills.

Different types of oil must also be tested. One metric to classify oil is the American Petroleum Institute (API) gravity, a measure of the density of crude oil relative to water. The API gravity is used for economic purposes and is related to the specific gravity (Head, Martin, & Larter, Biological Activity in the Deep Subsurface and the Origin of Heavy Oil, 2003). The equation used to relate specific gravity to API gravity can be seen in equation 1. In brief, crude oil with an API gravity >10 is lighter than water and floats, while crude oil with an API gravity <10 is heavier than water and sinks. Although API gravity is used to compare relative densities of petroleum, it may affect the rate of degradation. In addition to the API gravity, crude oils can differ in viscosity, color and solubility. (Screening-Level Hazard Characterization, 2011).

Equation 1: Relationship between API gravity and specific gravity (Chaudhuri, 2011)

$$API\ gravity\ = \frac{141.5}{Specific\ gravity} - 131.5$$

Health Risks

There are many health risks associated with crude oil that vary based on the concentration, length and type of exposure. For example, exposure can be through inhalation, ingestion or dermal contact (touching) (Screening-Level Hazard Characterization, 2011). For each route of exposure, the concentration of a given type of crude oil that is harmful will vary; and because crude oils differ in their relative concentrations of harmful compounds, such as benzene or benzo[a]pyrene, some crude oils will be more toxic than others.

The EPA has made some attempts at creating a 'generic concentration' of crude oil that should be avoided since it is impractical to test all oils for health effects. However this process is not straightforward. For example, dermal contact at concentrations above 2500mg/kg-day in male mice result in reduced body weight over a 28-day period but in female mice there is no noticeable adverse effect. It is only when the exposure drops to below 250mg/kg-day in male mice that there is no observable adverse effect. This level is referred to as the no-observable-adverse-effect level (NOAEL), and experiments like this show that the NOAEL is sex dependent, further compounding the difficulty in identifying a toxic 'generic concentration' for oil.

Another issue is the definition of toxicity. For example, a 90-day dermal study showed that dosages as low as 30 mg/kg-day caused hypertrophy and hyperplasia of cells (hypertrophy - an increase in cell size; hyperplasia – an increase in cell number) (Screening-Level Hazard Characterization, 2011) (Muscle Fiber Hypertrophy vs

Hyperplasia, 2009). While not harmful by themselves hyperplasia and hypertrophy can increase cancer risk (Non-Cancerous Breast Conditions, 2012).

Oil Cleanup

A variety of methods can be used to cleanup oil spills including containment, chemical, biological, and physical methods (Oil Spill Response Techniques, 2011). For example, gelling and dispersive agents are two chemical methods of cleanup: the former binds oil into a solid while the latter breaks oil apart (Gelling Agents, 2011). Their use is not equivalent - dispersants will not work as well on heavy crude oil as on light crude oil (Dispersing Agents, 2011).

Biological agents are usually bacteria, fungi or yeast, selected to speed up the rate of degradation by facilitating the breakdown of complex compounds to simpler compounds (Biological Agents, 2011). Physical methods of cleanup involve pressure washing, raking, bulldozing or wiping with sorbent. These techniques are usually faster acting but also more invasive than the chemical and biological techniques (Oil Spill Response Techniques, 2011).

The rate at which oil can be cleaned up is a function of the method used and the environmental conditions present. Our research focuses on phytoremediation – a biological method – and how it is impacted by environmental factors.

Phytoremediation

Phytoremediation is a relatively new field that focuses on the use of plants to address specific environmental problems without the need to excavate the contaminant material under consideration. It has gained in popularity over the past 20 years with the realization that plants have an extraordinary ability to clean up the environment (Pilon-Smits, Phytoremediation, 2004). Phytoremediation as applied to petroleum cleanup in salt marshes is attractive because of its low cost, low environmental impact, and

demonstrated ability to remove pollutants from the aqueous, soil, and biological compartments of ecosystems.

There are six basic ways plants can clean up a contamination: rhizodegradation, phytoextraction, phytovolatilization, phytodegradation, rhizofiltration, and rhizostabilization (Pilon-Smits, Phytoremediation, 2005). The first and most important for the degradation of TEH is rhizodegradation, a process that occurs in the rhizosphere. The rhizosphere is a small area (between 1-2 mm) surrounding the roots of a plant that is abundant in nutrients and respirations. It contains microorganisms, which breakdown contaminants, making the study of the rhizosphere especially important (Rhizosphere) (Rovira, 1969). Available data have shown that the larger the microbial population the faster the rate of degradation, hinting at the importance of the rhizosphere and the habitat it provides for the microbial populations (Gunther, Dornberger, & Fritsche, 1996). In the rhizosphere petroleum compounds can be transformed to simpler, less toxic molecules or mineralized completely to water and carbon dioxide. The rhizosphere also removes harmful petroleum hydrocarbons from soil and water by promoting volatilization through transpiration, and by accumulating organic compounds through lignification (Srujana & Anisa, 2011).

The second process, phytoextraction, refers to the process by which plants take up a contaminant. The ability of a contaminant to enter cells depends in great deal on its hydrophobicity (Pilon-Smits, Phytoremediation, 2005). Naphthalene, for example, has a K_{ow} of 3.29 and does not enter plants because it is too hydrophobic; benzene, in comparison, has a K_{ow} of 2.13 and potentially can be taken up by plants and stored inside cell walls (Naphhalene, 1-Methylnaphthalene, and 2-Methlynaphthalene) (EPA). Translocation of organic pollutants requires that the pollutant pass through the membrane between the root symplast and the xylem apoplast using diffusion (Pilon-Smits, Phytoremediation, 2005). The symplast is a continuous system of protoplasts located on

the inner side of the plasma membrane, while the apoplast is the outer pathway. The apoplast facilitates the transport of solutes and water (Cambell & Reece, 2002).

The third process, phytovolatilization, is when a water-soluble pollutant is released by the plant into the environment through volatilization. Once the pollutant is transported through the shoots and enters the leaves, its chemical characteristics determine whether it can leave the plant by volatilization. This process is variably successful and depends a great deal on the pollution that is being absorbed (Soil and Water Remdiation, 1998).

The fourth method of phytoremediation, phytodegradation, deals with the ability of plant cells to break down chemical compounds that enter the plant tissue. Plant cells are capable of breaking down many different compounds, including petroleum and aromatic compounds (Newman & Reynolds, 2004).

The fifth and sixth methods for phytoremediation, rhizofiltration and phytostabilization, take place in the root system. Rhizofiltration refers to the concentration of pollutants around the root system as they settle out of the dissolved phase, while phytostabilization is exudation of chemicals and enzymes from the plant into the surrounding soil, leading to reaction with and breakdown of the contaminant (Soil and Water Remdiation, 1998). Plants not only cleanup contaminants, they also slow groundwater hydraulics and reduce the spread of pollutants.

Phytoremediation Case Studies

Various plant species have been proven capable of remediating petroleum-contaminated soils in bench-top studies, including *Vicia faba*, Zea mays, *Triticum aestivuml*, and *Glycine max* (Diab, 2008) & (Njoku, Akinola, & Oboh, 2009). In greenhouse and field studies of the Indiana Harbor riparian zone, plantings of sedge (*Carex stricta*), switchgrass (*Panicum virgatum*), and gamagrass (*Tripsacum dactyloides*) were found to significantly reduce TPH in one year of growth (Kaimi, Mukaidani, &

Masahiko, 2007). In the salt marsh environment, phytoremediation of petroleum using the native *Spartina* would be most appropriate given the unique oxygen and salinity demands. Multiple studies have shown that *Spartina alterniflora* is tolerant of high oil concentrations (Bergen, Alderson, Bergfors, Aquila, & Matsil, 2000) & (Lin, Mendelssohn, Suidan, Lee, & Venosa, 2002).

Spartina alterniflora has been shown that in the presence of petroleum hydrocarbons, the gas exchange and photosynthesis rates decrease by 53 to 80% of controls (Pezeshki & DeLaune, 1991). The decreased gas exchange rate and photosynthesis rate usually is linked to a decrease in exudates released in the plant roots, which provide a healthy environment for bacteria.

Each plant affects the rate of degradation differently (Reilley, Banks, & Schwab, 1996). When discussing phytoremediation considerations include the plant's ability to degrade the compound, the lethal dosage, rate of degradation, and type of plant. Table 2-1: This table was created and presented to BP in an effort to receive funding for the research conducted in this thesis. Since BP is operating around the world, any oil spill must be considered in the context of the environmental location in which it occurs.

Influence of Environment on Phytoremediation

Salinity

The salinity of the water in which the oil spill occurs has an effect on the rate of degradation. There is an optimal salinity of approximately 6% or 60ppt, which is higher than any naturally occurring saline water (Coulon, Pelletier, Gourhant, & Delille, 2005). Ocean salinity is often in the range of 30-40ppt, as can be seen in Table 2-1; this salinity range is tolerable by *Spartina alterniflora* (Spartina alterniflora Loisel. Smooth Cordgrass). Figure 2-3 shows that salinity concentrations in the 30-40ppt range are the second most optimal salinity after 60ppt.

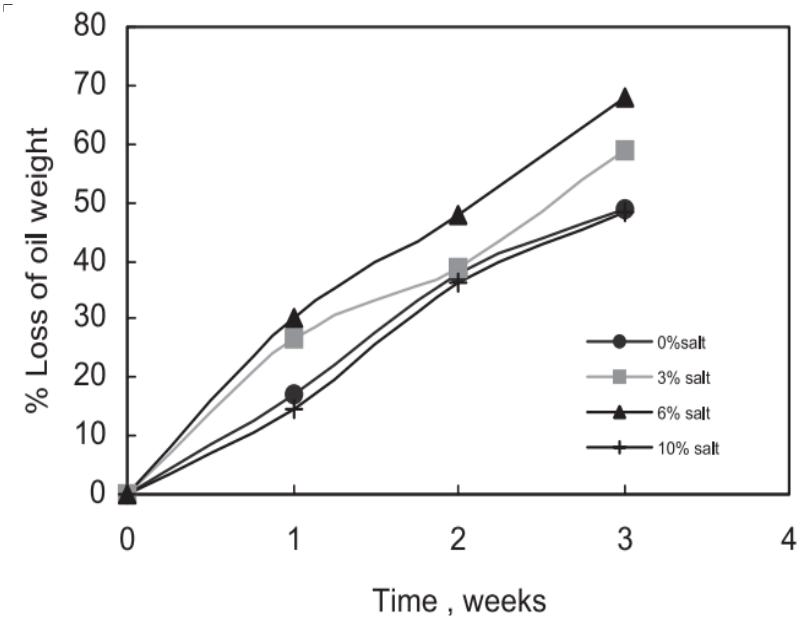


Figure 2-2: Shows the effect of salinity on the degradation of crude oil (Coulon, Pelletier, Gourhant, & Delille, 2005).

Soil

The type of soil that the oil contacts has an effect on how well the oil is degraded. It has been shown that soils higher in organic matter actually slow the rate of oil decay (Dowty, 1998). This counterintuitive finding may reflect the fact that hydrocarbon-consuming bacteria may preferentially consume natural organic matter over oil (Pezeshki, Hester, Lin, & Nyman, 2000). There are also studies to suggest that the type of soil (sand, clay, silt) affects the rate of degradation, soils containing larger pores being capable of breaking down hydrocarbons faster since the oil can percolate through the soil at a faster rate (Apitz & Myetes-Schulte, 1996). It is pertinent that a slower rate of degradation can be detrimental to plants since they are then exposed to oil for a longer time period, which may result in chronic problems.

Nutrients

Adequate nutrients during phytoremediation are very important since plants and microbes are under stress. Lower nutrients have been shown to occur in the presence of hydrocarbons, since petroleum hydrocarbons are low in nitrogen and phosphorous but high in carbons (Xu & Johnson, 1997). Crude oil also physically reduces the space available for water, which contains important nutrients for plants (Schwendinger, 1968).

Temperature and Season

Studies have shown that light contamination of crude oil to plants during anytime of the year does not affect plants in the long term (Baker, 1971). However, in the short term plants are more impacted by oil if it occurs during the growing season (Pezeshki, Hester, Lin, & Nyman, 2000). The effect of temperature variation from $4^{o}C$ - $30^{o}C$ ($39.2^{o}F$ - $86^{o}F$) on bacterial degradation of diesel oil has been studied, with maximum biodegradation occurring between $10^{o}C$ - $20^{o}C$ ($50^{o}F$ - $68^{o}F$) (Margesin & Schinner, 1997).

Saltmarshes

When trying to conduct remediation of an oil spill in a salt marsh, it is difficult to predict the movement and degradation of compounds since there are many complex physical-chemical reactions taking place. Tidal movement, storms, wind, and daily and seasonal cycles are all highly difficult environmental conditions to model in the lab settings and all can have a large impact on the rate of degradation. Figure 2-3 shows some of the conditions that can be present in a salt marsh and not only how these would be difficult to model, but also how they determine the kinds of plants that are present in an ecosystem.

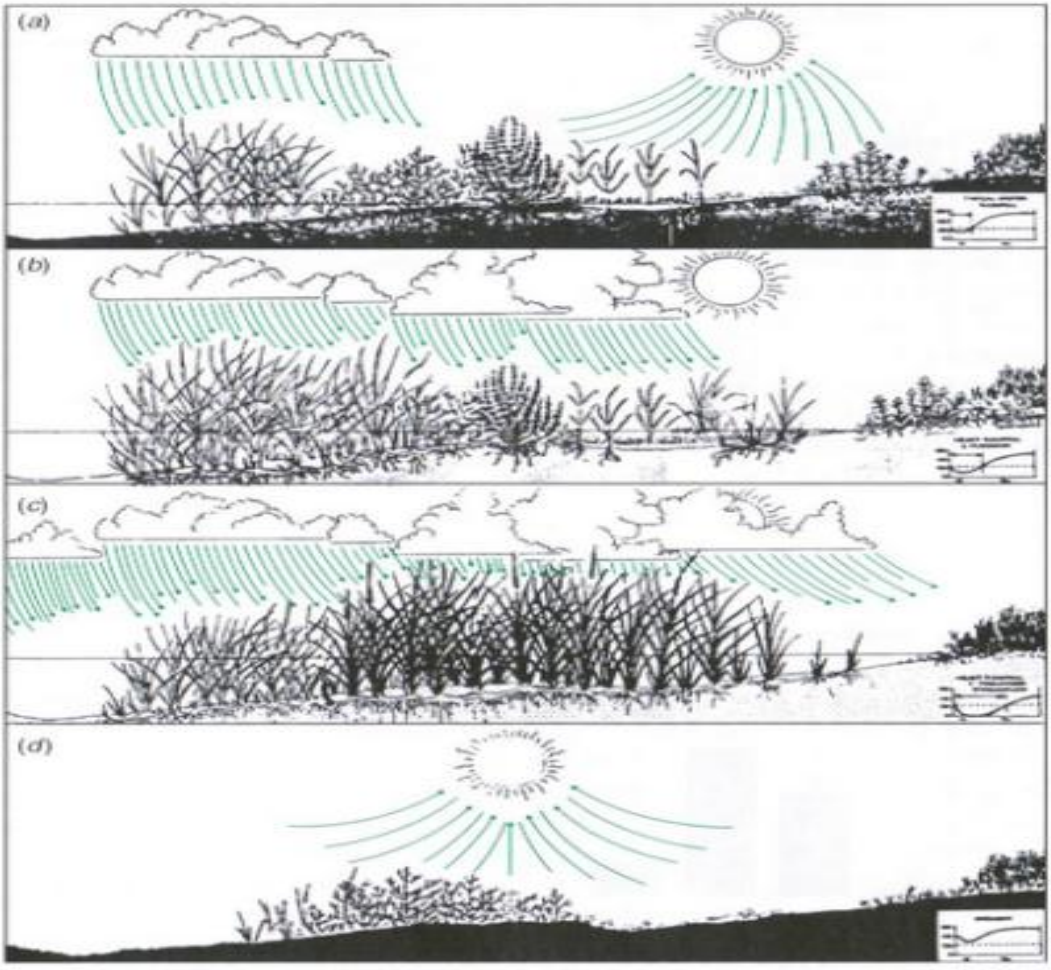


Figure 2-3: Saltmarsh grasses experiencing climatic changes (Keddy, 2010). Not only are there many different environmental conditions to try and model in salt marsh, but the plants can constantly be changing as well.

Grasses

Smooth Cordgrass *(Spartina alterniflora)*

Spartina alterniflora are located in the low marsh regions and can grow up to 250cm tall. The optimal salinity for them is between 10 and 20ppt, but they are capable of surviving up to 60ppt, higher than any major ocean waters (Spartina

alterniflora/hybrids, 2001). Figure 2-4 shows where *Spartina alterniflora* is located around the world. During September and October seedheads that are approximately a foot long emerge at the ends of the stems. A non-threatening rust-like fungi is common to the *Spartina alterniflora*. The optimum water depth for smooth cordgrass is between 1 and 18 inches. Smooth cordgrass can grow in a pH range of 3.7 to 7.9 and aerobic or anaerobic conditions (Smooth Cordgrass).

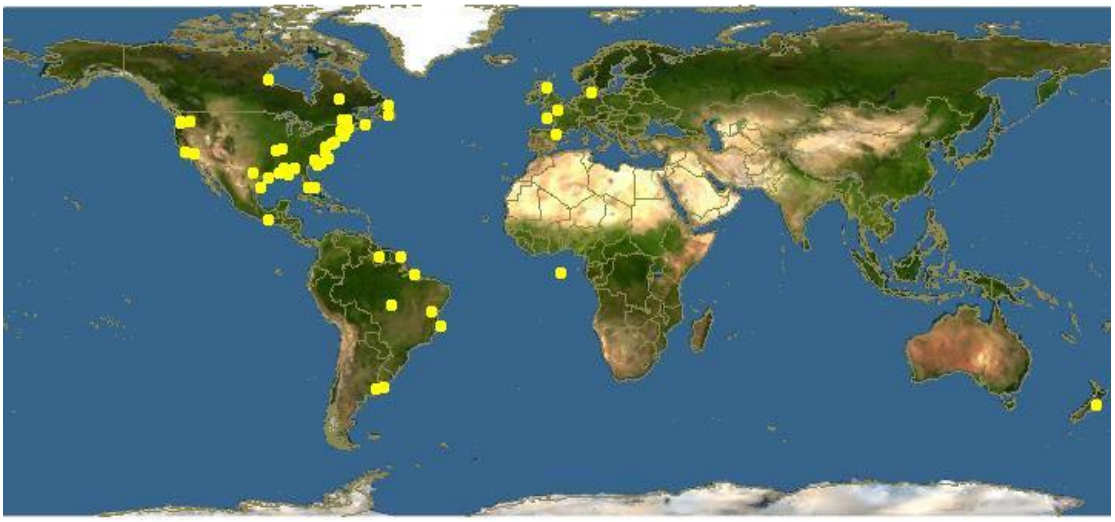


Figure 2-4: Shows the location of *Spartina alterniflora* globally (Sparina alterniflora).

Saltmeadow cordgrass *(Spartina patens)*

These plants are often located in similar areas as *Spartina alterniflora*; however, instead of being located in the lower marsh land they are located in the middle and upper marsh regions. They are capable of growing up to 80 cm tall and usually will not experience initial concentrations of a contamination. When attempting to establish Saltmeadow cordgrass they should contain 3-5 stems per pod when planting (Saltmeadow

Cordgrass). Figure 2-5 shows the locations of *Spartina patens* around the world, as noted earlier they are in similar locations as *Spartina alterniflora*. They are not as invasive as *Spartina alterniflora* and as a result have not spread around the world as extensively as *alterniflora's* have. One thing that can be noted is that the spread of smooth cordgrass was caused by ships coming into port and collecting seeds on the boat and then transporting them unintentionally.

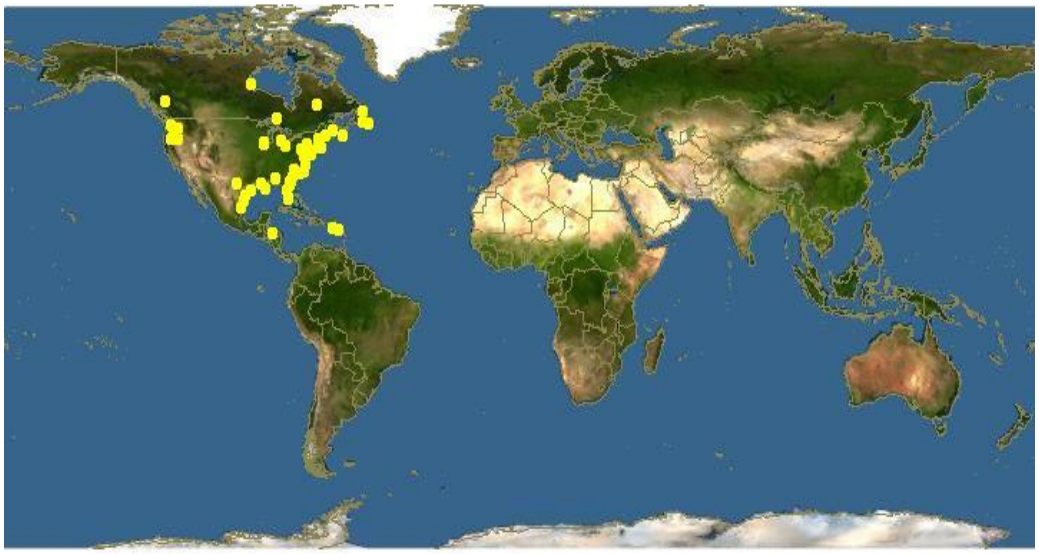


Figure 2-5: Shows a map of *Spartina paten* locations globally (Sparina alterniflora).

Small Cordgrass *(Spartina maritima)*

These plants are native to southern Europe and during the colonial period were transported to western Africa on boats. These plants can grow up to 70 cm tall and are not as widely spread as smooth cordgrass. Figure 2-6 shows the major locations where S*partina maritima* can be found around the world.

Figure 2-6: Shows the locations of *Spartina maritima* globally (Sparina alterniflora).

Scientists have found that due to the expression of different genes in *Spartina alterniflora* as compared to *Spartina maritima*, smooth cordgrass has become more of an invasive species while small cordgrass is on the decline. Thus it is predicted that *Spartina alterniflora* will continue to spread throughout the world while *Spartina maritimia* will lose habitat (Chelaifa, Mahe, & Ainouche, 2010).

Common Cordgrass *(Spartina anglica)*

This plant is a hybrid of *spartina x townsendii*, *Spartina maritima*, and *Spartina alterniflora*. It is now found throughout European estuaries and has taken over salt marshes in Europe and taken over *Spartina maritima*. This species is capable of growing up to 130cm tall and handling salinities up to 40ppt. Figure 2-7 shows the locations of

Spartina anglica around the globe. *Spartina anglica* are located in the intertidal zone similar to *Spartina alterniflora* (Nehring & Henning, 2009).

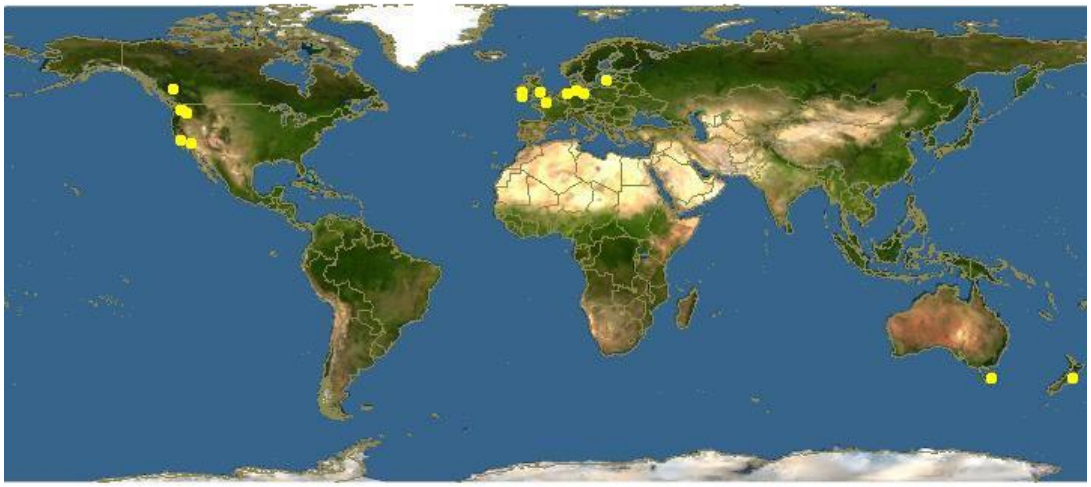


Figure 2-7: Shows the locations of *Spartina anglica* globally (Sparina alterniflora).

Plant Selection

For the phytoremediation experiments presented in this thesis, the species *Spartina alterniflora*, smooth cordgrass, was chosen. Figure 2-8 from Paul Keddy shows where *Spartina alterniflora* are located in the salt marsh land. As can be noted from the picture, *Spartina alterniflora* are a good selection for initial studies on the ability for plants to degrade oils since they will be the first species to be in contact with the oil. Studies have shown that smooth cordgrass are very hardy plants and capable of handling a variety of environments and conditions (Lin, Mendelssohn, Suidan, Lee, & Venosa, 2002). Since the environment in which *Spartina alterniflora* lives is so diverse, the study is only capable of capturing a fraction of the variables present.

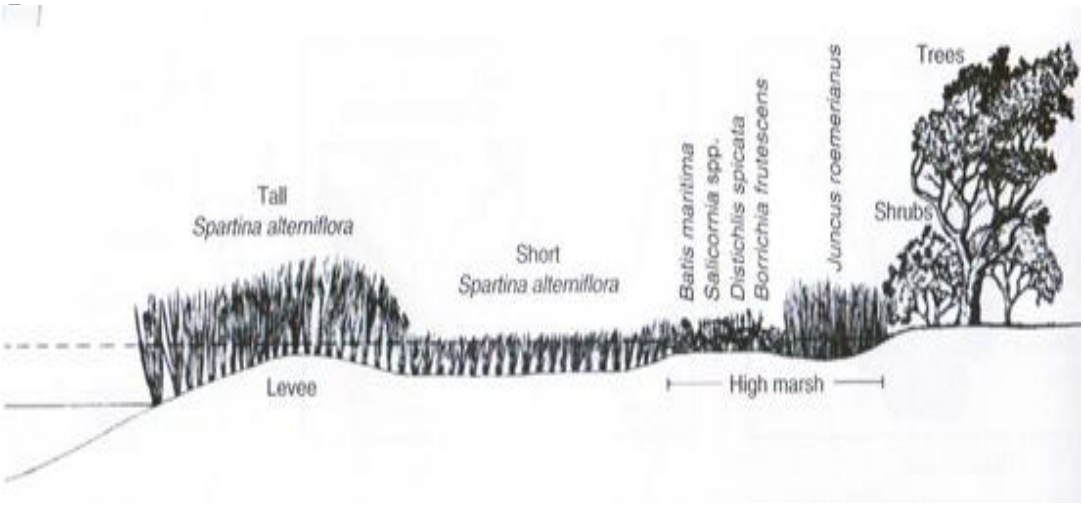


Figure 2-8: Shows the location of *Spartina alterniflora* in saltmarshes. Since *Spartina alterniflora* are hardy plants they are always located in the intertidal zone no matter the temperature. However, warmer temperatures will cause competition by other species with *Spartina alterniflora* for supra tidal space.

Modeling

While numerous models have been created to deal with the movement of oil between different phases without plants, the ability of plants to clean up soil, or phytoremediation of individual oils, no models have been created to show phytoremediation of three different types of oil in the same environment. A model created by Thomas et al. demonstrated phytoremediation for a single type of oil, in the soil, with varying root lengths. Their model focused on how oil is degraded in the soil due to the contact with the rhizosphere (Thomas, Lam, & Wolf, 2003). Nagheeby et al. created an impressive model that demonstrated two-phase transport of an oil slick in estuarine water, but they did not consider phytoremediation (Nagheeby & Kolahdoozan, 2010).

Most of the models that try to predict the movement of oil account for many more parameters than we have set forth in this paper. The model we present is designed to predict the most basic movement of hydrocarbons through four different phases. Some of the variables that have been ignored but are common in estuaries or during oil spills include tidal movement, photolysis, wind, dispersants and turbulent flow. These variables tend to be very important in the degradation or dispersion of oil so that the concentration of oil affecting a plant would not be as intense. As a result of excluding these variables from the model works in a way as a safety factor.

CHAPTER 3 - EXPERIMENTAL METHODS

Analytical Method

Multiple methods will be presented for the different aspects of the research that was undertaken. The "Plant Sampling" portion of the methodology will discuss acquiring the appropriate materials, setting up, and running the experiment. The Section "Soil Sampling" will discuss and explain how the TEH in the soils were found. The third Section "Hydrocarbon Extraction" discusses the method that was used to extract the hydrocarbons from the water samples. The fourth section "GC-FID" discusses the method used by the University (State) Hygienics Laboratory for Gas Chromatography Flame Ionization Detector (GC-FID). The Mathematica model description, parameters, and assumptions are described in the "Modeling" section.

The following flow chart shows the steps taken in the experiment. Detailed explanations of the flow chart can be found in later parts of the methodology.

1) Half of Organic Potting soil mixed with 5mg oil/g of soil

↓

2) Immediately take soil samples of oiled and non-oiled soil

↓

3) Send samples to the State Hygienic Laboratory at the University of Iowa

↓

4) After four months take soil samples of oiled and non-oiled soil, send samples to State Hygienic Laboratory at the University of Iowa and begin experiment

↓

5) Combine water, soils, oils, and plants in beakers

↓

6) Take water sample from beakers

↓

7) Perform hydrocarbon extraction on water samples

↓

8) Send extraction samples to Hygienics Laboratory for GC-FID analysis

↓

9) Repeat steps 6-8 at t = 30, 60, and 90 days

↓

10) At t = 90 days take soil samples from beakers with:

- No oil slick, no plants, and non-oiled and oiled soils
- No oil slick, plants, and non-oiled and oiled soils
- Oil slick with medium oil at a concentration of 50mg oil/g soil, plants, and non-oiled and oiled soil

Plant Sampling

One of the most difficult and important parts of the experiment was acquiring the appropriate crude oil and therefore this step was started immediately following the grant funding from BP. Once the oil was obtained the soil was purchased and the appropriate amount of oil, 5mg oil/g soil, was mixed with half of the soil. Both non-oiled and oiled soils were left for four months in similar conditions so that oiled soil could acclimate to the new conditions.

During the period when the soil was acclimating hundreds of *Spartina alterniflora* were ordered from various plant nurseries and a final batch of plants was selected based on the strength and uniformity between the plants. The plants that were selected were freshwater, which is one of the reasons that salinity was not part of the experiment. It

was assumed that introducing the plants to a saline environment could shock the plants and cause mortality that was not related to the oil concentration.

One hundred one liter beakers were purchased and then modified to have a glass side port on the side of the beaker at the 750mL mark. The side port contained a plastic stopper that could be penetrated with a syringe, but did not allow water to flow out. The port allowed sampling to occur without having to penetrate the oil layer and risk potentially skewing the data. One liter beakers were also selected due to their size which allowed noticeable root growth and due to the large opening which allowed plants to be removed at the end of the experiment so soil sampling could occur. Figure 2-9 through Figure 2-16 show a variety of side views for the different kinds of samples that were present throughout the experiment.

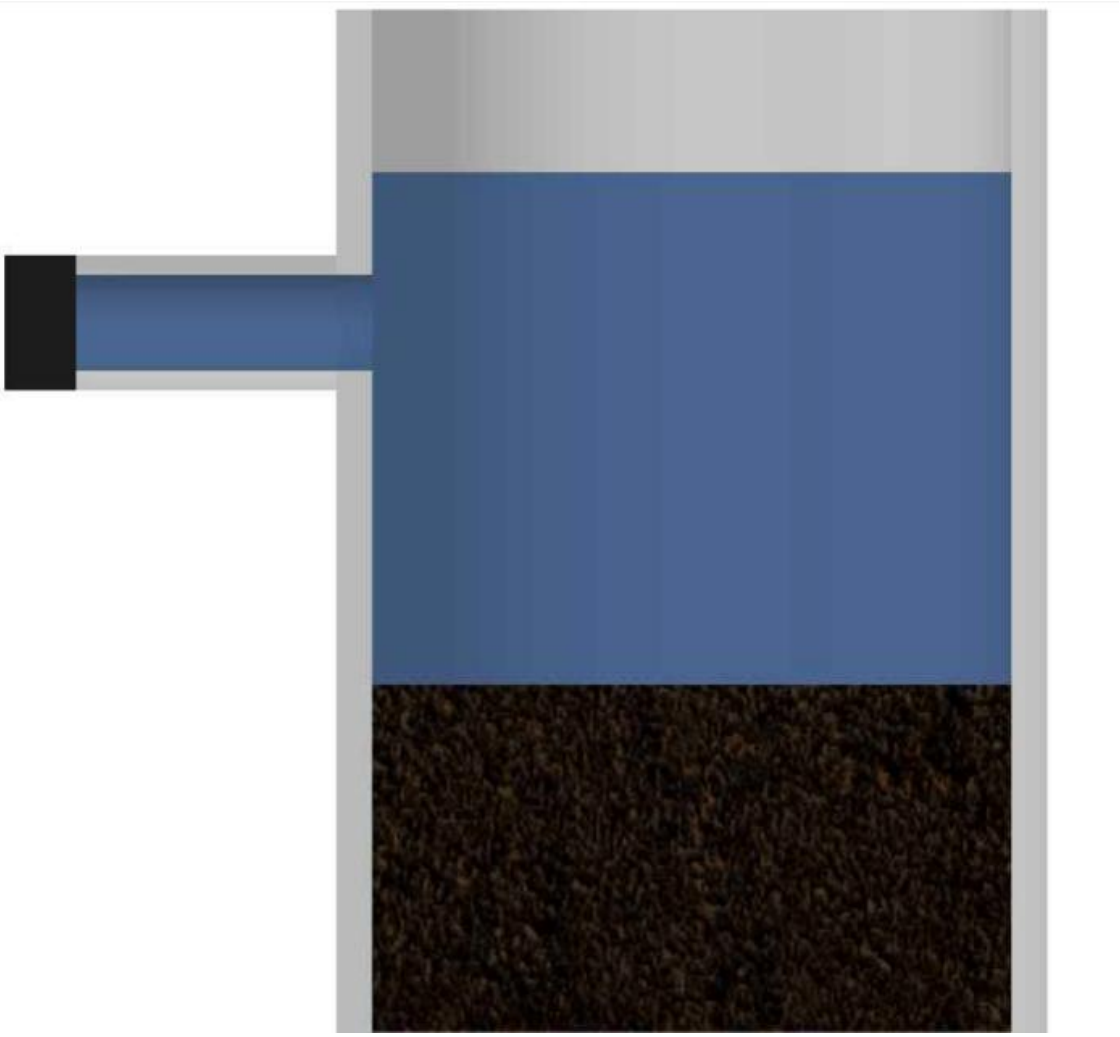


Figure 2-9: A control that does not contain an oil slick or any oil in the sediment. The container that is shown is a 1 liter beaker with a side port located about ¾ of the way up the beaker. The side port was created to allow for water sampling with the use of a sampling syringe. The bottom, brown phase is the uncontaminated sediment and the clear blue phase is the water. In future graphs soil that was not mixed with oil will be abbreviated N-S.

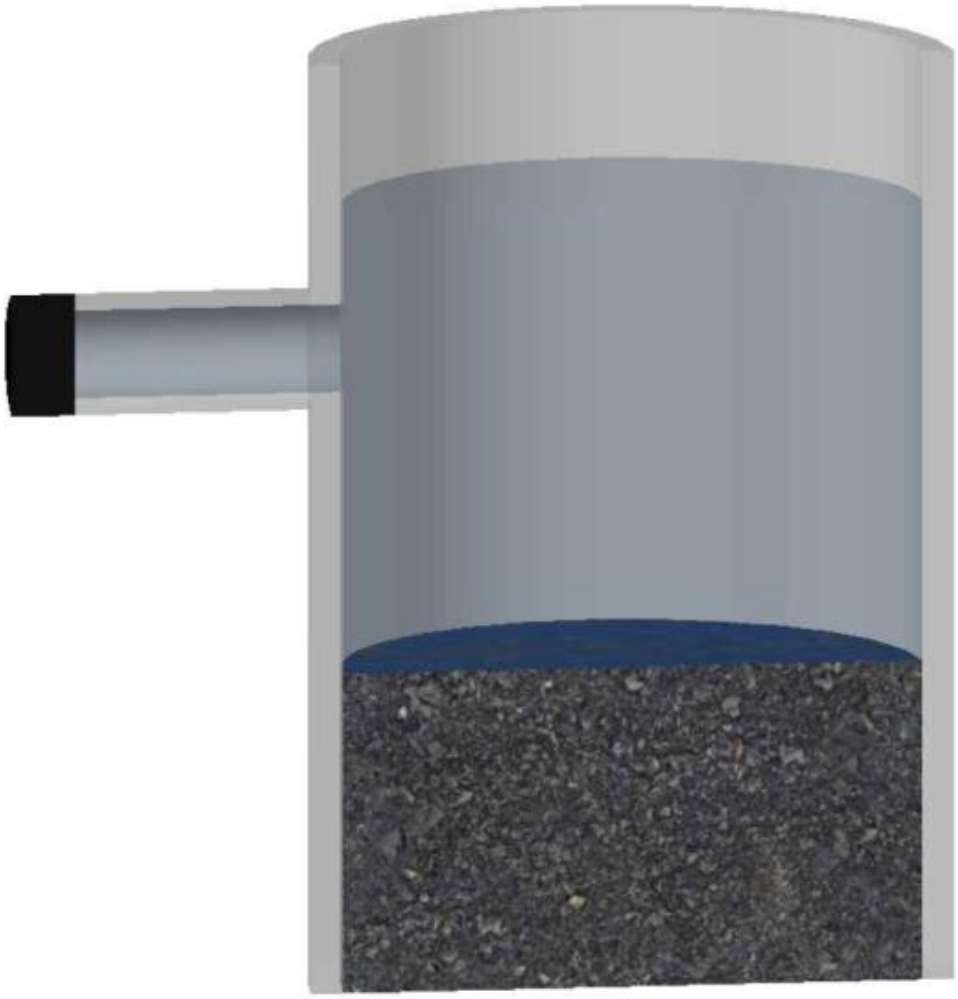


Figure 2-10: Treatment without plants and which does not contain an oil slick, but does contain oil in the sediment. The bottom, gray phase is a mix of 5 mg of Louisiana Sweet Crude oil per gram of organic potting soil the clear blue phase is water.

Figure 2-11: Treatment which contains an oil slick, but does not contain any oil in the sediment (organic potting soil). No plants are present. The bottom, brown phase is the sediment, the middle, clear blue phase is the water and the top, clear yellow phase is the oil slick.

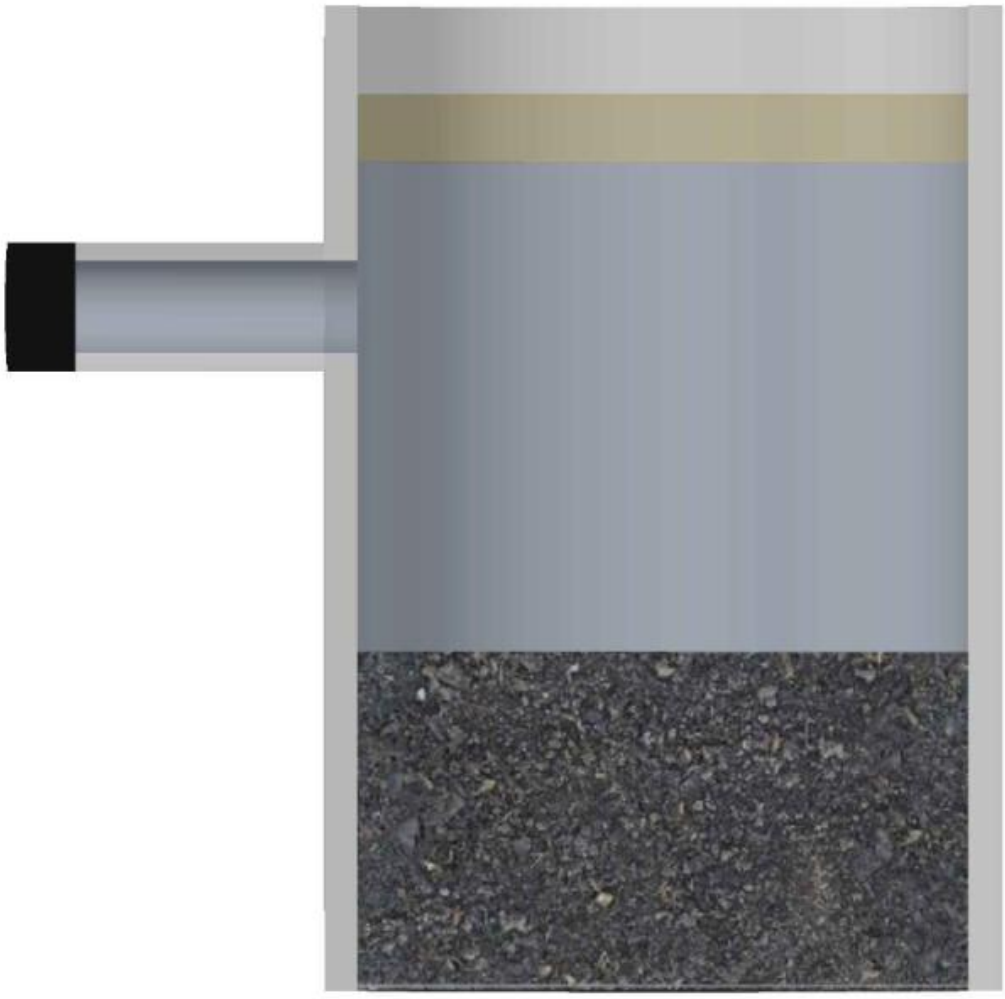


Figure 2-12: Treatment with soil that has been mixed with 5 mg of Lousiana Sweet Crude oil per gram of organic potting soil. The bottom, gray phase is the sediment, the middle, clear blue phase is the water and the top, clear yellow phase is the oil.

Figure 2-13: Control with plants, non-contaminated soil, and no oil slick. The bottom, brown phase is normal sediment, the clear blue phase is the water and the green is *Sparitina alterniflora*.

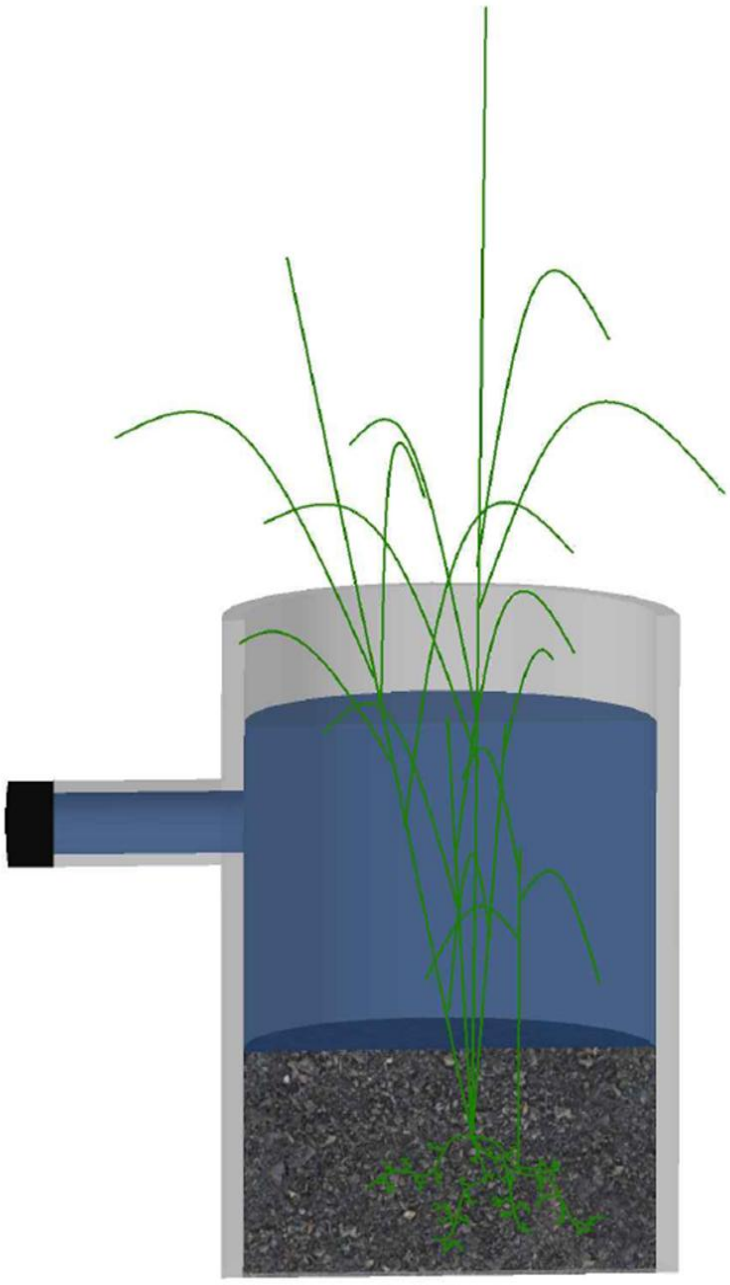


Figure 2-14: Treatment with plants, oiled soil, and no oil slick. The bottom, gray phase is contaminated sediment, the clear blue phase is the water and the green is *Sparitina alterniflora*..

Figure 2-15: Treatment with plants, oil slick, and clean sediment. The oil slick with the plants varied in concentration from 10mg oil/g soil to 150mg oil/g soil. In this figure the sediment at the bottom of the beaker has not been contaminated.

Figure 2-16: Treatment with plants, oil slick, oiled soil. The oil slick with the plants varied in concentration from 10mg oil/g soil to 150mg oil/g soil. In this figure the sample has contaminated sediment.

Following the four months of soil acclimation the experiment was started. Plants were placed in beakers with water, oil, and soil. Once the soil has been placed in the beaker and submerged with water it will be referred to as sediment. The beakers were then placed in cardboard boxes that were covered. Covering the boxes prevented photolysis from occurring and prevented algae from growing. The greenhouse was kept at 68^{o}F. The boxes had slits in the top for shoots to penetrate, but the opening was kept small again to reduce sunlight penetration and to reduce volatilization. Figure 2-17 provides a schematic of how the beakers were setup in the greenhouse and covered by the cardboard boxes.

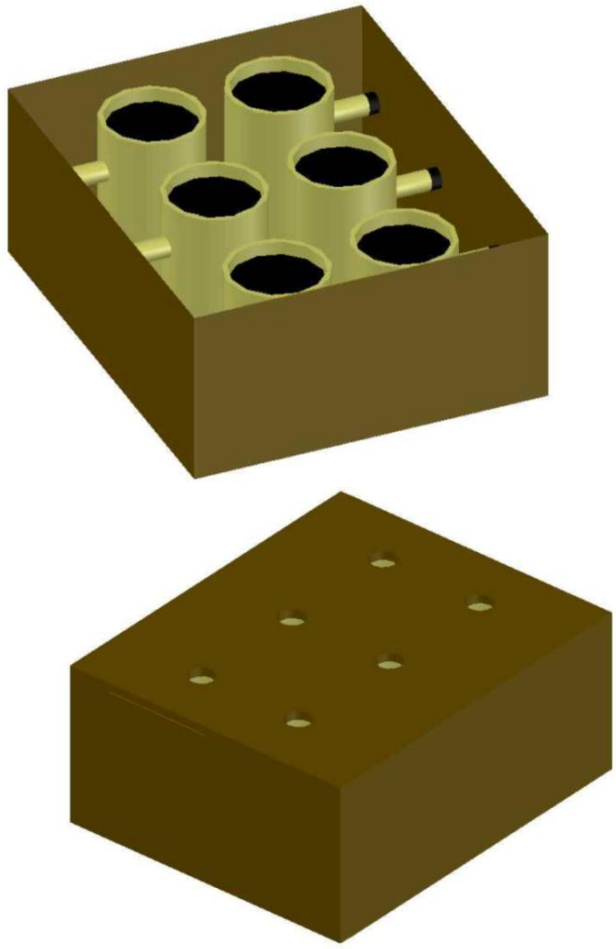


Figure 2-17: The top image demonstrates how the six beakers are situated in the cardboard box if the top were removed. The bottom picture actually shows the system with the lid closed and holes created to allow for the plants to grow.

Soil was filled to the 400ml line allowing for approximately 10 cm of water above the soil; this was chosen as readings suggested that optimal water depths for cord grass are approximately 2.5cm to 45cm (Materne, 2009). Since the soils found in salt marshes are not load bearing the soil was not compacted when the plants were added to the beakers. The normal soil found in salt marshes has a very low organic content, anaerobic and usually grows outside the tidal zone (Materne, 2009). We were unable to mimic these criteria in the experiment and instead purchased potting soil that that we could verify uniformity. Some of the soil was then contaminated with oil while some of the soil was left alone.

Soil was initially weighed and then added to the beakers followed by the addition of the cordgrass. Three shoots or one plug was added to each beaker. The amount of plants to add was decided based on a study that tested the spacing of plants and found that plugs are best planted with a spacing of 2'-3' (Materne, 2009). Once the cordgrass was established in the beaker water and oil was then added to the beaker. Plants were watered with ¼ *Hoagland* solution best mimicking the nitrogen found in a saltmarsh (Childers, McKellar, Dame, Sklar, & Blood, 1993). Table 2-2 shows the makeup of quarter strength Hoagland solution.

Table 2-2: Make-up of quarter-strength Hoagland nutrient solution for microcosms

Stock Solution	**Vol. Stock Solution per 12 L batch**
1M $Ca(NO_3)*4H_2O$	12 mL
2M KNO_3	9 mL
2M $NH_4H_2PO_4$	6 mL
MICRONUTRIENTS	6 mL
20mM Fe-EDTA	6 mL
1M $MgSO_4*7H_2O$	3 mL
1M NaOH	Add until pH = 6.8

The plants were weighed, watered, and photographed weekly. This allowed for calculations to be done determining the rate of biomass growth and the rate of volatilization. Every week the plants were given water so that the water level was kept at the 1000mL. The plants were placed in a greenhouse where the temperature was kept at 68^oF and no additional lighting was provided. The plants were initially placed in the North West facing greenhouse; however, due to infection of spider mites the plants were moved to the South West facing green house at 60 days.

Water samples were taken through the side ports at time 0, 30, 60, and 90 days. During water extractions, two different sampling syringes were used. Between samples the sampling syringes were wiped clean and rinsed with pure Hoagland solution. The sample volume was 5mL even though only 1 mL was used for hydrocarbon extraction. The samples were placed in 5ml vials and then stored in a fridge until extracted.

Soil Sampling

Soil sampling was conducted by initially taking two bags of all-purpose organic potting soil and adding a concentration of 5mg Louisiana Sweet Crude oil /g soil in one of the containers and then mixing thoroughly. Samples of the soil were then taken to the State Hygienic Laboratory at the University of Iowa and tested for hydrocarbon concentration. The soils then acclimated for four months before being retested for hydrocarbons and the experiment beginning.

After the conclusion of the 90 day experiment the plants were removed, any oil in the beaker was skimmed from the top, the water was poured out, and the soil was then collected and tested by the State Hygienic Laboratory at the University of Iowa for hydrocarbon concentration. This part of the experiment was run in hopes that it would be another means of relating the experiment to the Mathematica model. The soil samples were collected in glass jars provided by the hygienics lab, kept in a fridge, and then delivered as soon as possible.

The extraction process that was run by the hygienics lab on the soil samples can be seen in greater detail in Appendix A. The main steps involve the initial addition of sodium sulfate to the soil mixture. Following the addition of sodium sulfate methylene chloride, CH_2Cl_2, and ortho-terphenyl, $C_{18}H_{14}$, was added to the sample, mixed, and then sonicated. The solution was then decanted and the above steps were repeated two more times. Once enough liquid was collected it was run through a GC-FID and the results are recorded (Method for Determination of Extractable Petroleum Hydrocarbons by GC/FID).

Hydrocarbon Extraction

The method for hydrocarbon extraction was taken from the University (State) Hygienic Laboratory which was based off of EPA 3510: Extraction of Total Extractable Hydrocarbons in Water. Modifications had to take place due to the size of the sample

that was taken from the plants. As well, the cleaning method was modified due to the sampling size and the limited time allowed between extractions. Once the water was sampled from the plants all of the samples had to be extracted within one week according to the procedure, to reduce and degradation of hydrocarbons.

The cleaning method that took place for the equipment used during the extraction was the use of scrubbing the equipment with detergent and hot water. Then filling the equipment with approximately 50mL of dichloromethane (DCM) and shaking vigorously for approximately 1 minute and venting to prevent any pressure buildup. Finally the instrument was then rinsed with approximately 100mL of nanopure water and let to dry.

The necessary equipment and supplies that were needed to conduct the extraction were: 2L Separatory funnels with appropriate polytetrafluoroethylene washers, nuts, and rubber O-rings, 250mL pear shaped flasks, hot water bath, glass funnel, DCM-rinsed glass wool, disposable glass pipets, and 2mL screw top vials. The appropriate reagents were: sodium sulfate, DCM, oil spike, and nanopure water.

Once all of the equipment that was used in the experiment was cleaned with DCM the extractions could begin. If samples were not being extracted they were stored in a refrigerator at less than 4^{o}C and stored up to 1 week. Each set of 18 samples contained one blank and one spike. Three spikes were prepared by the University (State) Hygienics Laborator one for each type of oil present in the experiment (light, medium, and heavy crude oil).

The extractions began by placing 1mL of extracted water into 1000mL of nanopure water for all samples excluding the blank which does not receive the 1ml of sample. The mixture was then poured into a clean 2L separatory funnel. Sixty mL of DCM was then added to the separatory funnel, capped and shaken for 2 minutes. During the shaking process the funnel was vented often due to pressure buildup. After the two minutes of shaking the funnel sat for 10 minutes to allow DCM to separate from the water. DCM will naturally settle to the bottom of the funnel due to its density and water

solubility characteristics (Public Health Goals for Chemicals in Drinking Water Dichloromethane (Methylene Chloride, DCM), 2000).

After the 10 minutes the DCM was allowed to flow into the glass funnels that contained glass wool with sodium sulfate placed on top of the glass wool. The sodium sulfate was placed on the glass wool to absorb any water that may accidentally leak from the funnel. The glass funnels were placed inside the opening of the 250mL pear shaped flasks and allowed to drain into them. Only the DCM was allowed to drain into the flasks. The process of adding 60mL of DCM, shaking/venting for 2 minutes, sitting for 10 minutes, and then draining into the flasks was repeated two more times.

Once the third DCM extraction had occurred an additional 30mL of DCM was added to the sodium sulfate funnels and then the 250mL flask were taken to the hot water bath. The bath was set at 45^{O}C since the boil temp of DCM is approximately 40^{O}C and the GC-FID starting temperature will be 50^{O}C (Public Health Goals for Chemicals in Drinking Water Dichloromethane (Methylene Chloride, DCM), 2000). The liquid in the flask was then concentrated to 0.5mL. The sample extract was added to a 2mL screw top vial and an additional 0.5 mL of DCM was added to the flask, shaken, extracted, and placed in the screw top vial for a final volume of 1mL. The screw top vials were then labeled and stored in the fridge until all samples were finished extracting. Once all the extractions were done the samples were taken to the University (State) Hygieinics Laboratory for the samples to be run on the GC-FID and analyzed.

Gas Chromatograph – Flame Ionization Detector (GC-FID)

Once the sample extracts were created they were sent to the State Hygienic Laboratory at the University of Iowa to be run on a GC-FID and then analyzed. The State Hygienic Laboratory at the University of Iowa used EPA method 8015B to identify they hydrocarbon concentration in the sample. The following parameters shown in Table 2-3 were used to detect total extractable hydrocarbons.

Table 2-3: Provides the GC-FID parameters used by the University (State) Hygienic Laboratory.

Column 1	
Carrier gas (Helium) flow rate:	5 mL/min
Oven temperature program:	
Initial temperature:	50^{O}C, hold for 5minutes
Program:	50^{O}C to 300^{O}C at 10^{O}C/min
Final temperature:	300^{O}C, hold for 31 minutes
Injector temperature:	300^{O}C
Detector temperature	300^{O}C
Make-up gas:	25ml/min

Once the sample has been run the results are plotted out and the area under the curve was calculated. The area under the curve was then used to calculate the concentration of total extractable hydrocarbons in the sample.

Mathematica Model

The model created does not take into account the degradation rate of photolysis, oxidation, reduction, or possible increases in volatilization caused by any wind. While the model does not include some common advective and dispersive forces that are present in nature not including these forces works as a good "safety factor". Without these forces included in the model the result from the model are more conservative then

would be expected in nature which is good when dealing with a situation as devastating as an oil spill.

For this model many assumptions had to be made since the study that the model was based off of didn't constantly measure all of the phases that the model is predicting. The environment that was modeled in this experiment was a 1 liter beaker with 300 g of soil, *Spartina alterniflora*, 600ml of water, and varying oil concentrations.

Table 2-4: Rate consants used in the Mathematica model.

Rate Constants	Rate (1/days)	Source
K_L	0.05	(Gamst, Olesen, Jonge, Moldrup, & Rolston, 2001)
K_H	$4.83*10^{-4}$	(EPA)
K_O	0.05	(GSI Chemical Properties Database, 2010)
K_{bio}	2	Calibrated for model
K_{bios}	$1.7*10^{-6}$	(Al-Bashir, Cseh, Leduc, & Samson, 1990)

Equation 1 was used to model the movement of Total Extractable Hydrocarbons from the oil to the air phase. The assumption was made that the chemicals volatilize in the air relatively fast, with respect to the entire study and therefore reached equilibrium almost instantaneously. In equation 1 *p* is the vapor pressure of Naphthalene (Naphthalene, 2007), MWoil is the average molecular weight of medium oil (Boduszynski & Rechnsteiner, 1998), Ca is the concentration of TEH in the air, and kH is

the Henry's constant for Naphthalene (On-line Tools for Site Assessment Calculation, 2012).

$$Equation\ 1: \qquad Ca = \frac{MWoil * p}{kH}$$

Equation 2 was used to calculate the volume loss of oil that occurred as it dissolved in the water phase. From equation 1 it was found that the amount of oil that was lost due to evaporation was not important to consider in the remaining equations. That is why equation 4 does not contain the volume loss due to volatilization. In equation 2 the rate constant for the transfer of TEH from the oil phase to the water is ko (Naphthalene, 2010), t is representative of time, V_{O1} is the initial volume of oil, and V_O is the final volume of oil. It was assumed that the concentration of TEH in the oil would remain constant for the purposes of this model. In reality it would be expected that the light hydrocarbons would dissolve faster and therefore change the concentration of the TEH in the oil.

$$Equation\ 2: \qquad V_o = V_{o1} * e^{-k_o t}$$

Once the first three equations were solved equations 3 and 4 had to be solved simultaneously. Equation 3 is used to solve for the concentration of TEH in the water phase (Cw). Equation 4 is used to solve for the concentration of TEH in the soil, (Cs). In the equation 3 k_{bio} is the rate constant for biodegradation of the hydrocarbons, (GSI Chemical Properties Database, 2010). The rate transfer of TEH between the water and soil phase is given as kL (Gamst, Olesen, Jonge, Moldrup, & Rolston, 2001). K_{bios} is the rate constant for the biodegradation of TEH in the soil from microorganism (Al-Bashir, Cseh, Leduc, & Samson, 1990).

$$Equation\ 3: \quad V_w \frac{dCw}{dt} = C_o K_o V_o - C_w V_w K_{bio} - C_w V_w K_L$$

$$Equation\ 4: \quad V_s \frac{dCs}{dt} = C_w V_w K_L - C_s V_s K_{bios}$$

The mass in each phase was then checked by multiplying the concentration in that phase by the volume in that phase. Finally the total mass was summed and compared to the initial weight in the system. Equation 5 shows the mass balance of TEH in the system and was used to help check and plot values for concentrations.

$$Equation\ 5: \quad Mt = Ma + Mo + Ms + Mw$$

Once the program had been created and was running the model showed results that match the experimental data showing no statistically significant difference at a probability less than 0.05. The model being explored was for a medium crude oil with 50mg oil/gram of soil and for a control with no oil slick, but oiled soil.

CHAPTER 4 - RESULTS AND DISCUSSION

Experiment

The results from the experiment showed that with the help of the plants and associated microbes total extractable hydrocarbons (TEH) in the water rose to concentrations in the aqueous phase of approximately 500 mg/L and then declined to less than 100 mg/L within the 90 day experimental period. The results from the GC-FID provided by the State Hygienic Laboratory at the University of Iowa had a recovery rate greater than 89%. The experimental data suggest that the type of oil does not noticeably affect the TEH concentration in the water. Not only does the type of crude oil not matter, but based on the experimental setup the concentration of oil used in the experiment also does not matter, in part, because the spread of the oil was limited by the size of the beaker. The amount of surface area for the water and oil to interact was the same for all oil concentrations, and as a result, the TEH in the oil entered the water at approximately the same rate for all concentrations.

Figures 4-1 to 4-6 show the change in total extractable hydrocarbons present in the water for the different types and concentrations of oil. Figure 4-7 shows the TEH in the water for the samples. Figure 4-8 and figure 4-9 show the TEH concentrations in the soil over time. Throughout Figure 4-1 through Figure 4-9 the symbol O-S is used to represent soil that was oiled four months prior to the experiment beginning. Non-oiled soil is represented by N-S meaning that the soil was never oiled throughout the entire experiment.

The first data set presented is Figure 4-1 shows the change in total extractable hydrocarbons for all samples that had an oil slick concentration of 10mg of oil per gram of soil. This equates to approximately 4 grams of crude oil that was applied to each microcosm. The figure shows the three different types of crude oil (light, medium, and heavy) and their respective soil type. In Figure 4-1 and all preceding figures the

abbreviation N-S is used for samples that had “non-oiled soil” and O-S is used for the samples that had “oiled soil”.

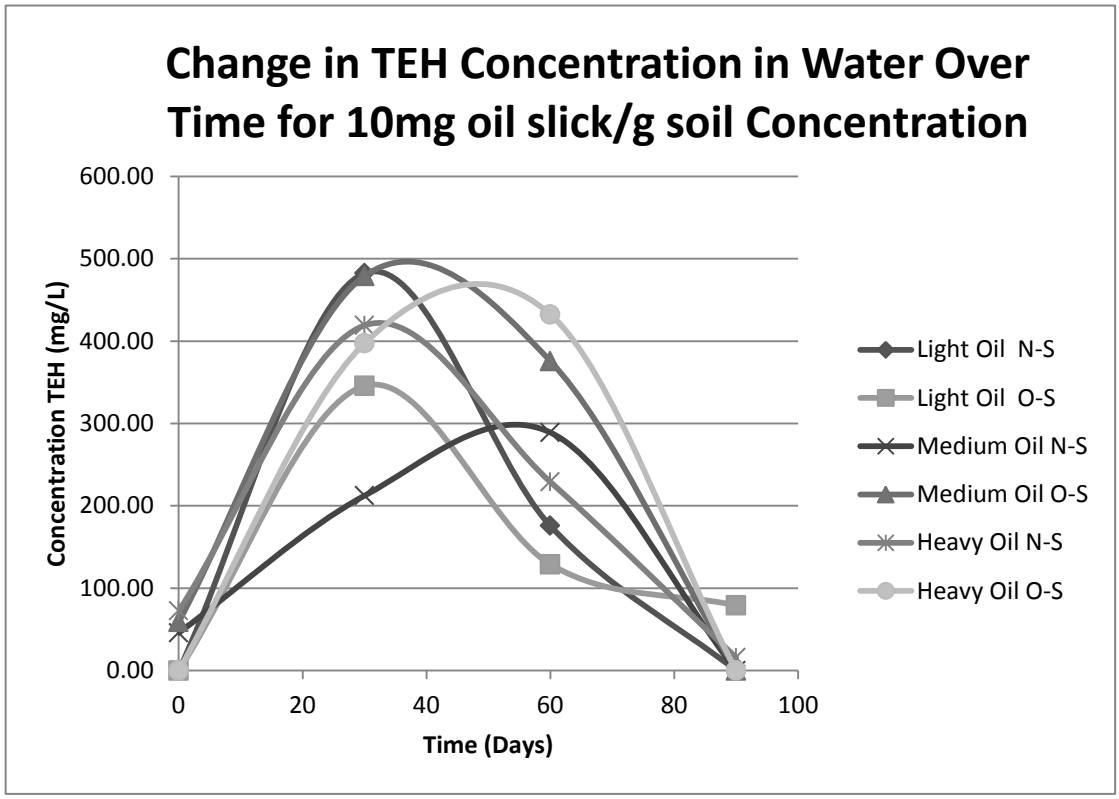


Figure 4-1: Compares the change in total extractable hydrocarbon concentration over time with respect to light, medium, and heavy crude oils at a concentration of 10mg of oil slick/g of soil. The figure also compares the non-oiled soil (N-S) and the oiled soil (O-S). The plot shows that the type of oil and the type of soil does not affect much the shape of the response curve or the rate at which the TEH in the water is degraded. The plot also shows that by 90 days relatively all TEH has disappeared from the water phase.

Figure 4-2 is a similar representation as figure 4-1 except that it shows the results for an oil slick concentration of 50 mg oil/g soil. This results in approximately 20 grams of crude oil that was applied to each microcosm. One of the data sets is thought to be an outlier due to its abnormally large TEH concentration for the extractions that occurred at time equal to 30 days. The outlier data is shown with a dotted line to help identify it from the other samples.

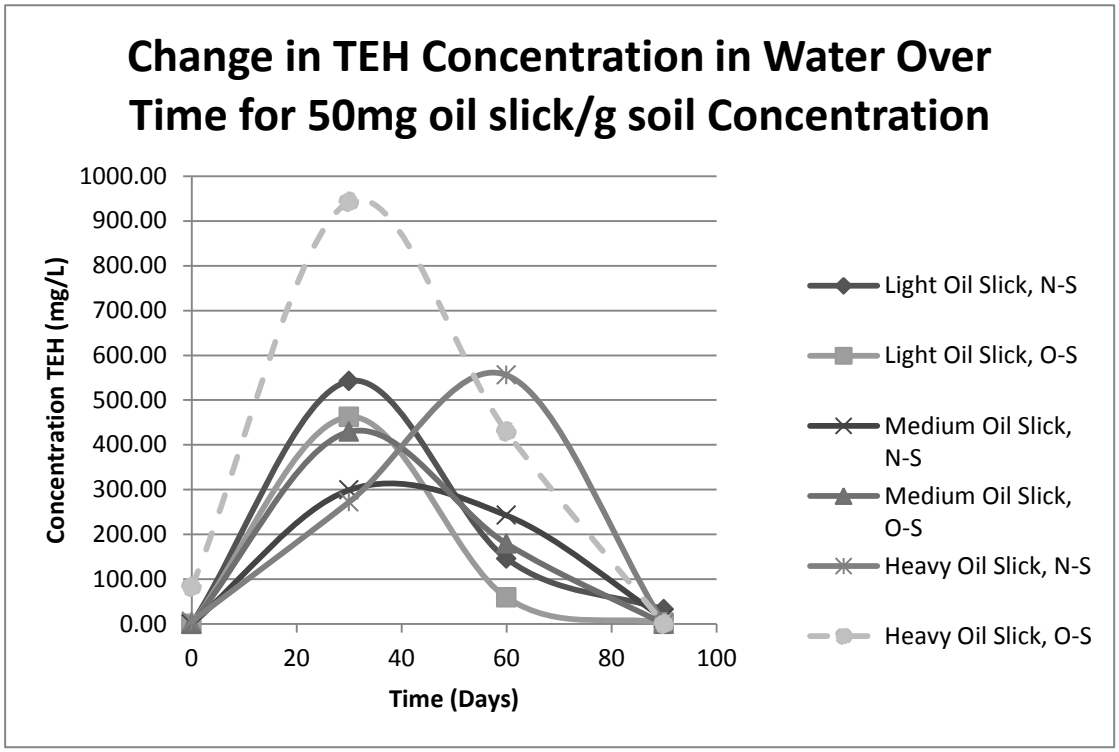


Figure 4-2: Shows the change in total extractable hydrocarbons for light, medium, and heavy crude oil for samples that contained an oil concentration of 50mg of oil slick/g of soil. The figure also compares the non-oiled soil (N-S) and the oiled soil (O-S). The dashed curve with the maximum point at 940 mg/L is believed to be in error (contaminated sample).

Figure 4-3 is similar to Figure 4-1 and Figure 4-2 except that all samples in Figure 4-3 contained an oil slick concentration of 150 mg of oil per gram of soil. This equates to approximately 60 grams of crude oil that was applied to each microcosm. At this high of a concentration in the slick, the amount of TEH added to the microcosms through the soil (O-S) is negligible. As a result N-S and O-S for light oil should be almost the same unless acclimation to low levels of oil in the soil caused greater rates of biodegradation by rhizosphere microorganisms; this hypothesis applies for the medium and heavy crude oil microcosms as well. As can be seen from Figure 4-3 this hypothesis shows some promise especially for the light crude oil slick. The medium and heavy crude oil slicks each have slight differences at time 30 days, but then show similar results for the other days.

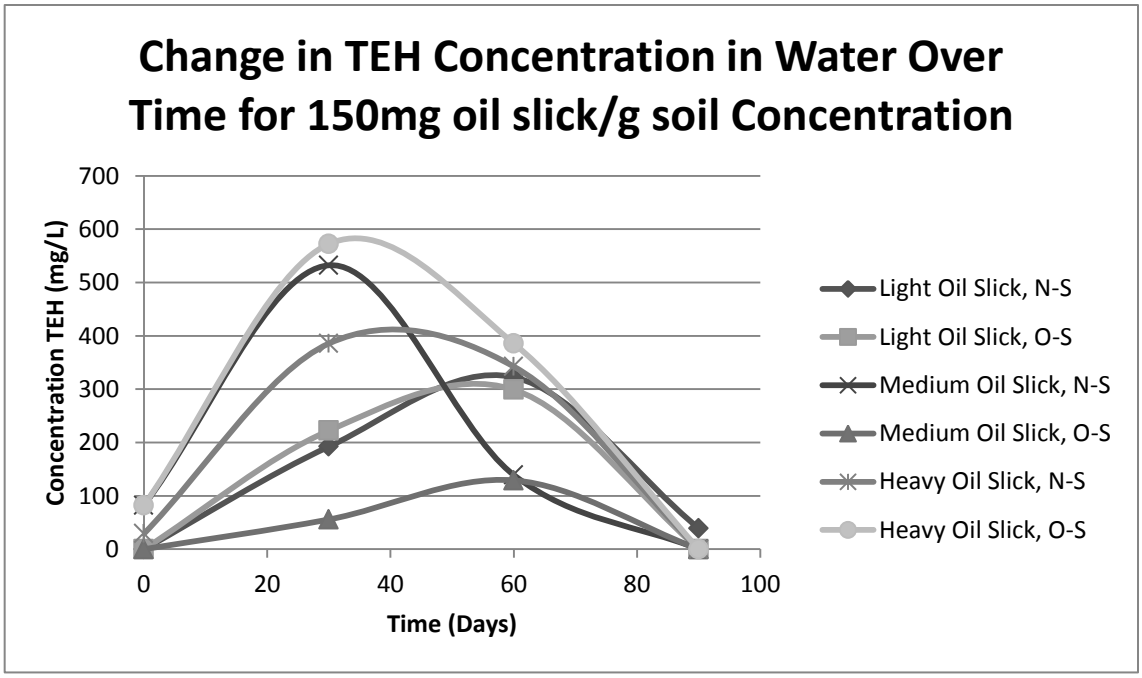


Figure 4-3: Shows the concentration of total extractable hydrocarbons for light, medium, and heavy crude oil samples that contain a concentration of 150mg of oil slick/g of soil. The figure also compares the non-oiled soil (N-S) and the oiled soil (O-S).

Figure 4-4 through Figure 4-6 shows the same data as Figure 4-1 through Figure 4-3; however, the data is presented in a different manner and compares the concentration of the oil slick and not the type of oil. Figure 4-4 shows the light crude oil with three different types of oil concentration. The highest concentration of 150mg of oil per gram of soil appear to reach a max concentration at time 60 days instead of at time 30 days, which occurs for the other lower oil concentrations.

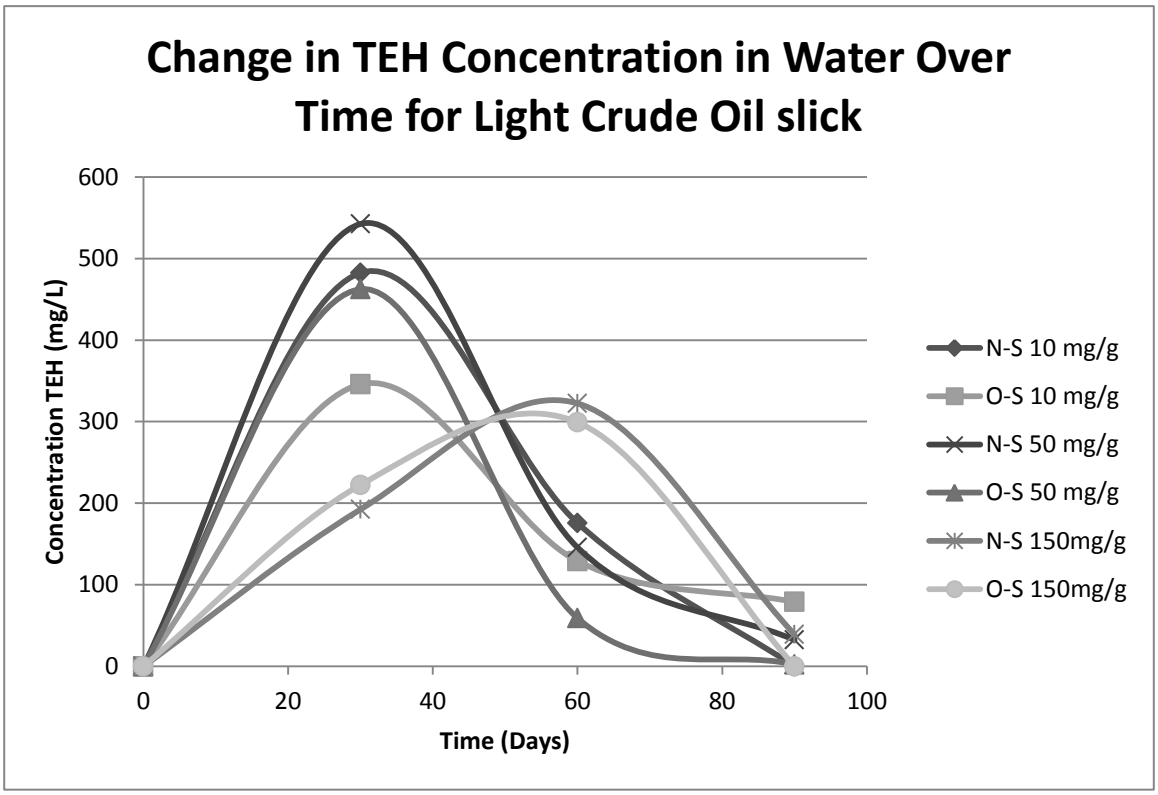


Figure 4-4: Shows the change in concentration of total extractable hydrocarbons for light crude oil with three different concentrations: 10 mg of oil/g of soil, 50 mg of oil slick/g of soil, and 150mg of oil/gram of soil. The figure also compares the non-oiled soil (N-S) and the oiled soil (O-S).

Unlike Figure 4-4 it is hard to draw any adequate conclusions from Figure 4-5. Figure 4-5 shows how the change in the concentration of oil affects the concentration of TEH in the water for an oil slick with medium crude oil. Again the planted microcosms demonstrate that the presence of plants helps to degrade the varying oil concentrations whether the soil has already experienced oil prior to the experiment beginning or not.

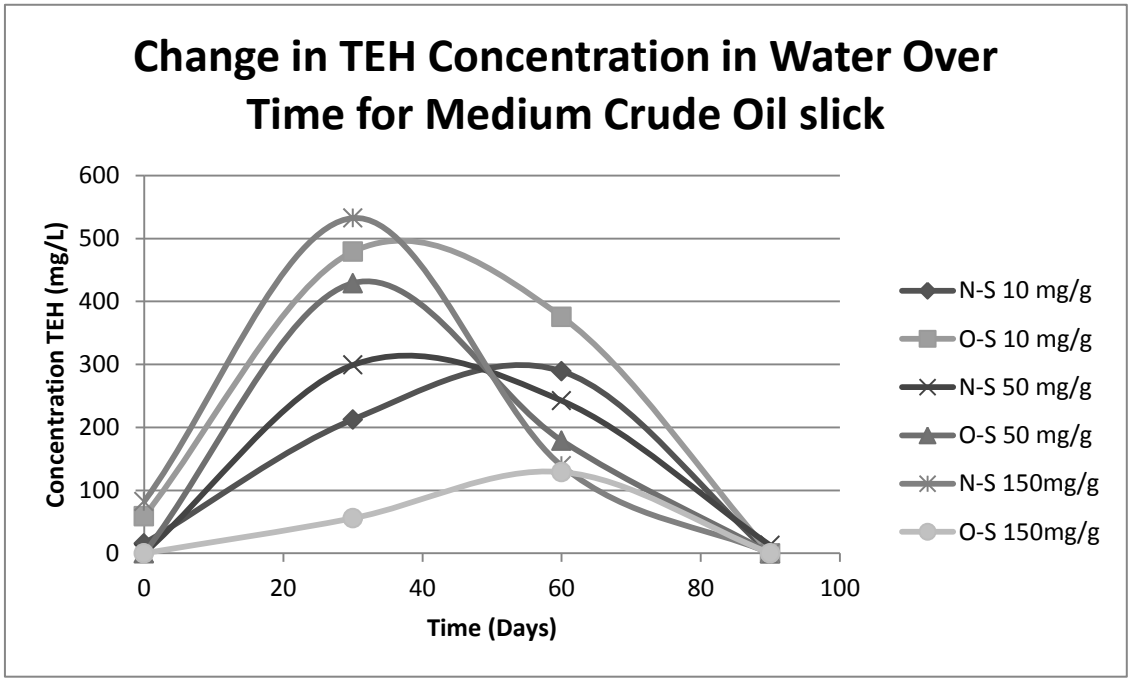


Figure 4-5: Shows the change in concentration of total extractable hydrocarbons from medium crude oil at concentrations of 10mg of oil slick/g of soil, 50 mg of oil/g of soil, and 150mg of oil/g of soil. The figure also compares the non-oiled soil (N-S) and the oiled soil (O-S).

Finally Figure 4-6 compares the different oil slick concentration for a heavy crude oil. In this sample there was an outlier during the 30 day sampling. Like the previous oils sampled the heavy oil usually has a peak concentration around 500 mg of TEH per liter of water. Similar to Figure 4-5 there are no strong conclusion that can be drawn from the data except that the plant and microbes do appear to clean up the oil within a 90 day period.

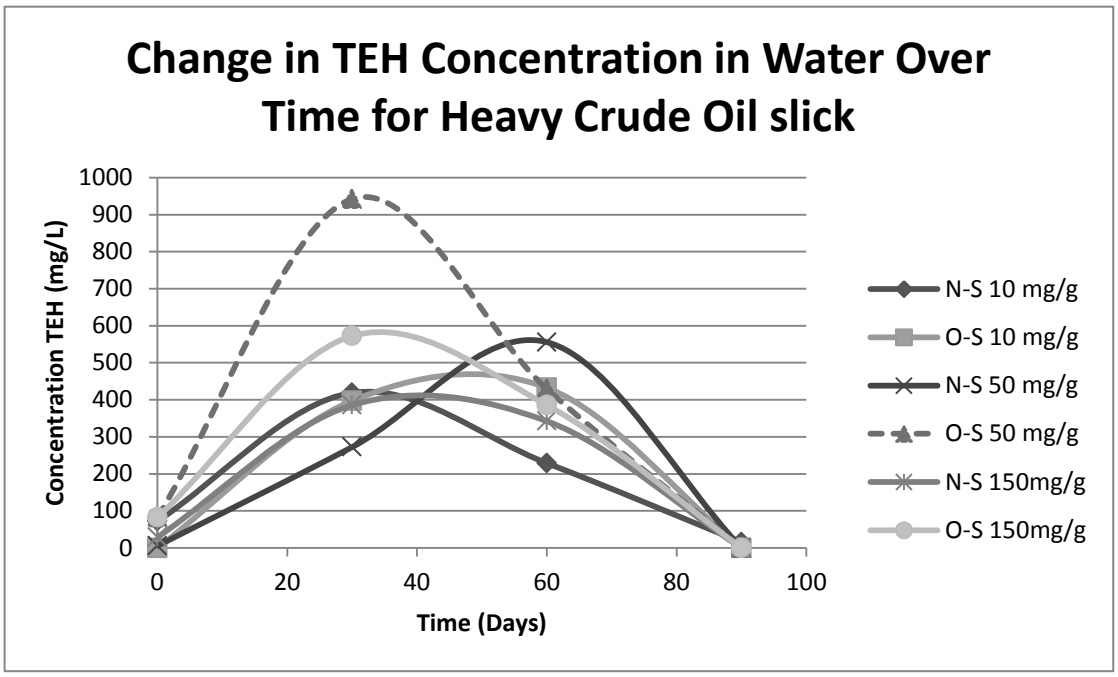


Figure 4-6: Shows the change in concentration of total extractable hydrocarbons from heavy crude oil at concentrations of 10mg of oil slick/g of soil, 50 mg of oil/g of soil, and 150mg of oil/g of soil. The figure also compares the non-oiled soil (N-S) and the oiled soil (O-S). The dashed line and the maximum concentration of 940 mg/L is believed to be in error (contaminated sample).

Figure 4-7 shows the controls and some treated samples over the 90 day time period. The graph is showing the concentration of TEH in the water for the different types of controls and treatments. Based on the results it is believed that there may have been some contamination with respect to the initial controls and the equipment not being cleaned enough at the beginning of the extractions. For example, the control microcosm (No Oil Slick, No Plants, N-S) has no oil added whatsoever, yet it ranges from 100 mg/L to 230 mg/L in TEH concentration during the experiment. This indicates that contamination of sampling syringes in the greenhouse could have added as much as 100-230 mg/L of TEH to the microcosms , but it is unlikely that contamination was so large because many samples were significantly <100mg/L TEH at the end of the experiment.

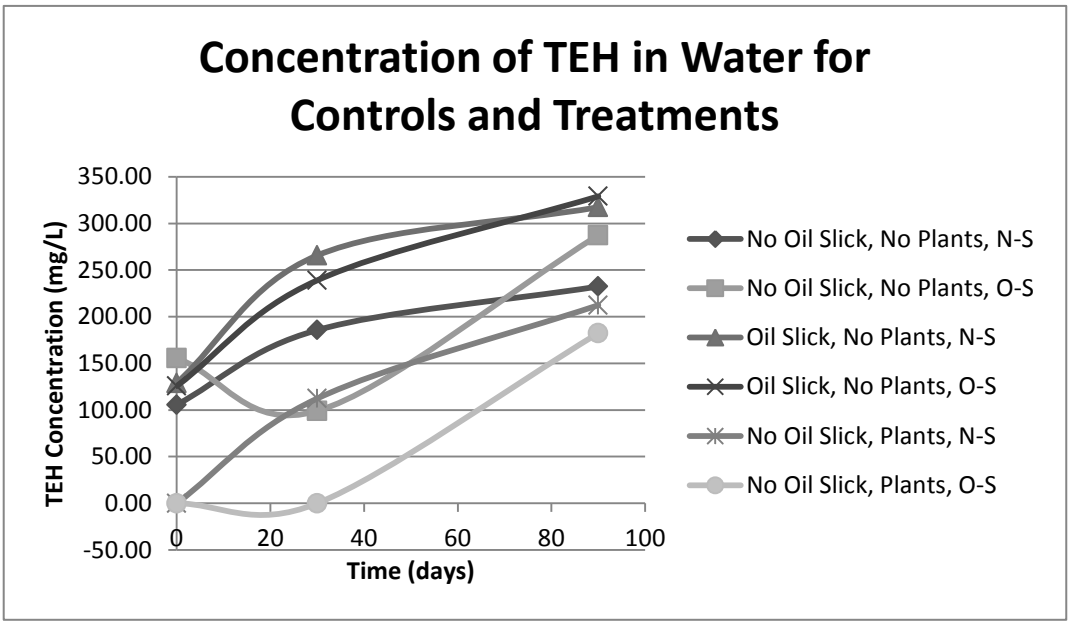


Figure 4-7: Shows the different controls and treatments used in the experiment and their relative change in concentration over time. N-S represents non-oiled soils and O-S represents oiled soils. It is expected that the TEH concentration in the samples with an oil slick and no plants would increase due to diffusion of TEH from the oil to the water. It is also expected that there could be a slight increase in TEH in the non-oiled plant samples due to the exudates released by the plants. However, we would not expect to see any increase in TEH in the non-oiled non-planted controls which means some contamination may have occurred.

The other control microcosm (No Oil Slick, Plants, N-S) has no oil added whatsoever, yet it ranges from 0 mg/L to 200 mg/L of TEH present in the water during the experiment. Again this shows that contamination of sampling syringes in the greenhouse could have added as much as 200 mg/L of TEH to the microcosms because it is unlikely that plant and soil dissolution products could have added so much.

As described in the methodology section soil samples were taken four months before the experiment began, the same time that Louisiana Sweet Crude oil was mixed with half of the soil. Soil samples were taken again at time 0 days, just before the experiment began and a final time 90 days later, when the experiment ended. Figure 4-8 shows the results from the first and second soil extractions, four months before the experiment began and when the experiment began respectively. The graph shows the change in total extractable hydrocarbons for the non-oiled soil and the oiled soil. As can be seen from the figure the non-oiled soil contained noticeable amount of hydrocarbons even though none were added. There was also no change in hydrocarbon concentration in the non-oiled soil. However, in the oiled soil there was a large TEH concentration and there was also a noticeable decrease in the TEH concentration. This would suggest that there might be bacteria degrading microorganisms present in the oiled soil.

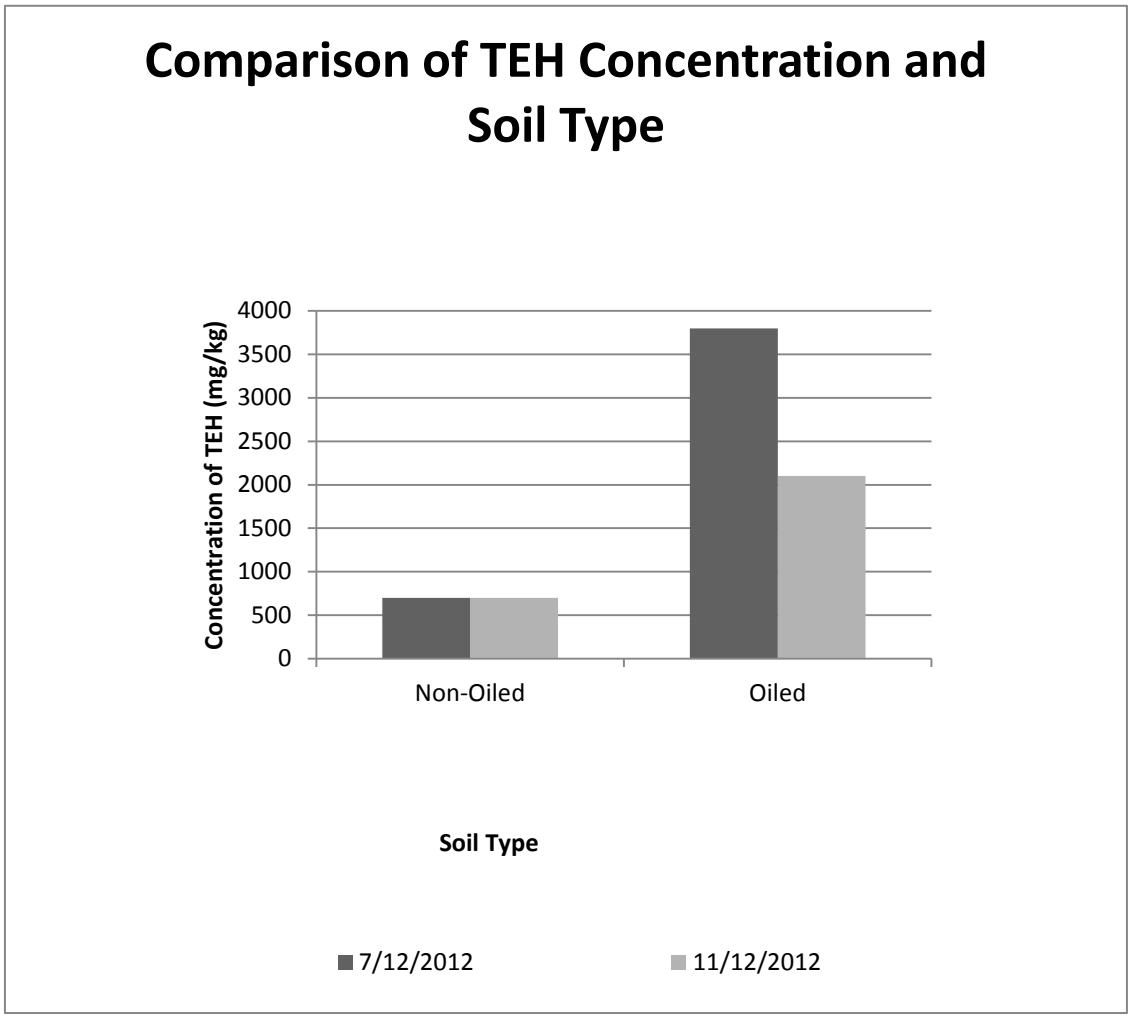


Figure 4-8: Shows the degradation of TEH after 4 months of soil acclimation. The two different soil samples were both organic potting soils, but one was mixed with 5mg oil/g of soil.

Figure 4-9 shows the final concentration of TEH in the soil samples taken when the experiment ended at 90 days. The graph represents three oiled soils and three non-oiled soils. The samples were taken from three different categories each containing an oiled and a non-oiled sample. The different soil samples are: (1) No oil slick, plants, and both oiled and non-oiled soil (2) no oil slick, no plants and both oiled and non-oiled soil (3) oil slick with medium crude oil at a concentration of 50 mg of oil per gram of soil, plants, and both oiled and non-oiled soil. The dotted line in Figure 4-9 shows concentration at time t = 0days.

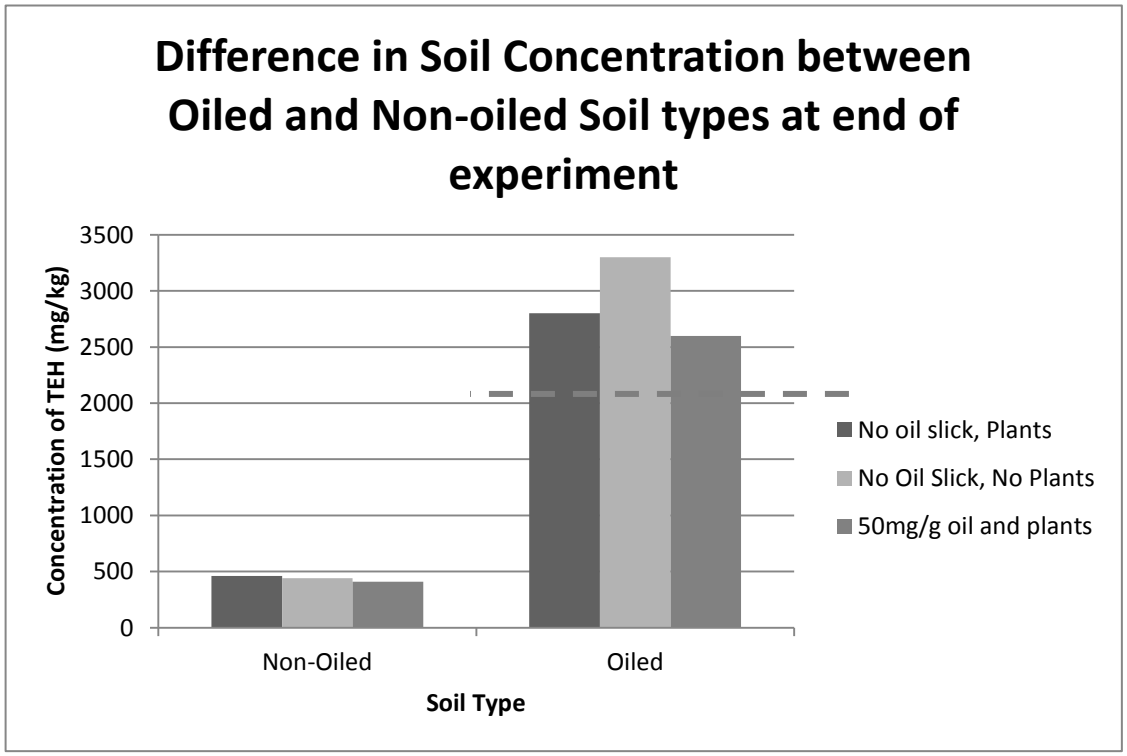


Figure 4-9: Shows the difference in TEH concentration in the soils between oiled and non-oiled soil at the end of the experiment. Six different samples were taken of non-oiled and oiled soils. The non-oiled samples showed a decrease in oil throughout the experiment while the oiled sampled actually appeared to increase.

There was noticeable variability in measurements of soil concentrations in Figure 4-9. It is believed that the soil may not have been mixed well enough initially, and that variability resulted from "hot spots" within the system. The soil shows an initial decay in TEH, Figure 4-8; however, the soil also shows an increase in TEH concentration, Figure 4-9, in soil that was oiled and placed in a beaker with no oil slick or plants. One would expect this concentration to stay relatively the same or possibly to decrease.

Model

The model was created to simulate the processes that occurred in the experimental microcosms. Once the model was compiled and executing and all the samples were analyzed, the model results were compared against the experimental data. Figure 4-10 shows the relationship between the model results and the results obtained during the experiment. Figure 4-10 only shows four data points from the continuous model which correspond to the sampling times in the microcosms. The four data points are at time 0, 30, 60, and 90 days. As can be seen in Figure 4-10 the model matches the experimental data very closely. Using a one tailed paired t-test between the experimental data and the model data shows no significant difference at the probability less than 0.05 significance level.

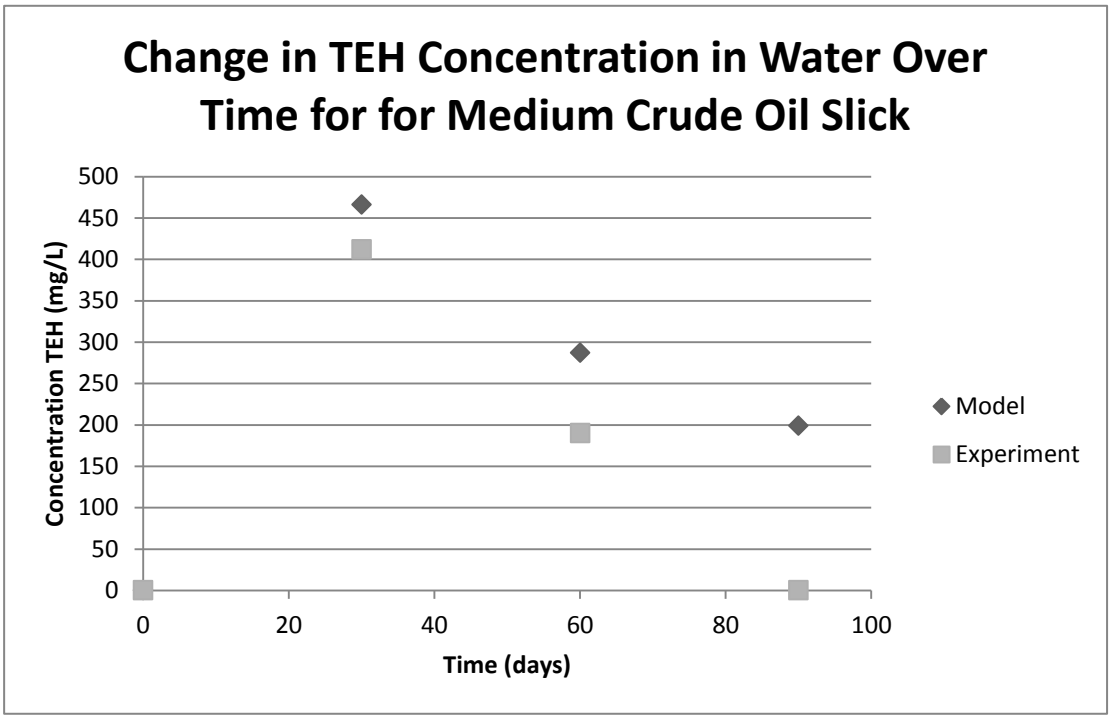


Figure 4-10: Shows the model results compared to experimental data for a crude oil slick with medium oil and a concentration of 50mg of oil/g of soil. Only four data points were selected from the model to demonstrate what was collected during the experiment.

Since a model was created, one has the ability to hypothesize what might be happening to the TEH concentration in the water between the extraction points shown in Figure 4-10. Figure 4-11 shows the results from the continuous model and what may have been missed between extractions at times 0, 30, 60, and 90 days. These model results suggest that more samples should have been taken in the initial 30 day period, where it is believed the TEH concentration in the water actually peaked.

Something else that should be noted is that based on the Mathematica model the initial assumption that the rate of diffusion of TEH into the water was limited by molecular diffusion is likely not correct. The rate constant for the model suggests that the

total extractable hydrocarbons are moving into the water at a faster rate likely due to mixing that occurred during sampling periods.

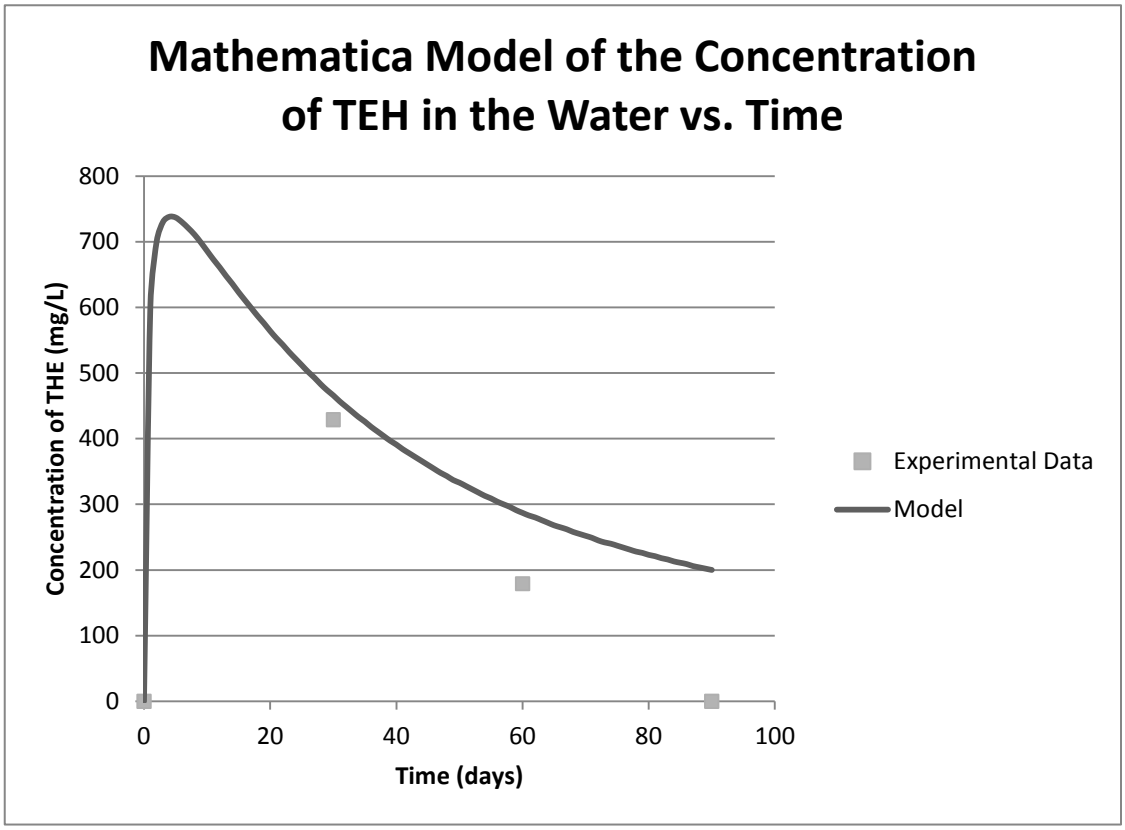


Figure 4-11: Shows the model results for the movement of TEH in the water throughout the experiment. The model is then compared to comparable experimental data. The data represented is medium grade crude oil at a concentration of 50mg of oil per gram of soil. It should be noted that the model will never reach a concentration of zero TEH.

The model mimics the results of the experiment with respect to the TEH concentration in the water for the samples. However, we were also curious if the model could represent the results from the controls that showed a continued increase in TEH throughout the length of the experiment. The model was changed to represent an

experimental situation where there was no oil slick present. It was then assumed that without the plants present there would be reduced bacterial degradation in the soil. The model results compared to experimental data can be seen in Figure 4-12. Figure 4-12 also shows experimental data for a control that contains plants, no oil slick, and non-oiled soil. Based on our earlier hypothesis bacteria would be present in this control and therefore the TEH concentration in the water would be closer to 0 mg/L throughout the experiment. Since this is not the case, the model simulation lends credence to the explanation that some contamination did occur during the experiment, quite possibly due to inadequate cleaning of the syringes at the greenhouse.

Table 4-1: Mass balance results from model of 50mg medium crude oil/g soil

MASS BALANCE TABLE

(50*mg Medium Crude Oil/g soil)*

	Time 0 days	**Time 90 days**
Phase	**Mass (g)**	**Mass (g)**
Air	0	1.10E-09
Oil	20	0.17
Water	0	0.10
Soil	0	0.86
Degraded by Bacteria in Water	0	18.87
Degraded by Bacteria in Soil	0	1.90E-05

Table 4-1 shows the mass balance results from the Mathematica model that was created for a 50 mg medium crude oil/g non-acclimated soil. The mass balance shows that the bacteria present in the water are the primary reason for the degradation of TEH in the microcosm. Had more soil samples been gathered then the TEH concentration in the soil, shown in the model, could have been proven statistically significant or modified to better represent the movement of hydrocarbons in the system.

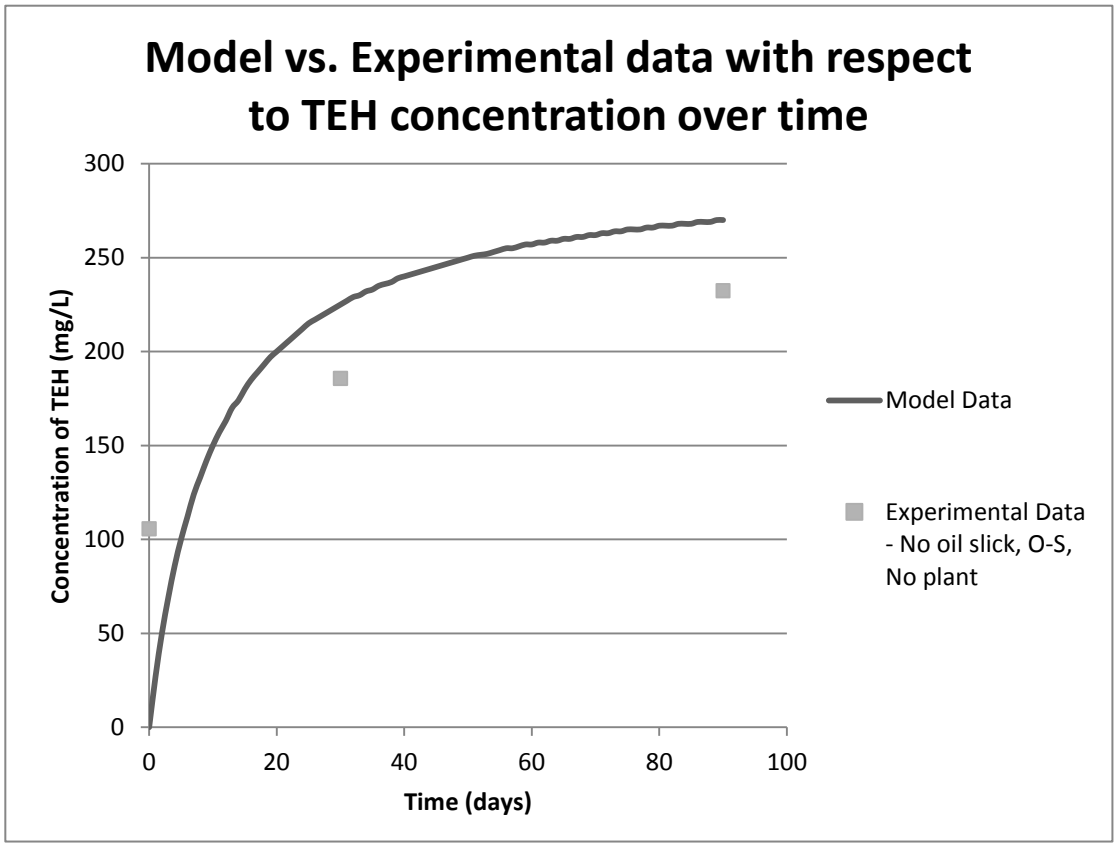


Figure 4-12: Shows the data collected from the model versus the experimental data that was collected. The model data shown is representative of a control that did not contain an oil slick or plants, but did contain oil in the soil. Additionally the model assumes that no hydrocarbons volatilize into the air. One set of experimental data presented shows the same information (No oil slick, O-S, and no plants present). The other data shown is for a control that also contained no oil slick, non-oiled soil, but did contain plants.

Decision tree

A decision tree was created by using the information that was gathered from the experiment and the model. With respect to oil concentrations that could cause mortality to the plants that concentration was not reached during the experiment and therefore the decision tree currently does not show a lethal dose. Previous research done by Elliott Beenk, at the University of Iowa, showed that a lethal dosage to *S. alterniflora* had been reached at oil concentrations of 250mg oil/gram soil. The decision tree was still created with the intent that it could be modified if further research is conducted that helps show the effects that other factors such as waves, wind, diurnal variation, and in general increased oil surface area exposure can have on plants and the concentration of TEH in the water. The entire decision tree can be seen under APPENDIX D: DECISION TREE.

A sample scenario is presented that shows the portion of the decision tree that would be used and how the decision tree works.

Scenario: A Light crude oil spill that affects a saltmarsh containing *Spartina alterniflora.* The oil slick is about 2mm thick and there has never been an oil spill in this location before.

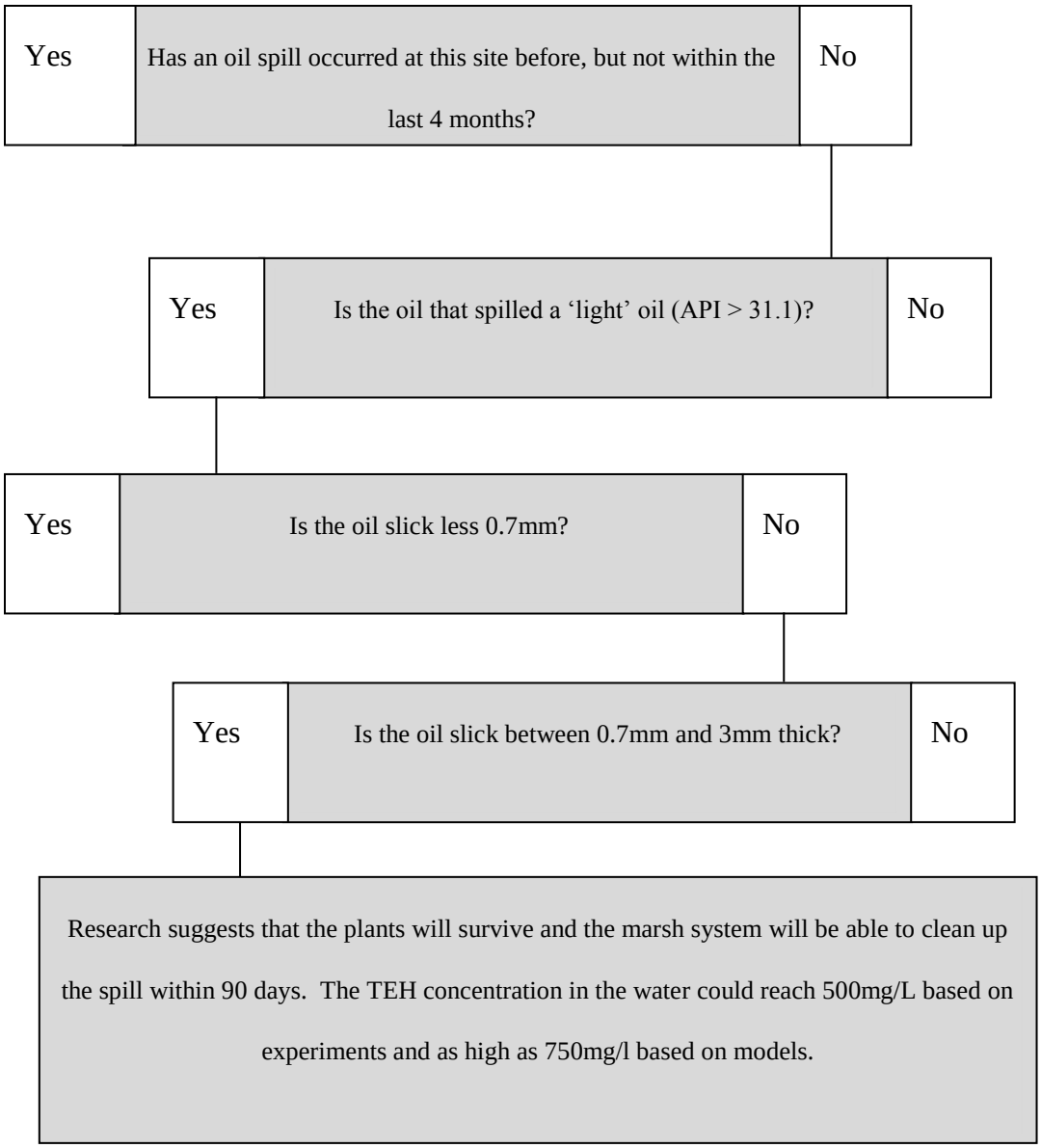


Figure 4-13: Sample case scenario, showing the use of the decision tree.

CHAPTER 4 - CONCLUSION AND RECOMMENDATIONS

The model and experimental data provided important insight into how total extractable hydrocarbons diffuse from an oil-slick phase to the water phase and are subsequently biodegraded in a plant/soil marsh system. The results identify relevant factors likely to be important in some scenarios, such as the rate of hydrocarbon movement and biodegradation, but it also identifies factors that could not be modeled well, such as the surface area interaction between water and oil, which effects the concentration of hydrocarbons that enter water. A physical model to predict the spread (areal extent) of the spill and the thickness of the slick were not included in the scope of this research, but other models exist in the literature which can simulate these processes.

With those caveats in mind, there are several important conclusions that can be drawn from this work. First, the data suggest that neither the type of oil nor its concentration significantly altered the rate of degradation in these quiescent microcosms. The practical application of this result is the importance of forcing oil into a smaller area once a spill occurs to reduce the areal impact.

Second, irrespective of the type of oil or the concentration of the oil, *Spartina alterniflora* and associated microbes show that they are capable of degrading oil to within 97% of its maximum concentration within a 90 day period. In these experiments, the lethal dosage for plants was not exceeded and although there was some decrease in biomass growth for some plants, there were no plant deaths. Based on the experimental data no conclusion can be drawn about the TEH concentration in water if a plant were to die.

Third, with respect to the concentration of TEH present in the soil samples no major conclusions can be drawn. The soil samples taken varied by up to 50 percent and as a result no conclusions can be drawn about TEH concentration in the soil. It is assumed that the reason for the variance in the soil samples was due to poor mixing of the

soil when the initial oil was added. The soil samples do successfully show that there was a small initial TEH concentration in both "clean" potting soils, in soil contaminated lightly with oil, and in soil that was not mixed with oil.

The experiment showed that the oil concentrations tested did not make a statistically significant difference on the TEH concentration in the water. Using a one tailed paired t-test between the experimental data and the model data there was no significant difference at the probability less than 0.05 significance level. This result may be secondary to area limitation. If the oil was allowed to spread out fully and was not limited by area, then more volume would be affected at similar concentrations. Thus having a larger surface area to allow the oil to fully mix could provide better results with respect to dissipating the volume and mass of oil faster.

Recommendations

The experiment provided insight into the degradation of oil by plants and bacteria. There are many variables present in saltmarshes and our study only focused on three – different soil conditions and acclimation to oil, oil types and densities, and TEH concentrations. In future studies, it would be valuable to model wave movement as well and to increase the available surface area so that oil concentration is not limited by the container but rather is limited by the quantity of oil used. Other variables that might impact oil degradation could be salinity concentrations and time of year. The latter are likely to be important since during the winter months less sunlight is available for the plants. With less sunlight, photosynthesis is reduced resulting in nutrient production decreases which lead to reduced exudates for bacteria in the rhizosphere.

Another area of study which would be interesting is the effect of temperature. Since deep water drilling occurs throughout the world and will likely expand as the Arctic ice cap melts, the impact of oil spills in cold climates should be modeled. I would also recommend that another study focusing on a variety of soil samples. Due to the low

number of soil samples in these experiments, the effect of soil type could not be studied. A final interesting experiment would be to relate the impact of the oil spill on aquatic life. Aquatic life can be affected in multiply ways because of oil two of them being a decrease in the dissolved oxygen in water when there is a noticeable oil slick on the surface and the TEH compounds entering the water that could be harmful to aquatic life.

BIBLIOGRAPHY

(2000). *Public Health Goals for Chemicals in Drinking Water Dichloromethane (Methylene Chloride, DCM).* Enviromental Protection Agency.

(2011). *Screening-Level Hazard Characterization.* U.S. Enviornmental Protection Agency Hazard Characterization Document.

Abuln, E., & Lissi, E. (1980). Partition Coefficient of Naphthalene between water and Cetyltrimethylammonium Bromide Micelles. *American Chemical Society*(84), 2605-2607.

Al-Bashir, B., Cseh, T., Leduc, R., & Samson, R. (1990). Effect of Soil/Contaminant interactions on the biodegradation of naphthalene in flooded soil under denitrifying conditions. *Applied Microbiology and Biotechnology, 34*, 414-419.

Apitz, S. E., & Myetes-Schulte, K. J. (1996). Effects of substrate mineralogy on teh biodegradability of fuel components. *Enviornmental Toxicology and Chemistry, 15*(11), 1883-1893.

Avens, H. J., Unice, K. M., Sahmel, J., Gross, S., Keenan, J. J., & Paustenbach, D. (2011). Analysis and Modeling of Airborne BTEX Concentrations from the Deepwater Horizon Oil Spill. *Environmental Science & Technology*(45), 7372-7379.

Baker, J. M. (1971). Seasonal Effects of Oil Pollution on Salt Marsh Vegetation. *Oikos, 22*(1), 106-110.

Bergen, A., Alderson, C., Bergfors, R., Aquila, C., & Matsil, M. (2000). *Restoration ofa Spartina alterniflora salt marsh following a fuel oil spill, New York City, NY.* Netherlands: Kluwer Academic.

Boduszynski, M. M., & Rechnsteiner, C. E. (1998). *Molecular Weight Distributions in Heavy Crudes.* Richmond: Chevron Research and Technology.

Biological Agents. (2011, 1 27). (U.S. Environmental Protection Agency) Retrieved 1 22, 2013, from Environmental Protection Agency: http://www.epa.gov/osweroe1/content/learning/bioagnts.htm

BP. (2011). *Annual Report and Form 20-F.* Washington, D.C.: BP.

BP Crude Assays. (2013). Retrieved March 1, 2013, from BP: http://www.bp.com/productslistsearch.do?categoryId=16002740&contentId=7020300

Caho, X., Shankar, J. N., & Wang, S. S. (2003). Waters, Development and APplication of Oil SPill Model for Singapore Coastal. *Journal of Hydraulic Engineering*, 495-503.

Cambell, N. A., & Reece, J. B. (2002). *Biology.* Benjamin Cummings: San Francisco.

Chabreck, R. H. (1972). *Vegetation, Water and Soil Characteristics of the Louisiana Coastal region.* L.A. Cooperative Fishery Unit: Louisiana State University and Agricultural and Mechanical College.

Chaudhuri, U. R. (2011). *Fundamentals of Petroleum and Petrochemical Engineering.* Boca Raton: CRC Press.

Chelaifa, H., Mahe, F., & Ainouche, M. (2010). Transcriptome divergence between the hexaploid salt-marsh sister species Spartina maritima and Spartina alterniflora (Poaceae). *Molecular Ecology, 10*, 2050-2063.

Childers, D. L., McKellar, H. N., Dame, R. F., Sklar, F. H., & Blood, E. R. (1993). A Dynamic Nutrient Budget of Subsystem Interactions in a Salt March Estuary. *Estuarine, Coastal and Shelf Science, 36*, 105-131.

Climate Zone. (2013, Januar 12). Retrieved from Climate Zone: http://www.climate-zone.com

Contreras, D. A. (2011). *Offshore Oil and Gas Development in the U.S.* New York: Nova Science Publishers, Inc.

Coulon, F., Pelletier, E., Gourhant, L., & Delille, D. (2005). Effects of Nutrient and Temperature on Degradation of Petroleum Hydrocarbons in Contaminated Sub-Antarctic Soil. *Chemosphere*, 1439-1448.

Diab, E. A. (2008). Phytoremediation of Oil Contaminated Desert Soil Using the Rhizosphere Effects. *Global Journal of Environmental Research*(2), 66-73.

Dispersing Agents. (2011, 1 27). (U.S. Environmental Protection Agency) Retrieved 1 22, 2013, from http://www.epa.gov/osweroe1/content/learning/disperse.htm

Dortch, Q., Parsons, M. L., Rabalais, N. N., & Turner, E. R. (1999). The Use of Diatom Remains as a Proxy of Historical Salinity Changes in Airplane Lake, Louisiana. *Louisiana Sea Grant College Program*, 65-78.

Dowty, R. A. (1998). *Wetland Restoration: the bioremediation of Small-scale Oil Spills in Fresh Marsh Environments and Adventitious Rooting Response in Marsh Vegetation.* Hammond, LA: Masters thesis, Southeastern Louisiana University.

EPA. (n.d.). *Technical Factsheet on: Benzene*. (National Primary Drinking Water Regulations) Retrieved January 29, 2013, from http://www.epa.gov/ogwdw/pdfs/factsheets/voc/tech/benzene.pdf

Field Guid to Virginia Salt and Brackish Marsh Plants. (n.d.). Retrieved May 2, 2012, from Integration & Application Network: http://ian.umces.edu/discforum/index.php?topic=174.0

Frick, C., Farrell, R., & Germida, J. (1999). *Assessment of Phytoremediation as an In-Situ Technique for Cleaning Oil-Contaminated Sites.* Calgary: Petroleum Technology Alliance of Canada.

Gamst, J., Olesen, T., Jonge, H. D., Moldrup, P., & Rolston, D. E. (2001). Nonsingularity of Naphthalene Sorption in Soil: Observations and the Two-Compartment Model. *Soil Sci. Soc. Am. J.*(65), 1622-1633.

Gelling Agents. (2011, 1 27). (U.S. Environmental Protection Agency) Retrieved 1 22, 2013, from http://www.epa.gov/osweroe1/content/learning/gelagnts.htm

GC/FID. (2009). (Library 4 Science) Retrieved March 9, 2013, from http://www.chromatography-online.org/directory/methdcat-2/page.html

GSI Chemical Properties Database. (2010). (GSI Environmental) Retrieved February 12, 2013, from http://www.gsi-net.com/en/publications/gsi-chemical-database.html

Gunther, T., Dornberger, U., & Fritsche, W. (1996). Effects of Ryegrass on Biodegradation of Hydrocarbons in Soil. *Pergamon, 33*(2), 203-215.

Haerens, M. (Ed.). (2010). *Offshore Drilling.* Farmington Hills: Greenhaven Press.

Head, I. M., Jones, M. D., & Larter, S. R. (2003, November 20). Biological activity in the deep subsurface and the origin of heavy oil. *Nature, 426*(6964), 344-352.

Head, I. M., Martin, J. D., & Larter, S. R. (2003). Biological Activity in the Deep Subsurface and the Origin of Heavy Oil. *Insight*.

Hydric Soils - Introduction. (n.d.). (Natural Resources Conservation Service) Retrieved 1 23, 2013, from http://soils.usda.gov/use/hydric/intro.html

Kaimi, E., Mukaidani, T., & Masahiko, T. (2007). *Screening of Twelve Plant Specie for Phytoremediaion of Petroleum Hydrocarbon-Contaminated Soils.* Hiroshima: Plant Production.

Kalf, D. F., Crommentuijn, T., & van de Plassche, E. J. (1997). *Envrionemental Quality Objectives for 10 Polycyclic Aromatic Hydrocarbons (PAHs).* Ecotoxicology and Environmental Safety.

Keddy, P. A. (2010). *Wetland Ecology Principles and Conservation* (2nd ed.). New York: Cambridge University Press.

Kjerfve, B. (1976). CIRCULATION AND SALINITY DISTRIBUTION IN COASTAL LOUISIANA BAYOUS. *Marine Science*, 1-10.

Lin, Q., Mendelssohn, I. A., Suidan, M. T., Lee, K., & Venosa, A. D. (2002). The dose-response relationship between No. 2 fuel oil and the growth of the salt marsh grass, Spartina alterniflora. *Marine Pollution Bulletin, 44*, 897-902.

Linthurst, R. A. (1980). A growth comparison of Spartina alterniflora Loisel. ecophenes under aerobic and anaerobic conditions. *American Journal of Botany, 67*(6), 883-887.

Margesin, R., & Schinner, F. (1997). Effect of temperature on oil degradation by a psychrotorophic yeast in liquid culture and in soil. *FEMS Microbiology Ecology, 24*(3), 243-249.

Materne, M. (2009, August 6). *Smooth Cordgrass, Plant Guide.* Retrieved August 3, 2011, from United States Department of Agriculture Natural Resoureces Conservation Service: http://plants.usda.gov/plantguide/pdf/pg_spal.pdf

Muscle Fiber Hypertrophy vs Hyperplasia. (2009, January 17). Retrieved January 31, 2013, from http://www.steroidology.com/muscle-fiber-hypertrophy-vs-hyperplasia/

(n.d.). *Method for Determination of Extractable Petroleum Hydrocarbons by GC/FID.* Retrieved from http://www.tn.gov/environment/ust/docs/oil.pdf

Nagheeby, M., & Kolahdoozan, M. (2010). Numerical Modeling of Two-Phase Fluid Flow and Oil Slick Transport in Estuarine Water. *Environmental Science and Technology*, 771-784.

Napthalene, 1-Methylnaphthalene, and 2-Methlynaphthalene. (n.d.). (Agency for Toxic Substances and Disease Regulations) Retrieved January 29, 2013, from http://www.atsdr.cdc.gov/toxprofiles/tp67-c6.pdf

Naphthalene. (2007, Novemeber 6). (EPA) Retrieved December 11, 2012, from http://www.epa.gov/ttnatw01/hlthef/naphthal.html

Naphthalene. (2010). (GSI Environmental Inc.) Retrieved December 11, 2012, from http://www.gsi-net.com/en/publications/gsi-chemical-database/single/383.html

Neaubauer, S. C. (2008). Contributions of mineral and organic components to tidal freshwater marsh accretion. *Esuarine Coastal and Shelf Science*, 78-88.

Nebel, B. J., & Kormondy, E. J. (1981). *Enviromental Science, The Way The World Works.* Englewood Cliffs: Prentice-Hall.

Neff, J. M., McKelvie, S., & Ayers, Jr., R. C. (200). *Environmental Impacts of Synthetic Based Drilling Fluids.* New Orleans: U.S. Department of the Interior Minerals Management Service.

Nehring, S., & Henning, A. (2009, February 07). *NOBANIS - Invasive Alien Species Fact Sheet-Spartina Anglica*. (Online Database of the European Network on Invasive Alien Species) Retrieved February 20, 2013, from http://www.nobanis.org

Newman, L. S., & Reynolds, C. M. (2004). Phytodegradation of organic compounds. *Biotechnology, 15*(3), 225-230.

Njoku, K. L., Akinola, M. O., & Oboh, B. O. (2009). Phytoremediation of Crude Oil Contaminated Soil: The Effect of Growth of Glycine Max on the Physico-chemistry and Crude Oil Contents of Soil. *Nature and Science, 7*(10), 79-87.

Non-Cancerous Breast Conditions. (2012, August 24). (American Cancer Society) Retrieved January 31, 2013, from American Cancer Society: http://www.cancer.org/healthy/findcancerearly/womenshealth/non-cancerousbreastconditions/non-cancerous-breast-conditions-hyperplasia

Oil Spill Response Techniques. (2011, 1 27). (U.S. Environmental Protection Agency) Retrieved 1 22, 2013, from http://www.epa.gov/osweroe1/content/learning/oiltech.htm

On-line Tools for Site Assessment Calculation. (2012, Novemeber 09). (EPA) Retrieved December 7, 2012, from http://www.epa.gov/athens/learn2model/part-two/onsite/esthenry.html

Page, C. A., Bonner, J. S., Sumner, P. L., & Autenrieth, R. L. (2000). Solubility of Petroleum Hydrocarbons in Oil/Water Systems. *Marine Chemistry*, 79-87.

Pezeshki, S. R., & DeLaune, R. D. (1991). *Effect of Crude Oil on Gas Exchange Functions of Juncus Roemerianus and Spartina Alterniflora.* Baton Rouge: Wetland Biogeochemistry Institute, Center for Coastal, Energy, and Environmental Resources, Louisiana .

Pezeshki, S., Hester, M., Lin, Q., & Nyman, J. (2000). Th effects of oil spill and clean-up on dominant US Gulf coast marsh macrophytes: a review. *Environmental Pollution*(108), 129-139.

Pilon-Smits, E. (2004). *Phytoremediation.* Fort Collins: Annual Review Plant Biology.

Pilon-Smits, E. (2005). Phytoremediation. *Plant Biology,* 15-39.

Potential for Human Exposure. (n.d.). (Naphthalene, 1-Methylnaphthalene, and 2-Methylnaphthalene) Retrieved December 11, 2012, from http://www.atsdr.cdc.gov/toxprofiles/tp67-c6.pdf

Reilley, K. A., Banks, M. K., & Schwab, A. P. (1996). Dissipation of Polycyclic Aromatic Hydrocarbons in the Rhizosphere. *Environmental Quality, 25*, 212-219.

Rhizosphere. (n.d.). (Dictionary.com Unabridged) Retrieved January 29, 2013, from http://dictionary.reference.com/browse/rhizosphere

Rice, T. *Phytoremediation.* RDG Planning and Design.

Rovira, A. D. (1969). Plant Root Exudates. *The Botanical Review, 35*(1), 35-57.

Salt Marsh Food Web. (n.d.). Retrieved March 3, 2012, from http://saltmarshlife.com/salt-marsh/ecology.html

(n.d.). *Saltmeadow Cordgrass.* United States Department of Agricultre Natural Resources Conservation Services.

Sanghi, R., & Singh, V. (2012). *Green Chemistry For Environmental Remediation.* Salem: Scrivener Publishing LLC.

Schnoor, J. L. (1996). *Environmental Modeling, Fate and Transport of Pollutants in Water, Air, and Soil.* New York: John Wiley & Sons, Inc.

Schwendinger, R. B. (1968). Reclamation of soil contaminated with oil. *The Institue of Petroleum, 54,* 182-197.

Settlemyre, J. L., & Gardner, L. R. (1977). Suspended Sediment Flux Through a Salt Marsh Drainage Basin. *Estuarine and Coastal Marine ZScience, 5*, 653-663.

(n.d.). *Smooth Cordgrass.* United States Department of Agriculture Natural resources Conservation Services.

Soil and Water Remdiation. (1998). (Bioremediation) Retrieved February 19, 2013, from Bioremediation: http://bioremediation.tripod.com/

Sparina alterniflora. (n.d.). (T. P. Corporation, Producer) Retrieved March 10, 2012, from Discover Life: http://www.discoverlife.org

Spartina alterniflora/hybrids. (2001). Retrieved March 5, 2012, from San Francisco Estuary Invasive Spartina Project: http://www.spartina.org/species/spartina-alterniflora-hy_v2.pdf

Spartina alterniflora Loisel. Smooth Cordgrass. (n.d.). Retrieved February 20, 2012, from United States Department of Agriculture: http://plants.usda.gov/java/profile?symbol=spal

Srujana, K., & Anisa, K. B. (2011). Phytoremediation approaches to PAH contaminated soil. *Indian Journal of Science and Technology, 4*(1), 56-63.

Tankersley, J. (2010, June 20). *A closer look at deep-water drilling*. Retrieved April 10, 2012, from Los Angeles times: http://articles.latimes.com/2010/jun/10/nation/la-na-oil-spill-qa-20100610

Thomas, G., Lam, T., & Wolf, D. (2003). A Mathematical Model of Phytoremediation for Petroleum-Contaminated Soil: Model Development. *International Journal of Phytoremediation, 5*(1), 41-55.

Wolfram, S. (1999). *The Mathematica Book* (4th ed.). Cambridge: Wolfram Media.

Xu, J. G., & Johnson, R. L. (1997). *Nitrogen dynamics in soils with different hydrocarbon contents planted to barley and field pea.* Vegreville, Alberta, Canada: Environmental TEchnologies Division.

Yaws, C. L. (2010, February 1). *Calculating H2O Solubility, Henry's Law Constant for Cycloalkanes in Crude*. Retrieved January 12, 2012, from Available: http://www.ogj.com/articles/print/volume-108/issue-4/technology/calculating-h-sub.html

Zekri, A. Y., & Chaalal, O. (2005). Effect of Temperature on Biodegradation of Crude Oil. *Energy Sources*, 233-244.

APPENDIX A: METHOD USED BY STATE HYGIENIC LABORATORY AT THE UNIVERSITY OF IOWA

METHOD FOR DETERMINATION OF EXTRACTABLE PETROLEUM HYDROCARBONS BY GC/FID

1. Scope and Application

1.1 Analytes

1.1.1 This method is designed to measure the concentration of Extractable Petroleum Hydrocarbons in water and soil. This corresponds to an alkane range of C12-C40.

1.1.2 The method is designed to measure mid to late-range petroleum products, such as diesel and spent motor oil, where contamination extends beyond diesel range organics. If, based on a review of the chromatogram, the presence of these product types is suspected, additional efforts may be performed including but not limited to, analysis of additional reference materials. These additional efforts are not contained within this method.

1.2 Quantitation Limits

1.2.1 Quantitation limits are based on 100 ug/ml of diesel/and or motor oil in the extract and are 0.10 mg/L for waters and 4.0 mg/kg for soils.

1.3 Dynamic Range

1.3.1 Dilutions should be performed as necessary to put the chromatographic envelope withinthe linear range of method. This is approximately equivalent to 100 ug/mL to 5000 ug/mL of oil in the final extract.

1.4 Experience

1.4.1 This method is based on a solvent extraction, Gas Chromatography (GC) procedure. This method should be used by, or under supervision of, analysts experienced in the use of solvent extractions and gas chromatographs. The analysts should be skilled in the interpretation of gas chromatograms and their use as a quantitative tool.

2. Method Summary

2.1 One liter of water or 25 grams of soil is spiked with a surrogate compound and extracted withmethylene chloride. The extract is dried and concentrated to a volume of 1.0 mL. An (optional)internal standard is added to each extract, and 2 uL of extract is injected into a capillary column gas chromatograph equipped with a flame ionization detector (FID). Quantitation is performed by comparing the total chromatographic area to the response of oil/diesel.

2.2 This method is based in part on USEPA Methods 8000 and 8100, SW-846, "Test Methods for Evaluating Solid Waste", 3rd Edition (1), Method OA-2 (2) and work by the EPA Total Petroleum Hydrocarbons Methods Committee (3).

3. Definitions

3.1 Extractable Petroleum Hydrocarbons (EPH): All chromatographic peaks eluting in the same retention time window as a representative diesel/oil standard mix.

3.2 Diesel/Oil Standard mix: An aliquot of commercial motor oil (10W30) obtained from a local outlet mixed 1 to 1 with an aliquot of diesel fuel(fuel oil #2) also obtained from a local outlet.

3.3 Surrogate Control Sample: A reagent water or method blank sample spiked with the surrogate compound used in the method. The surrogate recovery is used as a laboratory control. See 7.4.2.

3.4 Laboratory Control Sample: A reagent water or method blank sample spiked with diesel fuel (fuel oil #2) as a quality control check. The spike recovery is used as a laboratory control and must be greater than 50%. See 7.4.5.

3.5 Other terms are as defined in SW-846.

4. Interferences

4.1 Other organic compounds; including chlorinated hydrocarbons, phenols and phthalate esters are measureable. As defined in the method, the EPH results include these compounds.

4.2 Method interferences are reduced by washing all glassware with with hot soapy water and then rinsing it with tap water, methanol, and methylene chloride. Reagent blanks must be analyzed with each batch or for every 20 samples to demonstrate that the samples are free from method interferences.

4.3 High purity reagents, such as Burdick and Jackson GC2 methylene chloride or Baker capillary grade methylene chloride, must be used to minimize interference problems.

4.4 Contamination by carryover can occur whenever high-level and low-level samples are sequentially analyzed. Whenever an unusually concentrated sample is encountered, it should be followed by an analysis of a solvent blank to check for cross-contamination.

5. Safety Issues

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined. However, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of Occupational Safety and Health Administration (OSHA) regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data Sheets (MSDS) should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety should be available and should be identified for use by the analyst.

6. Apparatus

6.1 Glassware

6.1.1 All specifications are suggested only.

6.1.2 4 oz. amber glass wide mouth jars.

6.1.3 Separatory funnel - 2000 mL with Teflon stopcock. 6.1.4 Concentrator tube. Kuderna-Danish - 10 mL graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground glass stopper is used to prevent evaporation of extracts.

6.1.5 Evaporative flask, Kuderna-Danish - 500 mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.

6.1.6 Snyder column, Kuderna-Danish - three ball macro (Kontes K-503000-0121 or equivalent). Rotary evaporation set-up may also be used alternatively.

6.1.7 Vials - Amber glass, 10 to 15 mL capacity, with Teflon-lined screwcap. One mL glass vials with teflon-lined cap.

6.1.8 Reaction flask - Pyrex glass, 15 to 25 mL round bottom flask with standard tapered joint, fitted with a water cooled condenser and U-shaped drying tube containing granular calcium chloride.

6.1.9 Disposable pipets: Pasteur

6.2 Boiling chips - Approximately 10/40 mesh. Heat to 400oC for 30 minutes or Soxhlet extract with methylene chloride.

6.3 Microsyringes: 1 uL, 5 uL, 10 uL, 25 uL and 100 uL

6.4 Water bath - Heated with concentric ring cover, capable of temperature control (+/-2oC). The bath should be used in a hood.

6.5 An analytical balance capable of accurately weighing 0.0001g should be used for standards. A top-loading balance capable of weighing to the nearest 0.1 g should be used for sample analysis.

6.6 Gas Chromatography

6.6.1 Gas Chromatograph: Analytical system complete with gas and all required accessories, including a flame ionization detector, column supplies, gases and syringes. A data system capable of determining peak areas using a forced baseline and baseline projection is required. A data system capable of storing and reintegrating chromatographic data is recommended.

6.6.2 Columns

6.6.2.1 Column 1: 12M x 0.2mm ID x 0.33 micron film thickness DB-1, or equivalent.

6.6.2.2 Other columns may be used - capillary columns are required. See 9.2.2 for GC criteria.

6.7 Sonication

6.7.1 Ultrasonic cell disrupter: A horn-type sonicator equipped with a titanium tip should be used. A Heat Systems Ultrasonics, Inc. Model W-385 (475 watt) sonicator or equivalent (power wattage must be a minimum of 375 with pulsing capability and No. 200 1/2" Tapped Disrupter Horn) plus No. 207 3/4" Tapped Disrupter Horn, and No. 419 1/8" Standard tapered Microtip probe.

6.7.2 A Sonabox is recommended with the above disrupter for decreasing sound (Heat Systems-Ultrasonics, Inc., Model 432 13 or equivalent).

6.8 Soxhlet extraction apparatus is described in Method 3540.

6.9 Nitrogen evaporator with high purity nitrogen gas source.

7. Reagents and Standards

7.1 Reagent water: Carbon filtered deionized water

7.2 Methylene chloride, hexane, acetone - pesticide grade or equivalent.

7.3 Sodium sulfate - (ACS) granular, anhydrous. Purify by heating at 400° C for 4 hours in a shallow tray.

7.4 Stock standard solution - Prepare the following stock standards. Unless noted, all are prepared in the methlyene chloride listed in 7.2. Standard preparartion should follow guidelines in Method 8000.

7.4.1 Optional Stock Internal Standard: 1000 ug/mL 5 a -androstane.

7.4.2 Recommended Surrogate Standard: 2000 ug/mL ortho-terphenyl (OTP). A working solution is made at 20 ug/mL in acetone (a water soluble solvent). Other appropriate surrogates may be used.

7.4.3 Diesel/Oil Standard: 1 to 1 Commercial diesel/motor oil mix at approximately 100 ng/uL up to 5,000 ng/uL.

7.4.4 Stock Laboratory Control Sample - 5000 ug/mL diesel. A working solution is made at 1000 ug/ml in methylene chloride.

8. Sample collection, Preservation, Containers, and Holding Times

8.1 Water samples are collected in an one liter glass container, acid preserved and soils in a glass jar. The samples are stored at 4° C from the time of collection until extraction. Extraction must be performed on waters within seven days and soils within 14 days. All analysis must take place within 40 days.

9. Procedure

9.1 Sample preparation

9.1.1 Waters are extracted according to SW-846 Method 3510 (Separatory Funnel LiquidLiquid Extraction) or Method 3520 (Continuous Liquid-Liquid Extraction). Soil samples are extracted using Method 3550 (Sonication). Method 3540 (Soxhlet Extraction) may also be used.

9.1.2 Water extraction - Separatory Funnel

9.1.2.1 Measure a 1-L portion of the sample and transfer to the 2-L separatory funnel. If the sample is in a 1 liter or smaller bottle, mark the water meniscus on the side of the sample bottle for later determination of the sample volume. If the sample is in a larger bottle, use a 1 liter graduated cylinder. Pour the sample into a 2 liter separatory funnel. For blanks and quality control standards, pour 1 liter of carbon filtered water into the separatory funnel.

9.1.2.2 Check and note the initial pH.

9.1.2.3 Add 1 mL of ortho-terphenyl surrogate standard at 20 ug/mL.

9.1.2.4 For every batch or 20 samples extracted, prepare duplicate laboratory control samples by adding 1 mL of 1000 ug/mL diesel (laboratory control standard) to each of
two blank matrices. Daily or for every 20 samples, prepare a blank/surrogate control standard using 1 L of carbon filtered water.

9.1.2.5 For samples that were mixed before extraction, add 60 mL CH_2Cl_2 to the sample bottle to rinse the inner walls. Do NOT cap and shake the bottle, rinse the glass only; transfer the solvent to the separatory funnel. Extract the sample by shaking it for two minutes with frequent ventilation.

9.1.2.6 Allow the layers to separate. If there is an emulsion, break it. If the emulsion cannot be broken (recovery of <80% of the

methylene chloride, corrected for water solubility of methylene chloride), transfer the sample, solvent, and emulsion into the extraction chamber of a continuous extractor and proceed as described in 9.1.3.

9.1.2.7 Drain the bottom layer (CH2Cl2) into a 250 mL beaker.

9.1.2.8 Repeat the extraction twice more using a 60 mL aliquot of CH2Cl2 each time. Collect the solvent in the same beaker described in

9.1.2.7. Record the volume recovered.

9.1.2.9 Put a plug of glass wool in a funnel and fill about 2/3 full with Na2SO4. Rinse the funnel and Na2SO4 with 30-40 mL of CH2Cl2, discard. Pour the extract through the Na2SO4 into a 500 mL Kuderna-Danish (K-D) evaporative concentrator. Rinse the beaker then the Na2SO4 with small amounts of CH2Cl2. Add these rinses to the K-D.

9.1.2.10 Add a boiling chip to the K-D and attach a 3 ball Snyder to the top. Pre-wet the column by adding about 1 mL of CH2Cl2 to the top. NOTE: The concentration step is critical; losses can occur if care is not taken.

9.1.2.11 Place the K-D in a heated water bath set at 95° C so that the receiver tube is immersed in hot water and the entire lower rounded surface is bathed in steam. At a

proper rate of distillation, the balls of the column will actively chatter, but the chambers will not flood. When the apparent volume reaches 5-10 mL, remove the K-D from the bath and allow it to cool completely.

9.1.2.12 If the extract is highly colored or a precipitate forms during concentration, the final volume should be higher (5-10 mL).

9.1.2.13 After the K-D has cooled, rinse the Snyder column and middle flask with a small amount of CH2Cl2. Transfer the extract to a calibrated 15 mL centrifuge tube, rinsing with a small amount of CH2Cl2. Be sure to rinse all of the ground glass joints well, as compounds collect on the ground glass.

9.1.2.14 Carefully concentrate the extract to 1.0 mL under a gentle stream of nitrogen using the N-evap apparatus. If the extract is highly colored, forms a precipitate, or stops evaporating, the final volume should be higher (5-10 mL). Transfer to a labeled 4 mL (or 12 mL) vial with Teflon lined cap, mark the meniscus.

9.1.2.15 Record the prep information for the extraction and concentration steps. The sample extract is ready for analysis (See Section 9.2 through 9.6).

9.1.3 Water extraction - Continuous liquid liquid extraction

9.1.3.1 Mount the continuous extractor on appropriate racks.

9.1.3.2 Put 250 mL CH2Cl2 in a round bottom flask and add a few boiling chips. Add 300 mL of CH2Cl2 to the extractor flask.

9.1.3.3 When pouring water into the extractor, minimize the disturbance of the solvent layer and avoid getting water into either sidearm by pouring the water down the back of the extractor.

9.1.3.4 Check and note the pH.

9.1.3.5 For samples in 1 liter of smaller bottles, mark the meniscus on the side of the sample bottle and pour approximately 1 liter of the sample into the extractor flask.

Measure the exact volume by adding tap water to the bottle to the marked level and measuring the volume with a graduated cylinder. For samples in bottles larger than 1 liter, measure 1 liter of the sample in a graduated cylinder. Record the volume.

9.1.3.6 Add enough carbon filtered water to the extractor flask to allow the solvent in the removable sidearm to just begin to drip into the round bottom flask. Record the total volume carbon filtered water that was added on the prep sheet.

9.1.3.7 Remove the condenser from the rack and wipe the lower joint and lip with a tissue soaked with solvent. Place the condenser on the top of the extractor. Turn on the cool water supply and check the flow indicators.

9.1.3.8 Turn on the heating mantle. Record the starting time on the prep sheet. Check after 15 minutes to be sure that the solvent in the round bottom flask is boiling, that solvent is dripping from the lip on the condenser, and that the volume of the solvent in the round bottom flask is still about 240 mL.

9.1.3.9 Check all extractor joints for leaks with a Kimwipe. Allow the extraction to proceed for 18-24 hours.

9.1.3.10 Turn off the heating mantle and allow the apparatus to cool (30-60 minutes) with water flowing through the condenser.

9.1.3.11 The solvent contained in the round bottom flask is the extract. Transfer the extract to a 400 mL beaker, rinsing with a small amount of CH2Cl2. If the volume of solvent is less than about 250 mL, record the solvent volume.

9.1.3.12 Go to 9.1.2.9 and proceed with the prep.

9.1.4 Soil preparation - Sonication

9.1.4.1 Remove large rocks or other foreign materials and mix the sample well. Chop any vegetation into small pieces. 9.1.4.2 Weigh 25 g of the original sample into a 250 mL centrifuge bottle. Add 25 g of dried Na2SO4 and stir the mixture well with a steel spatula. The sample should have a grainy texture - if it forms a large clump, add more Na2SO4 and note it on the prep sheet.

9.1.4.3 Add 100 mL of CH2Cl2 to all samples.

9.1.4.4 Add 1 mL of 20 ug/mL ortho-terphenyl to all samples and standards. Mix the samples immediately.

9.1.4.5 Add 1 mL of 1000 ug/mL diesel (laboratory control standard) to the duplicate laboratory control standards. These standards should contain 25 g of Ottawa Sand. In addition, prepare a reagent

blank/surrogate control standard containing 1 mL of 20 ug/mL ortho-terphenyl.

9.1.4.6 Sonicate the samples for 1.5 minutes at an output setting of 10 with the 3/4 inch sonicator horn 1/2 inch below the surface of the solvent. The sonicator should be in the 1 second pulse mode, with the duty cycle set at 50%. Centrifuge the samples for
3-5 minutes at 35 RPM.

9.1.4.7 Decant the solvent layer into a rinsed 400 mL beaker.

9.1.4.8 Repeat the extraction twice more using 100 mL aliquots of CH2Cl2 each time. Collect these extracts in the same beaker described in 9.1.4.9.

9.1.4.9 Record the total volume of the solvent that is recovered.

9.1.4.10 Go to 9.1.2.9 and proceed with the prep.

9.1.5 Dilution Technique

9.1.5.1 This is used for product or waste samples which are soluble in methylene chloride.

9.1.5.2 Weigh 1 g of sample into a 10 mL volumetric flask. Dilute to 10 mL with CH2Cl2. Store in a 12 mL vial.

9.2 Gas Chromatography

9.2.1 Conditions (Recommended): Set helium column flow to 1 mL/min. Set column temperature to 35° C for 14 minutes, then 10° C/min to 210° C , then raise to 320° C at 40/min and hold 10 min. The FID temperature should be set at 300° C and the injector to 250°
C. These conditions may vary depending upon equipment.

9.2.2 Performance Criteria: GC run conditions and columns must be chosen to meet the following criteria:

9.2.2.1 Resolution from the solvent front and o-terphenyl of C22.

9.2.2.2 The column must be capable of separating typical oil components from the surrogate and internal standards.

9.3 Calibration

9.3.1 Calibrate the GC with an initial five point calibration using the commercial diesel/oil standard (7.4.4). Tabulate the area response of the diesel/oil standard. The ratio of the response to the amount injected, defined as the response factor (RF), can be calculated for the standard at each concentration. If the percent relative standard deviation (%RSD) is less than 25% over the working range, linearity through the origin can be assumed, and the continuing calibration response factor can be used in place of a calibration curve. Response Factor = Total area of commercial diesel/oil x I.S. amount (mg/mL) / Total diesel/oil standard amount (mg/mL) x I.S. area Note: I.S. = Internal Standard (optional)
Alternately, external standard calibration may be used (See SW-846 Method 8000).

9.3.2 The working response factor or calibration curve must be verified on each working day by the injection of a continuing calibration standard (CCS), mid-point. If the response for this standard varies from the

predicted response by more than +/-25%, a new calibration curve must be prepared.

Percent Difference = R1 - R2 / Ravg x 100

where: R1 = Average RF from the calibration curve

R2 = Response Factor from CCS

Ravg = (R1 + R2)/2

9.4 Retention Time Window Definition

9.4.1 Before establishing windows, be certain that the GC system is within optimum operating conditions. Make three injections of the method standard throughout the course of a 72-hour period. Serial injection over less than a 72-hour period result in retention time windows that are too tight.

9.4.2 Calculate the standard deviation of the three absolute retention times for the surrogate and/or internal standard.

9.4.2.1 The retention time window for individual peaks is defined as plus or minus three times the standard deviation of the absolute retention time for each component.

9.4.2.2 In those cases where the standard deviation for a particular analyte is zero, the laboratory should use +/-0.05 min as a retention time window.

9.4.3 The laboratory must calculate retention time windows for each standard on each GC column and whenever a new GC column is installed. The data must be retained by the laboratory.

9.5 Gas Chromatograph Analysis

9.5.1 Samples are analyzed by GC/FID. Suggested injection volumes are 2 uL using the conditions established in 9.2.

9.5.2 For internal standard calibration, 5-a androstane internal standard is spiked into each sample and standard at a concentration of 20 ug/mL of sample extract. 20 uL of 5-a Androstane stock at 1000 ug/mL may be spiked into the 1 mL final volume or a corresponding amount may be added to an aliquot of the final extract.

9.5.3 If initial calibration (9.3.1) has been performed, verify the calibration by analysis of a mid-point CCS (9.3.2). The midpoint standard must also be run once every ten runs and at the end of each sequence.

9.5.4 Calculate the percent difference of the response factor from the mean response factor as in 9.3.2. If the response factors have a percent difference >+/-25%, the instrument must be recalibrated (9.3.1).

9.5.5 A methylene chloride blank must be run in every sequence to determine the area generated on normal baseline bleed under the conditions prevailing in the 24 hour period. This area is generated by projecting a horizontal baseline between the retention times observed for C12 and C40. This area is subtracted from the EPH area generated in the same manner for the samples.

Methylene chloride blanks should also be run after samples suspected of being highly concentrated to prevent carryover.

9.5.6 If the product concentration exceeds the linear range of the method in the final extract, the extract must be diluted and reanalyzed.

9.6 Calculations

9.6.1 Internal Standard Calibration: The concentration of Extractable Petroleum Hydrocarbons in the sample is determined by calculating the absolute weight of analyte chromatographic peaks eluting in the defined retention time window of oil, using the calibration curve or the response factor determined in 9.3.2. Refer to 9.4. The concentration of Extractable Petroleum Hydrocarbons is calculated as follows:

Aqueous/Soil samples:

Cs = (Ax / As) x (Cis / RF) x (Vt / Vs) x D

Where:

Cs = Concentration of Extractable Petroleum Hydrocarbons (mg/L or mg/kg).

Ax = Response for the Extractable Petroleum Hydrocarbons in the sample, units in area.

RF = Response Factor from continuing calibration (See 9.3.1).

As = Response for the internal standard, units same as for Ax.

Cis= Concentration of Internal Standard (mg/mL).

Vt = Volume of Final extract (mL).

D = Dilution factor

Vs = Volume of sample extracted in L or kg.

9.6.2 Alternately, external standard calibration may be used (See SW-846 Method 8000).

10. Quality Control

10.1 The laboratory must establish the ability to generate acceptable accuracy and precision. This should include the analysis of QC check samples plus the calculation of average recovery as outlined in Method 8000, Section 8.0.

10.2 The laboratory must, on an ongoing basis, demonstrate through the analysis of quality control check standards that the operation of the measurement system is in control.

10.3 After successful calibration (Section 9.3), analyze a Surrogate Control Sample. This standard is also the reagent blank sample and is analyzed with every analytical batch or sequence. The surrogate recovery should be within established limits (Table 1) and the sample should not have Extractable Petroleum Hydrocarbons above the practical quantitation limit.

10.4 Every batch or 20 samples, duplicate Laboratory Control samples must be analyzed. The accuracy and precision of the duplicate standards must be within established limits (Table 1).

10.5 Each laboratory should generate control limits based on the average recovery +/-3 standard deviations.

10.6 If any of the criteria in 10.3 and 10.4 are not met, the problem must be corrected before samples are analyzed.

10.7 Calculate the surrogate standard recovery in each sample. If recoveries are outside established limits, verify calculations, dilutions and standard solutions. Verify instrument performance.

10.7.1 High recoveries may be due to a coeluting matrix interference; examine the sample chromatogram.

10.7.2 Low recoveries may be due to the sample matrix.

10.8 Field blanks, duplicates and matrix spikes are recommended for specific sampling programs. Matrix spikes should use the spike levels specified for laboratory control samples.

11. References

1. USEPA "SW-846 Test Methods for Evaluating Solid Waste", 3rd Edition; Methods 8000, 8100, 3510, 3520, 3540, and 3550.

2. "Method OA-2: Extractable Petroleum in Products: Revision January 10, 1990; University Hygienic Laboratory, Iowa City, Iowa.

3. "Method for Determination of Extractable Petroleum Hydrocarbons (EPH) in Soil and Water" - Draft - February 28, 1990; prepared for Total Petroleum Hydrocarbons Method Committee by Midwest Research Institute.

4. Zillis, K., M. McDevitt, and J. Parr; "A Reliable Technique for Measuring Petroleum Hydrocarbons in the Environment," presented at the conference on Petroleum Hydrocarbons and Organic Chemicals in Groundwater, NWWA, Houston, Texas, November 1988. 5. "Leaking Underground Fuel Tank (LUFT) Field Manual," State Water Resources Control Board, State of California, Sacramento, CA, May, 1988.

6. Fitzgerald, John "Onsite Analytical Screening of Gasoline Contaminated Media Using a Jar Headspace Procedure" in Petroleum Contaminated Soils, Vol. 2, 1989.

7. Senn, R.B., and M.S. Johnson, "Interpretation of Gas Chromatographic Data in Subsurface Hydrocarbon Investigations," Ground Water MonitoringReview, 1987.

8. Hughes, B.M., D.E. McKenzie, C.K. Trang, L.S.R. Minor, "Examples of the Use of an Advanced Mass Spectrometric Data Processing Environment for the Determination of Sources of Wastes" presented at 5th Annual Waste Testing and Quality Assurance Symposium, July 24-28, 1989.

9. ASTM "Standard Methods for Comparison of Waterborne Petroleum Oils by Gas Chromatography," 3328-78.

APPENDIX B: RESULTS TABLES

Table B-1: Data used to show the change in total extractable hydrocarbons for light, medium, and heavy oil at a concentration of 10mg of oil/g of soil.

	Light Oil	Light Oil	Medium Oil	Medium Oil	Heavy Oil	Heavy Oil
Day	**N-S**	**O-S**	**N-S**	**O-S**	**N-S**	**O-S**
0	<1	<1	46	59	72	<1
30	482	346	212	479	419	397
60	176	129	289	376	229	432
90	<1	79	<1	<1	16	<1

Table B-2: Data Used to show the change in total extractable hydrocarbons for light, medium, and heavy oil at a concentration of 50mg of oil/g of soil.

	Light Oil	Light Oil	Medium Oil	Medium Oil	Heavy Oil	Heavy Oil
Day	**N-S**	**O-S**	**N-S**	**O-S**	**N-S**	**O-S**
0	<1	<1	<1	<1	6	82
30	542	462	299	429	272	942
60	146	59	242	179	556	429
90	32	2	12	<1	<1	<1

Table B-3: Data Used to show the change in total extractable hydrocarbons for light, medium, and heavy oil at a concentration of 150mg of oil/g of soil.

	Light Oil	Light Oil	Medium Oil	Medium Oil	Heavy Oil	Heavy Oil
Day	**N-S**	**O-S**	**N-S**	**O-S**	**N-S**	**O-S**
0	<1	<1	82	<1	29	82
30	192	222	532	<100	386	572
60	322	299	139	129	342	386
90	39	<1	<1	<1	<1	<1

Table B-4: Data used to demonstrate the change in total extractable hydrocarbons for varying concentrations over time with respect to light crude oil

	N-S	O-S	N-S	O-S	N-S	O-S
Day	**10**	**10**	**50**	**50**	**150**	**150**
0	<1	<1	<1	<1	<1	<1
30	482	346	542	462	192	222
60	176	129	146	<100	322	299
90	<1	79	32	2	39	<1

Table B-5: Shows the data used to demonstrate the change in total extractable hydrocarbons for varying concentrations over time with respect to Medium crude oil

	N-S	O-S	N-S	O-S	N-S	O-S
Day	**10**	**10**	**50**	**50**	**150**	**150**
0	16	59	<1	<1	82	<1
30	212	479	299	429	532	56
60	289	376	242	179	139	129
90	<1	<1	12	<1	<1	<1

Table B-6: Shows the data used to demonstrate the change in total extractable hydrocarbons for varying concentrations over time with respect to heavy crude oil

	N-S	O-S	N-S	O-S	N-S	O-S
Day	**10**	**10**	**50**	**50**	**150**	**150**
0	72	<1	6	82	29	82
30	419	397	272	942	386	572
60	229	432	556	429	342	386
90	16	<1	<1	<1	<1	<1

APPENDIX C: SAMPLE GC-FID RESULTS

State Hygienic Laboratory
at the University of Iowa

Quantitation Report

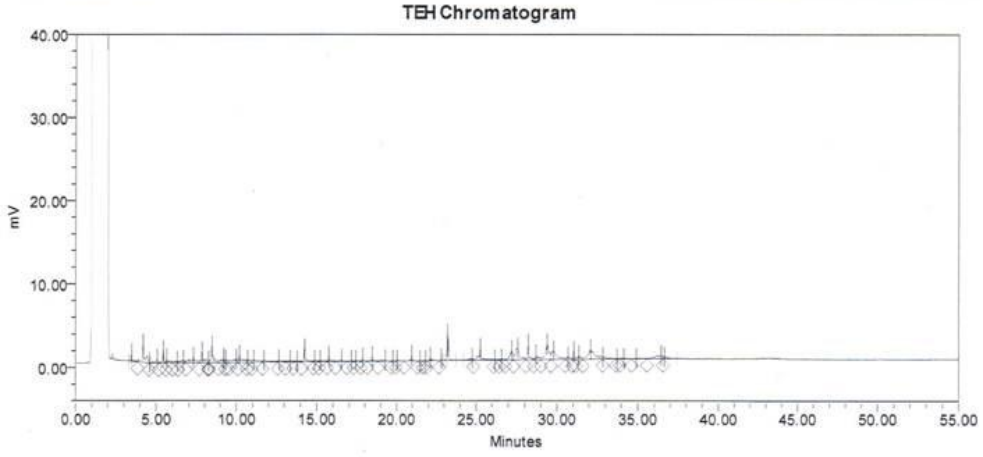
Bedroc 5890 II TEH Analysis in H2O

SampleName = 68930 Ui Eng.
Injection Id = 18972
Vial # = ~~37~~ 48
Inj. VoL = 2.00 ul
Run Time = 55.0 Minutes
Project Name = BEDROC_TEH_2011_02
System Name = TEH1

Result Id = 18999

Sample Set Name = 2012 11 28 333 PM
Date Acquired = 11/30/2012 1:27:25 AM CST
Date Processed: 11/30/2012
Acquired By = pma1
Acq. Method Set: diesel_full
Processing Method = Mid oil
Report Method Name = Client_Sample_H2O

TEH Chromatogram



Group Peak Results

	Name	RT	Area	Height	TEH_Final_Result Water_ug/L	Quantitation_Limit Water_ug/L
1	Mid oil	4.21	420515	36071	225114.8	100000

Report Method: Client_Sample_H2O Printed 7:00:19 AM U 11/30/2012 Page: 1 of 1

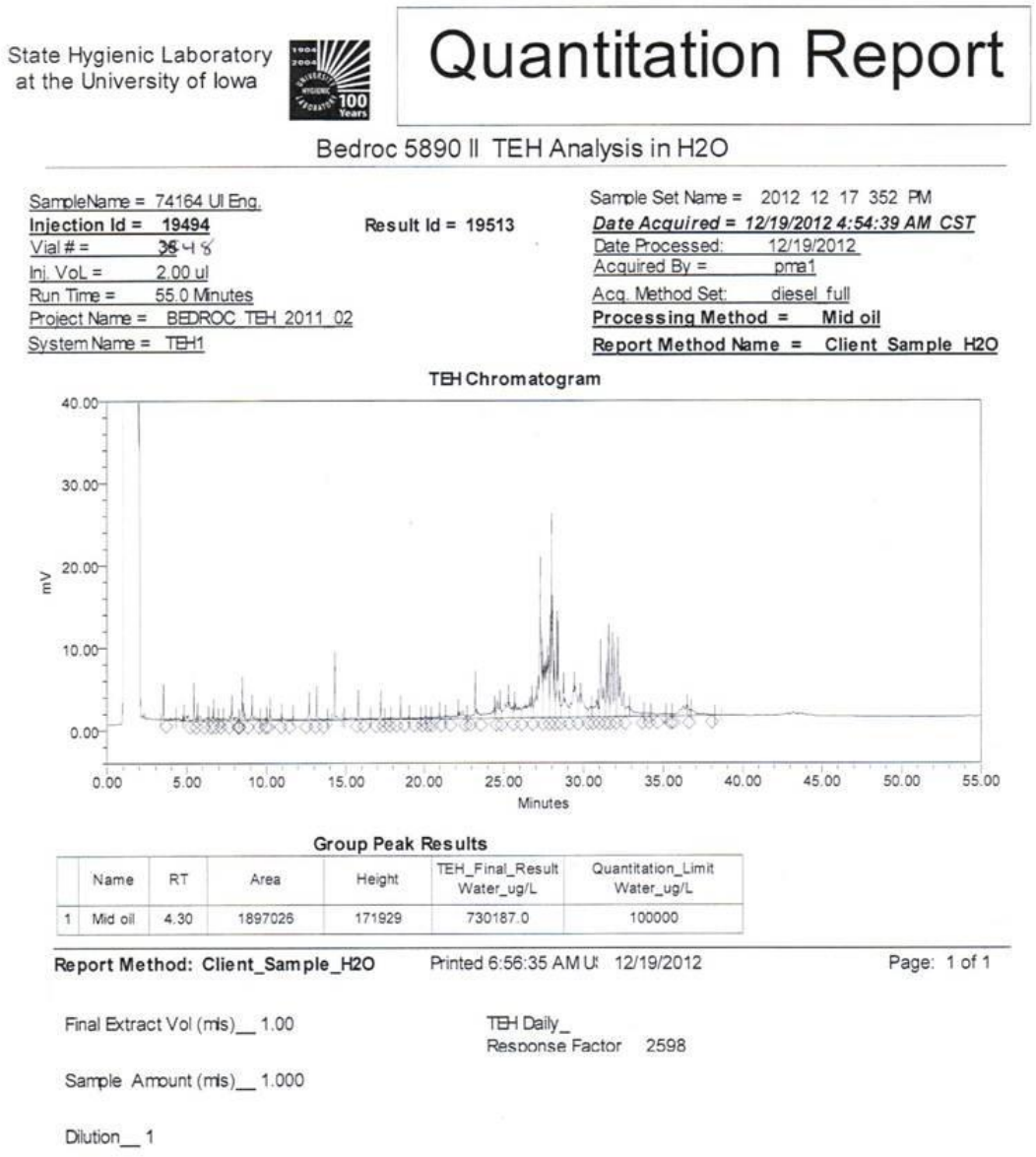
Final Extract Vol (mls)__ 1.00

TEH Daily_
Response Factor 1868

Sample Amount (mls)__ 1.000

Dilution__ 1

Figure C-1: Shows GC-FID extraction results from sample 48 for time 0days.


State Hygienic Laboratory
at the University of Iowa

Quantitation Report

Bedroc 5890 II TEH Analysis in H2O

SampleName = 74164 UI Eng.
Injection Id = 19494
Vial # = 36
Inj. VoL = 2.00 ul
Run Time = 55.0 Minutes
Project Name = BEDROC TEH 2011 02
System Name = TEH1

Result Id = 19513

Sample Set Name = 2012 12 17 352 PM
Date Acquired = 12/19/2012 4:54:39 AM CST
Date Processed: 12/19/2012
Acquired By = pma1
Acq. Method Set: diesel full
Processing Method = Mid oil
Report Method Name = Client Sample H2O

TEH Chromatogram

Group Peak Results

	Name	RT	Area	Height	TEH_Final_Result Water_ug/L	Quantitation_Limit Water_ug/L
1	Mid oil	4.30	1897026	171929	730187.0	100000

Report Method: Client_Sample_H2O Printed 6:56:35 AM U 12/19/2012 Page: 1 of 1

Final Extract Vol (mls)__ 1.00

TEH Daily_
Response Factor 2598

Sample Amount (mls)__ 1.000

Dilution__ 1

Figure C-2: Shows GC-FID extraction results for sample 48 at time 30days.

State Hygienic Laboratory at the University of Iowa

Quantitation Report

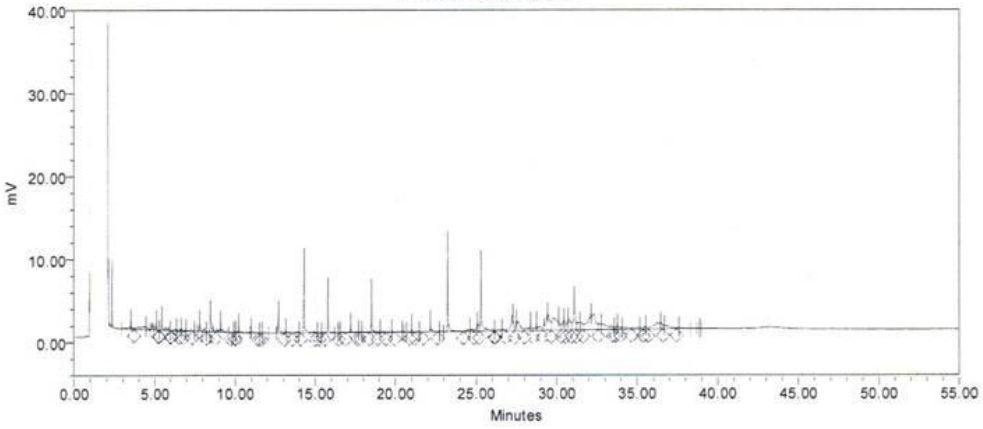
Bedroc 5890 II TEH Analysis in H2O

SampleName = 77499 UI eng
Injection Id = 20139
Vial # = ~~16~~ 48
Inj. VoL = 2.00 ul
Run Time = 55.0 Minutes
Project Name = BEDROC TEH 2011 02
System Name = TEH1

Result Id = 20189

Sample Set Name = 2013 01 18 018 PM
Date Acquired = 1/19/2013 2:58:01 AM CST
Date Processed: 1/22/2013
Acquired By = pma1
Acq. Method Set: diesel full
Processing Method = Mid oil
Report Method Name = Client Sample H2O

TEH Chromatogram



Group Peak Results

	Name	RT	Area	Height	TEH_Final_Result Water_ug/L	Quantitation_Limit Water_ug/L
1	Mid oil	4.44	697088	83676	437045.5	100000

Report Method: Client_Sample_H2O Printed 6:43:46 AM U: 1/22/2013 Page: 1 of 1

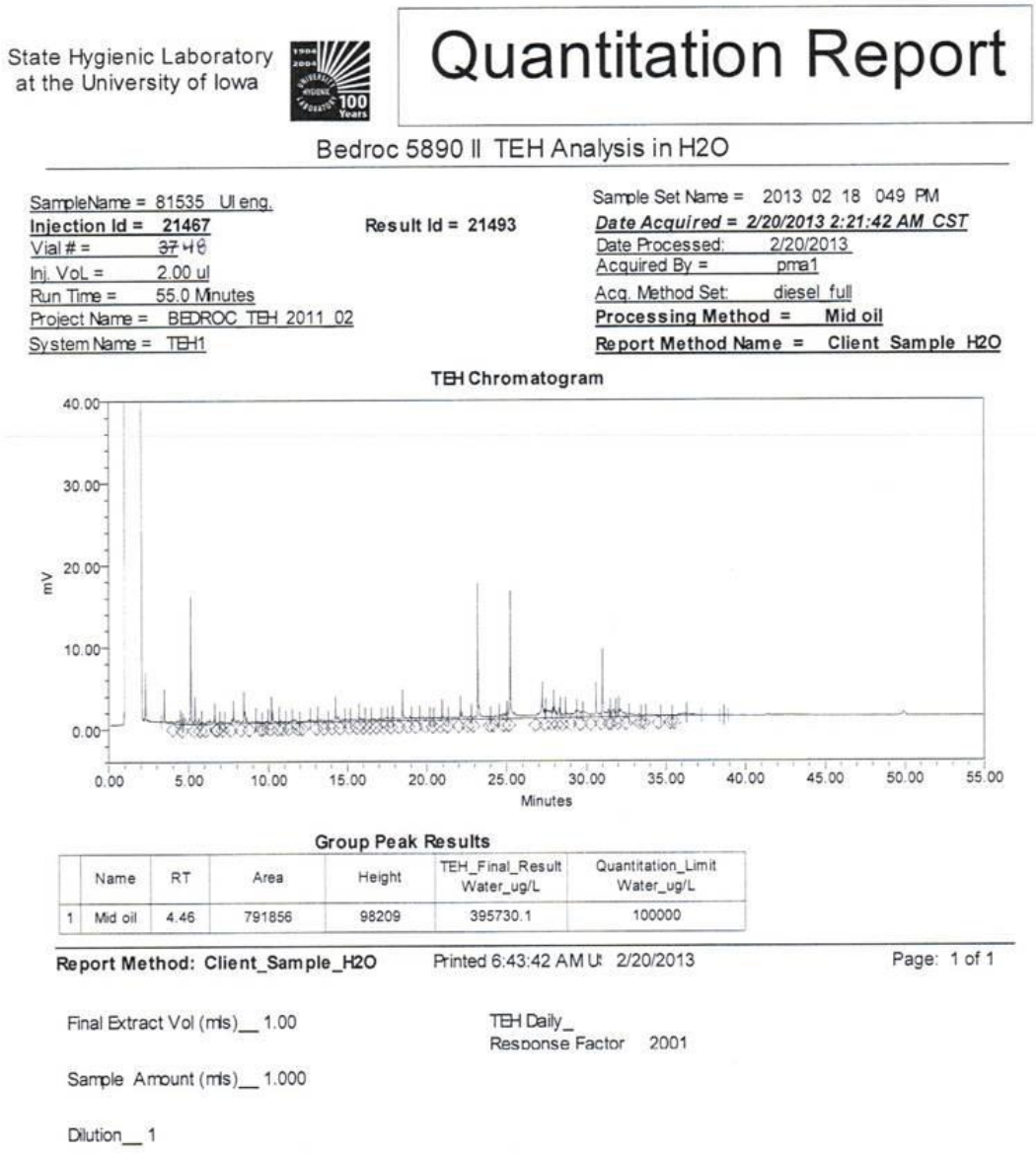
Final Extract Vol (mls)__ 1.00

TEH Daily_
Response Factor 1595

Sample Amount (mls)__ 1.000

Dilution__ 1

Figure C-3: Shows GC-FID extraction results for sample 48 at time 60 days



State Hygienic Laboratory
at the University of Iowa

Quantitation Report

Bedroc 5890 II TEH Analysis in H2O

SampleName = 81535 UI eng.
Injection Id = 21467
Vial # = ~~37~~ 48
Inj. VoL = 2.00 ul
Run Time = 55.0 Minutes
Project Name = BEDROC TEH 2011 02
System Name = TEH1

Result Id = 21493

Sample Set Name = 2013 02 18 049 PM
Date Acquired = 2/20/2013 2:21:42 AM CST
Date Processed: 2/20/2013
Acquired By = pma1
Acq. Method Set: diesel full
Processing Method = Mid oil
Report Method Name = Client Sample H2O

TEH Chromatogram

Group Peak Results

	Name	RT	Area	Height	TEH_Final_Result Water_ug/L	Quantitation_Limit Water_ug/L
1	Mid oil	4.46	791856	98209	395730.1	100000

Report Method: Client_Sample_H2O Printed 6:43:42 AM U: 2/20/2013 Page: 1 of 1

Final Extract Vol (mls)__ 1.00

TEH Daily_
Response Factor 2001

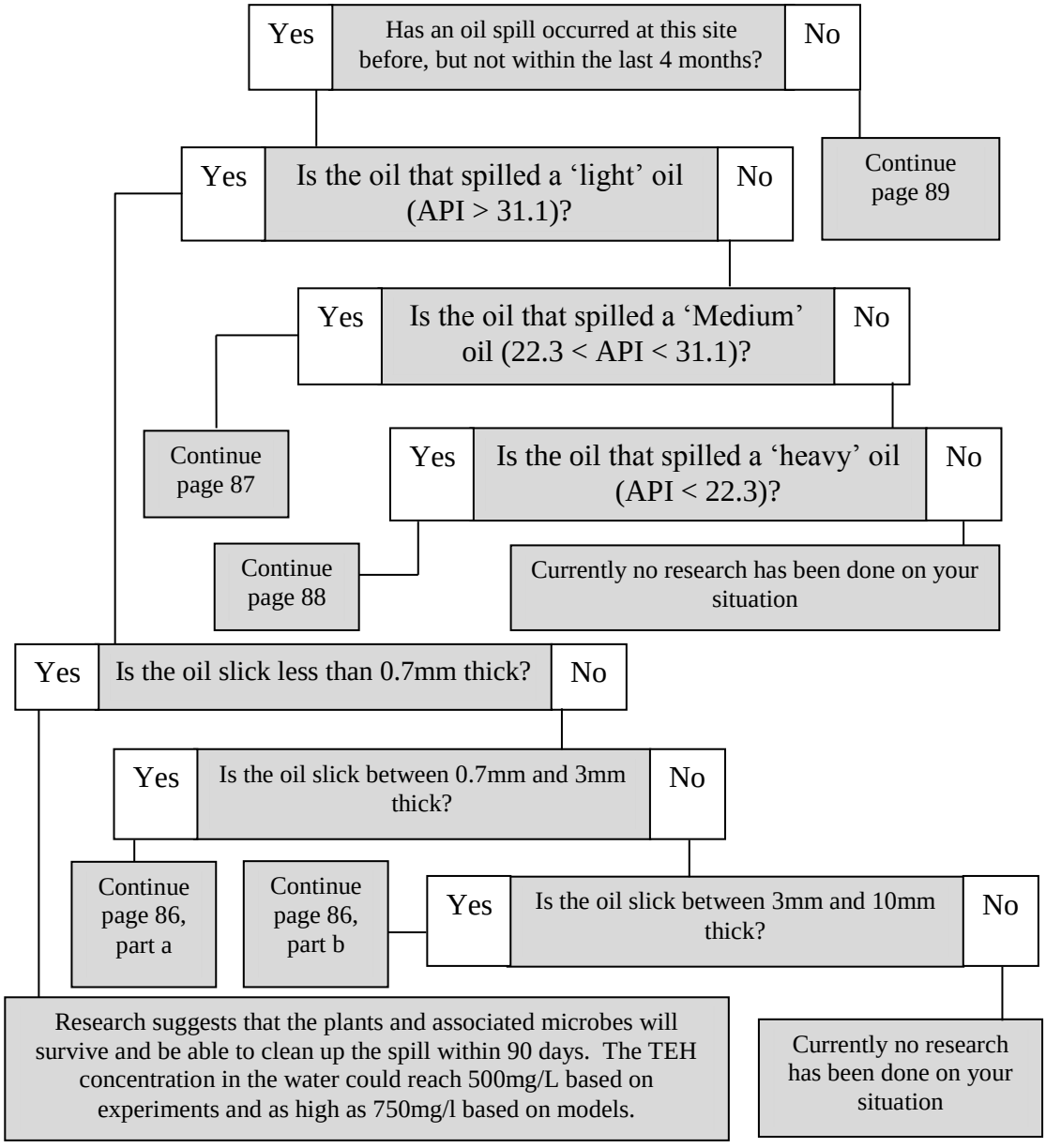
Sample Amount (mls)__ 1.000

Dilution__ 1

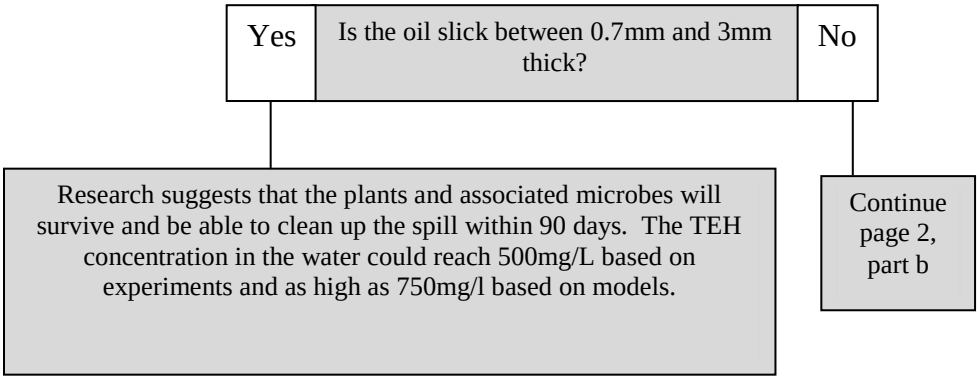
Figure C-4: Shows GC-FID extraction results for sample 48 at time 90 days

APPENDIX D: DECISION TREE

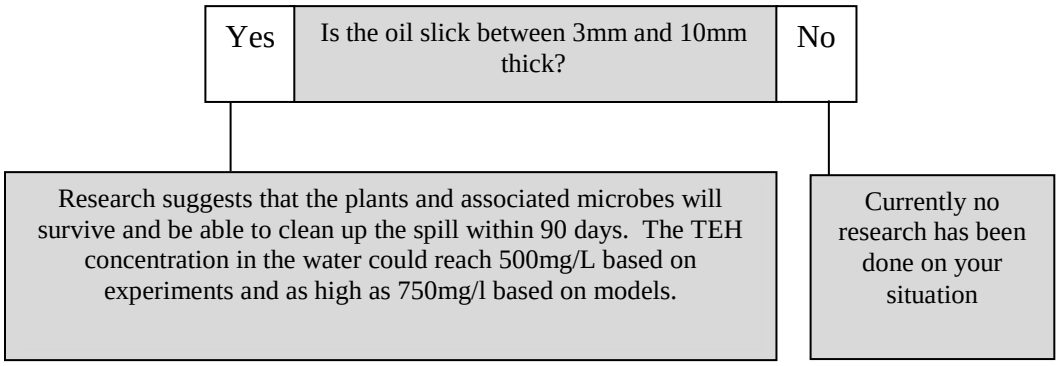
This decision tree has been created for saltmarshes that contain *Spartina alterniflora* and are going to be affected by an oil spill. The decision tree should not be the only reason for acting as there are variables that were not taken into account during this experiment. The decision tree also does not discuss the health effects that the spill may have on aquatic or human life. The decision tree has been setup to allow for changes as more research is discovered in this field.



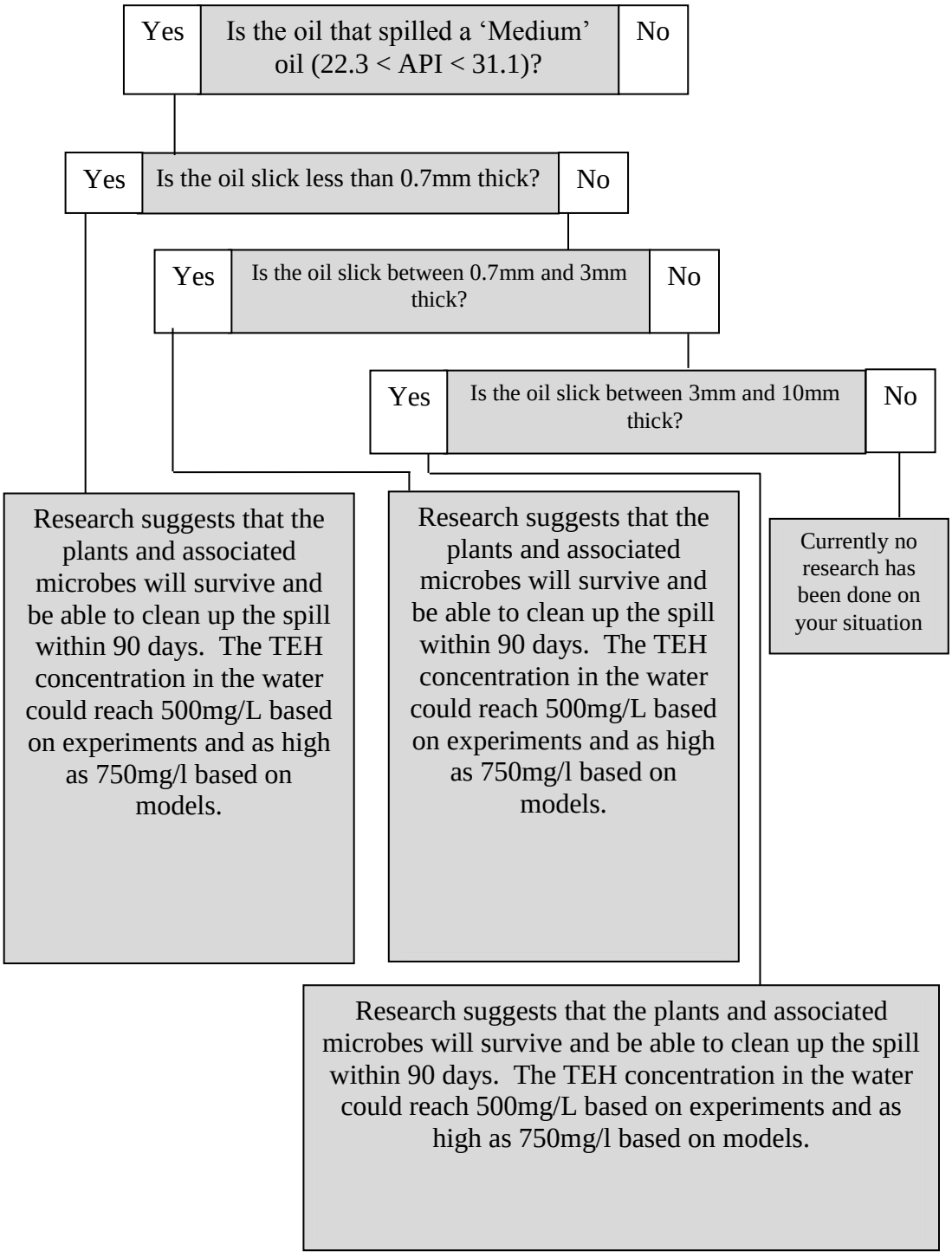
Part a) Begin here for site that has previously been contaminated with oil and a light crude oil.



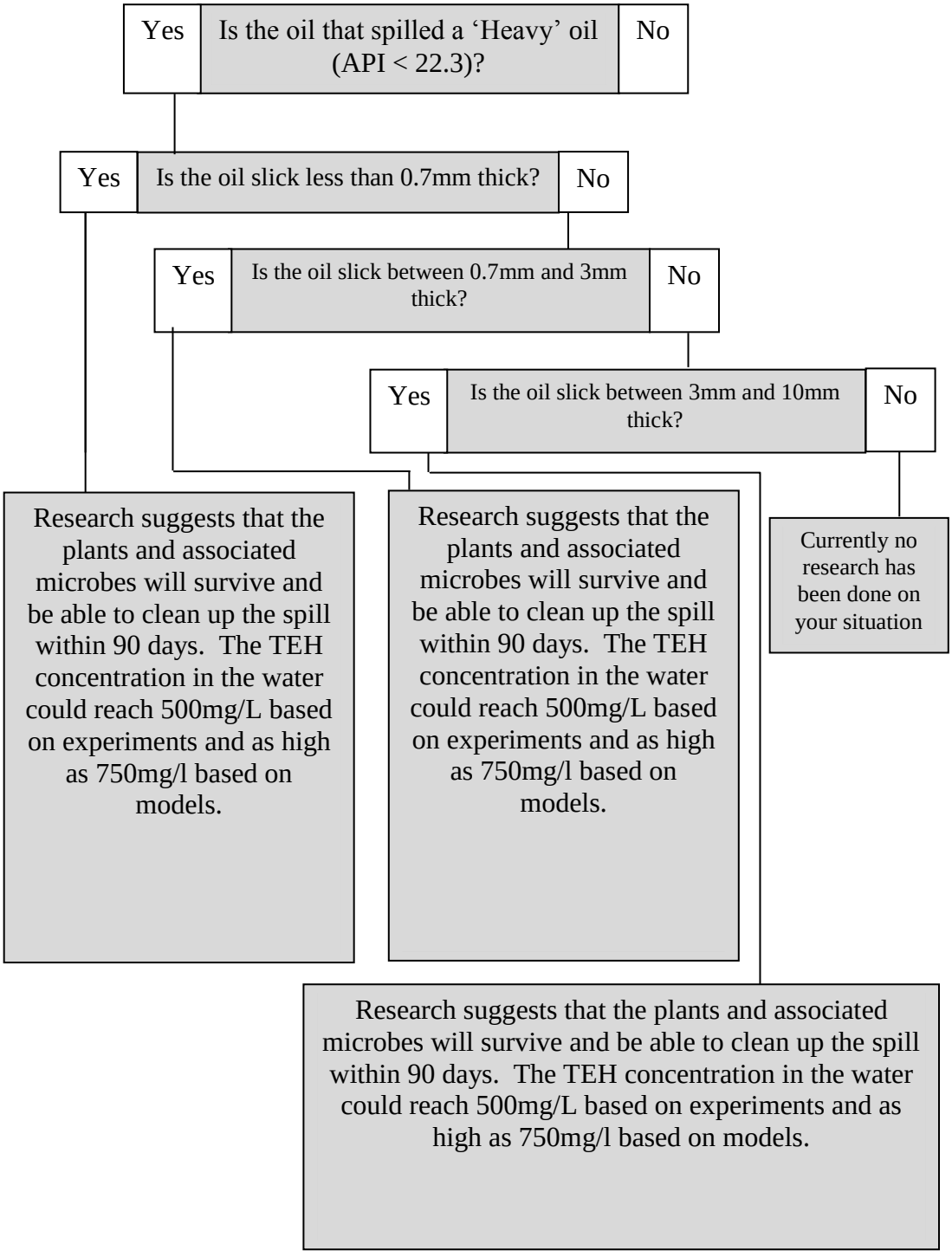
Part b) Begin here for site that has previously been contaminated with oil and a light crude oil



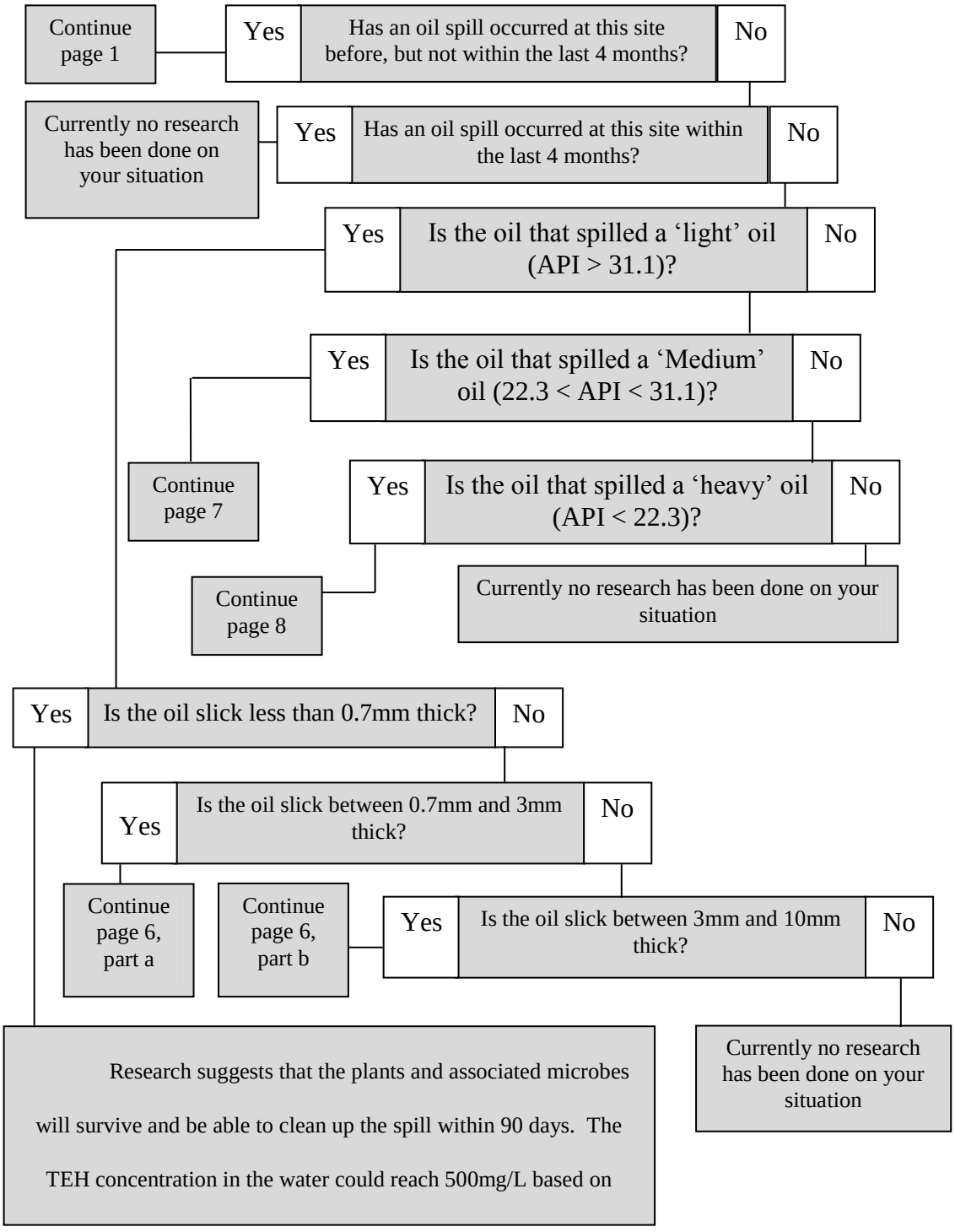
Begin here for site that has previously been contaminated with oil and the oil that spilled is a medium crude oil



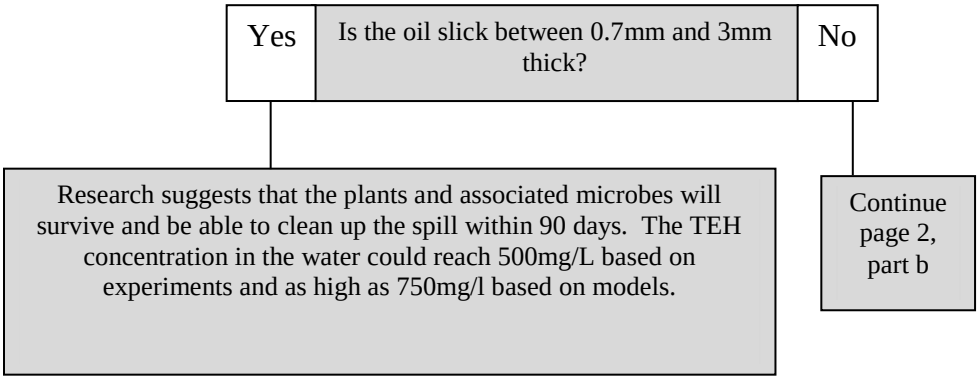
Begin here for site that has previously been contaminated with oil and the oil that spilled is a heavy crude oil



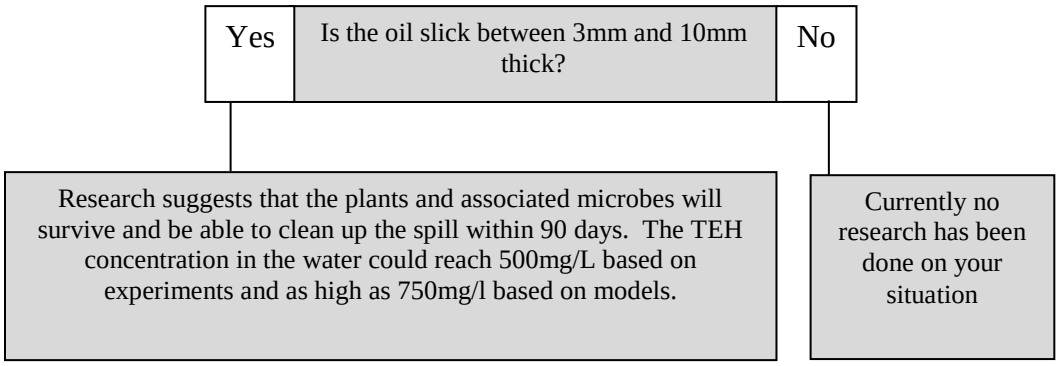
Begin here if the oil spill is occurring in a location where an oil spill hasn't occurred before



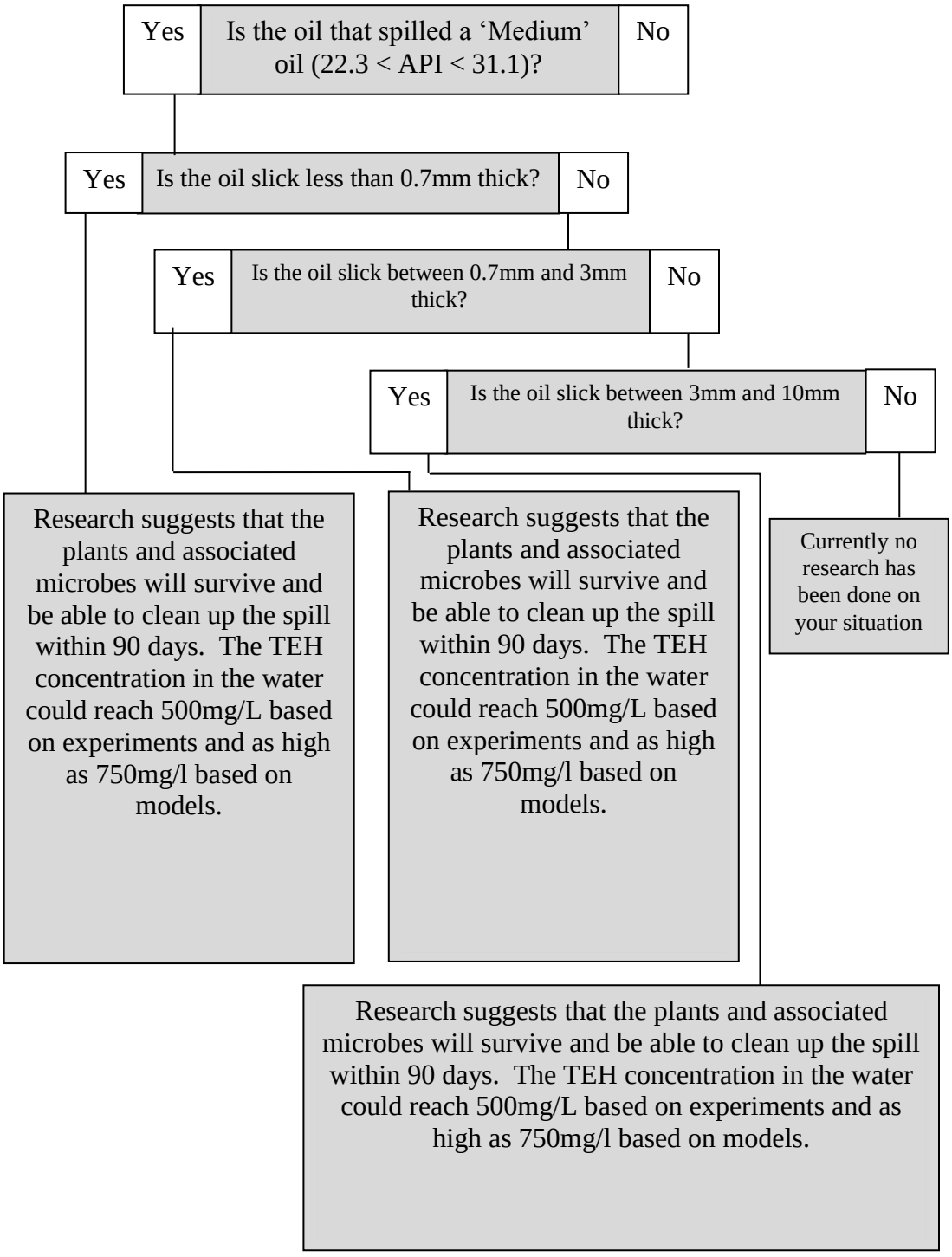
Part a) Begin here for site that has previously been contaminated with oil and a light crude oil.



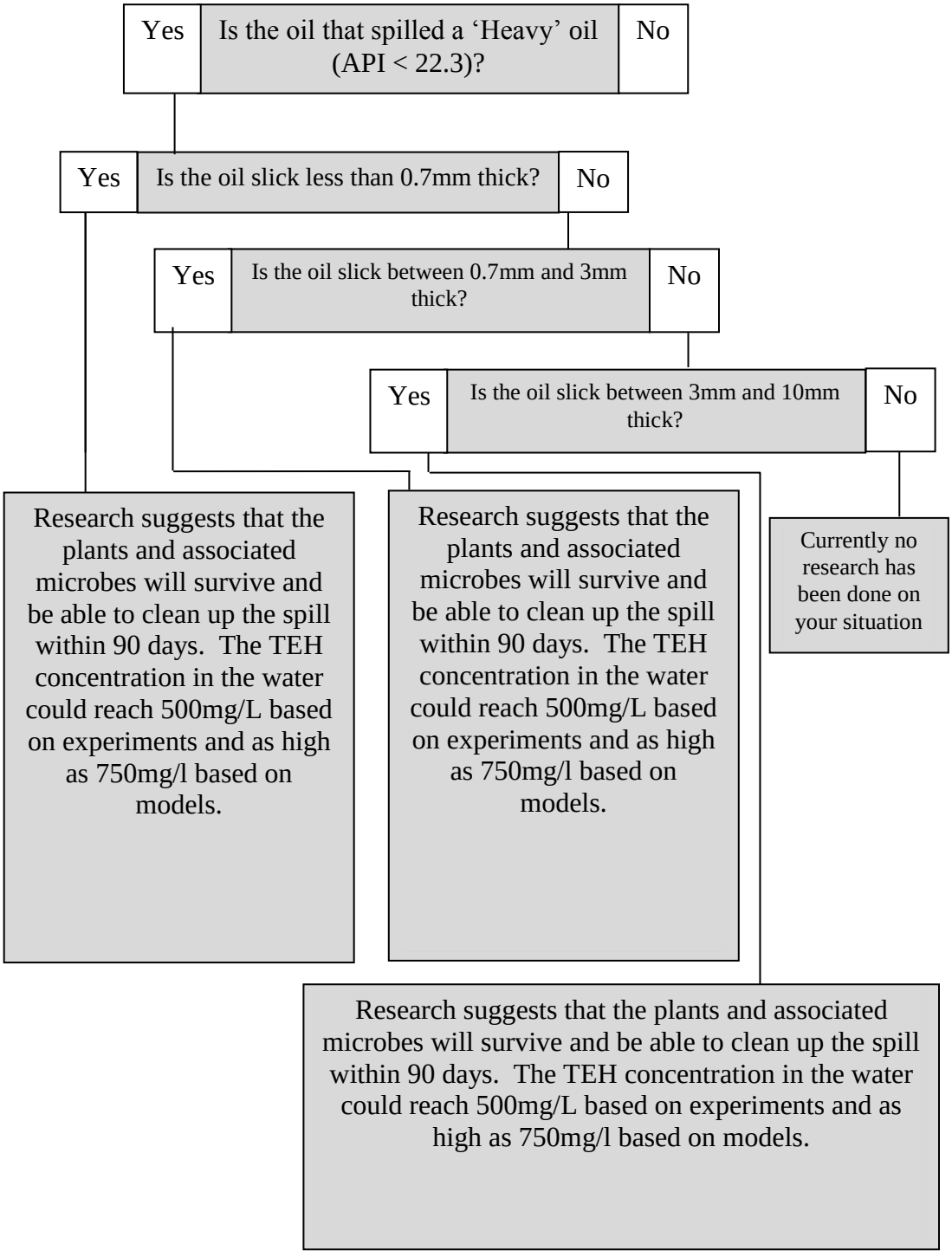
Part b) Begin here for site that has previously been contaminated with oil and a light crude oil



Begin here for site that has previously been contaminated with oil and the oil that spilled is a medium crude oil



Begin here for site that has previously been contaminated with oil and the oil that spilled is a heavy crude oil



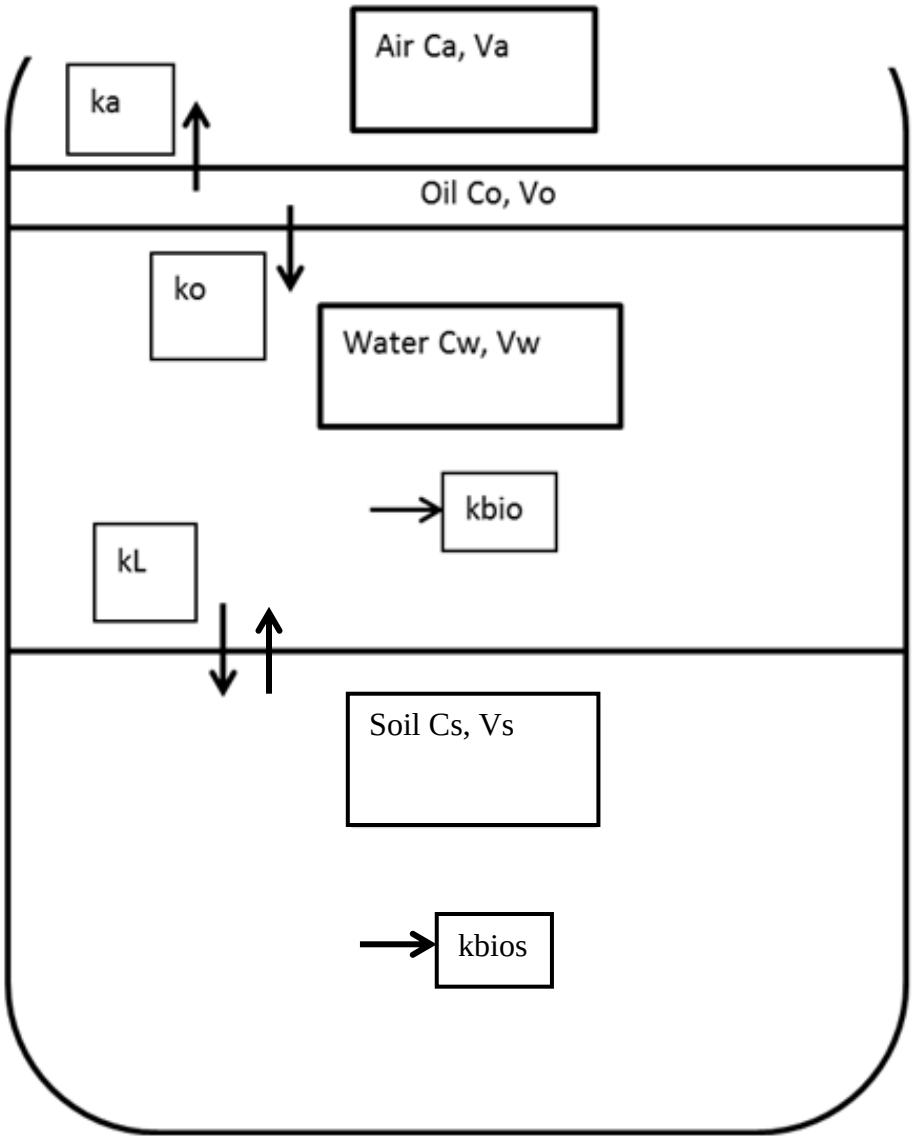
APPENDIX E: MATHEMATICA

Phytoremediation of Total Extractable Hydrocarbons From Crude Oil with the use of Spartina alterniflora

By : Luke Smith

A mathematica model has been created to explain the movement of total extractable hydrocarbons (TEH) in a study that tested the plant, Spartina alterniflora's,ability to degrade oil. The goal of the model is to be able to calculate the concentration and mass of TEH in each phase of the system at any time.

Introduction



Assumptions:

All volumes remain constant except for oil which was show to dissipate
Only one type of oil is present
the oil has an API greater than 10
Constant temperatuer of 23 C
Constant Pressure
Oil does not come into direct contact with soil
Constant concentration of hydrocarbons in the air
No photolysis is occuring
Oxidation and redution pathways are negilgable
Bacteria is present in the water phase and resonsable for some degradation

Variables:

Mt = Total Mass of TEH in the system (g)
MS = Mass of soil in the system (g)
Co = Concentration of TEH in the oil (g/L)
Cw = Concentration of TEH in the water at time t (g/L)
Ca = Concentration of TEH in the air at time t (g/L)
Cs = Concentration of TEH in the soil at time t (g/L)
Cwend = integration of Cw with respect to t
Csend = integration of Cs with respect to t
Vs = Volume of the soil (L)
Vo = Volume of the oil (L)
Vo1 = Initial Volume of the oil (L)
Vw = Volume of the water (L)
Va = Volume of the air (L)
kL = Rate constant of TEH dissolving from water to soil (1/day)
ko = Rate constant of TEH dissolving from oil to water (1/day)
ka = Rate constant of TEH dissolving from oil to air (1/day)
kbio = Rate constant for biodegradation of TEH in the water (1/day)
kbios = rate constant for biodegradation of TEH in the soil (1/day)
Ms = Mass of TEH in the soil phase (g)
Mo = Mass of TEH ni the oil phase (g)
Ma = Mass of TEH in the air phase (g)
Mw = Mass of TEH in the water phase (g)

Amount of Oil Present

Mass of soil in the system in grams

```
MS = 300 ;
```

(*assume 50mg of oil/g of soil *)

Total mass of TEH in the system, in grams

```
Mt = .050 * MS
```

```
15.
```

Calculate specific gravity of oil assuming API of 32.35

```
SG = 141.5 / (32.35 + 131.5)
```

```
0.863595
```

```
Rationalize[SG]
```

$$\frac{2830}{3277}$$

Calculate density of oil

```
ρwater = 1000
```

```
1000
```

- g/L

```
ρoil = ρwater * SG
```

```
863.595
```

g/L

Assume the concentration of oil in the oil phase is equal to the density of the oil

```
Co = ρoil
```

```
863.595
```

g/L

Calculate the volume of oil

```
Vol = Mt / ρoil
```

```
0.0173693
```

- L

Average molecular weight in crude oil, based on Chevron research and Technology

```
MWoil = 200 ;
```

- grams/mol

Inputs

volume of water

```
Vw = .400;
```

- L

volume of oil

```
Voi
```

```
0.0173693
```

- L

volume of air

```
Va = .025;
```

- L

volume of soil, (density for soil from Neubauer, Scott)

```
Vs =  .4
```

```
0.4
```

- L

Total Volume of system in L

```
Vt = Vo + Va + Vw + Vs
```

```
0.825 + Vo
```

surface area incontact with the water

```
A = 3.1415 * 16;
```

- cm2

Vapor Presssure for naphthalene (EPA, Naphthalene)

```
VP = 1.08 * 10^-4
```

```
0.000108
```

- atm

Rate constant for TEH in oil to dissolve in water, found from GSI Chemical

```
ko = 0.864 / A;
```

```
ko = .05
```

```
0.05
```

- 1/days

Rate constant for TEH in water to dissolve into soil, found from Gamst et. al.

```
kL = 1.2*10^-3;
```

```
kL = .05
```

```
0.05
```

- 1/day

Rate constant for biological degradation, found from Naphthalene

```
kbio = 2;
```

- 1/day

Rate constant for biological degradation in soil, value found from Al-Bashir

```
kbios = 1.7*10^-6;
```

- 1/day

Henry's constant taken from the EPA

```
kh = 4.83*10^-4;
```

- (atm*m^3)/mol

Partitioning coefficient , value found from Abuln

```
kp = 2500;
```

- L/kg

Suspended solids concentration in water, based on saltmarsh values from Settlemyre

```
M = 3.93*10^-4 ;
```

- kg/L

Height of water in the beaker

```
H = 40;
```

- cm

Depth of soil in the beaker

```
d = 10 ;
```

- cm

TEH in the Air and Oil

Air

Assume the chemicals volatilize into the air relatively fast and therefore reach equilibrium almost instantaneously

```
Clear[t]
```

```
Ca = (MWoil * VP) / (kh * 10^6)
```

```
0.0000447205
```

- g/L

Oil

The change in the volume of oil is related to the mass of oil that is transfered to the water phase. It is assumed that the concentration of the oil remains relatively constant and that the dissolution between water and oil effects the volume more than the concentration. The reason for this assumption is due to observation of oil loss during experiments.

Change in Oil volume

```
Vo = Voi * E^(-ko * t)
```

$$0.0173693\, e^{-0.05\, t}$$

Concentration of TEH in Water and Soil

Assume water is never in direct contact with air

Fraction dissolved

```
fd = 1 / (1 + (kp * M))
```

```
0.504414
```

Fraction particulate

```
fp = 1 - fd
```

```
0.495586
```

Clear values

```
Clear[Cw]
Clear[Cs]
Clear[t]
```

Solve for Cw and Cs between time 0 and t

```
Clear[Cs, Cw]
solu5 =
 Solve[{Integrate[1, Cs] == Integrate[((kL * Cw * Vs) / Vw) - (kbios * Vs * Cs / Vw), {t, 0, t}],
   Integrate[1, Cw] == Integrate[((ko * Co * Vo) / Vw) - kL * Cw - kbio * Cw, {t, 0, t}]},
  {Cs, Cw}]
```

$$\left\{\left\{Cs \to \frac{538\,020.\, e^{-0.05\,t}\left(-1. + 1.\, e^{0.05\,t}\right) t}{(0.487805 + 1.\, t)\,(588\,235. + 1.\, t)},\ Cw \to -\frac{1.\,\left(-37.5 + 37.5\, e^{-0.05\,t}\right)}{1. + 2.05\, t}\right\}\right\}$$

Select concentration of TEH in the soil and water

```
Csend = Cs /. solu5[[1]]
```

$$\frac{538\,020.\, e^{-0.05\,t}\left(-1. + 1.\, e^{0.05\,t}\right) t}{(0.487805 + 1.\, t)\,(588\,235. + 1.\, t)}$$

```
Cwend = Cw /. solu5[[1]]
```

$$-\frac{1.\,\left(-37.5 + 37.5\, e^{-0.05\,t}\right)}{1. + 2.05\, t}$$

Mass of TEH in Phases with respect to time

Air

```
Clear[t]
Ma = Va * Ca / 1000
```

$$1.11801 \times 10^{-9}$$

Oil

```
Clear[t]
Mo = Co * Vo
```

$$15.\, e^{-0.05\, t}$$

Water

```
Clear[t]
Mw = Cwend * Vw
```

$$-\frac{0.4\, \left(-37.5 + 37.5\, e^{-0.05\, t}\right)}{1. + 2.05\, t}$$

Soil

```
Clear[t]
Ms = Csend * Vs
```

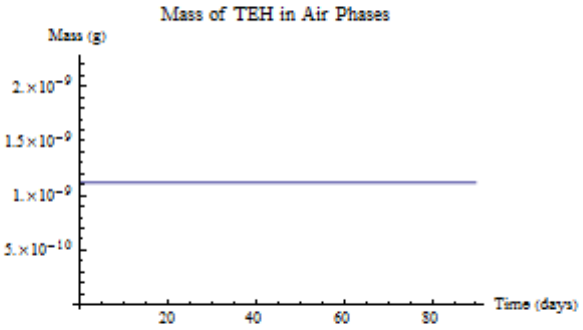
$$\frac{215\,208.\, e^{-0.05\, t}\, \left(-1. + 1.\, e^{0.05\, t}\right)\, t}{(0.487805 + 1.\, t)\, (588\,235. + 1.\, t)}$$

Plots of Mass vs. Time

Air

```
In[48]:= Plot[Ma, {t, 0, 90}, PlotLabel → "Mass of TEH in Air Phases",
          AxesLabel → {"Time (days)", "Mass (g)"}]
```

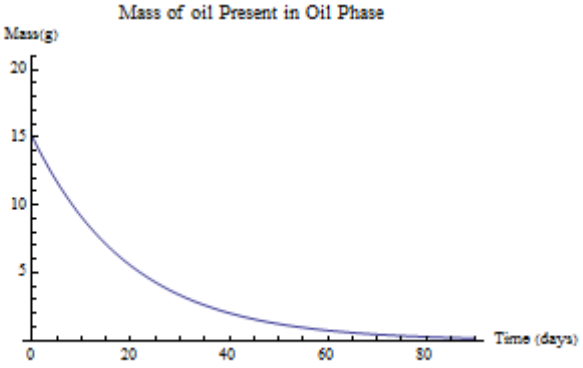
Out[48]=



Oil

```
In[49]:= Plot[Mo, {t, 0, 90}, PlotRange → {0, 21}, PlotLabel → "Mass of oil Present in Oil Phase",
          AxesLabel → {"Time (days)", "Mass(g)"}]
```

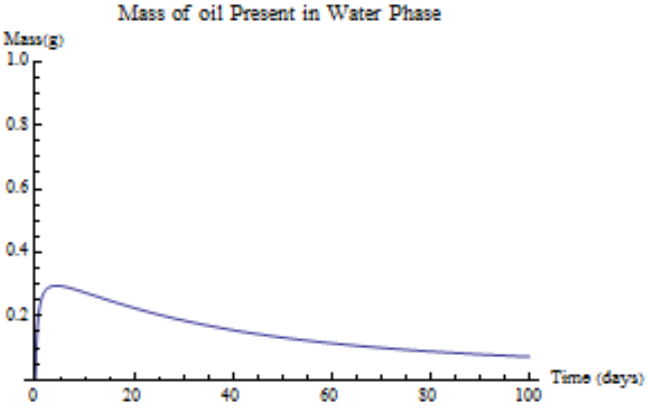
Out[49]=



Water

```
In[50]:= Plot[Cwend, {t, 0, 100}, PlotRange → {0, 1},
          PlotLabel → "Concentration of TEH Present in Water Phase",
          AxesLabel → {"Time (days)", "Concentration(g/L)"}]
         Plot[Mw, {t, 0, 100}, PlotRange → {0, 1},
          PlotLabel → "Mass of oil Present in Water Phase",
          AxesLabel → {"Time (days)", "Mass(g)"}]
```

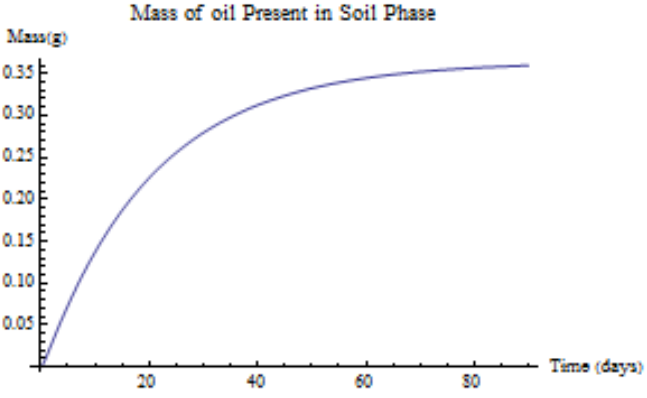
Out[51]=



Soil

```
In[52]:= Plot[Ms, {t, 0, 90}, PlotLabel → "Mass of oil Present in Soil Phase",
          AxesLabel → {"Time (days)", "Mass(g)"}]
```

Out[52]=



Total Mass of TEH after 90 Days

Air

t = 90

Ma = Va * Ca / 1000

90

1.11801×10^{-3}

- g

Oil

t = 90

Mo

90

0.166635

- g

Water

t = 90

Mw

90

0.0799642

- g

Soil

t = 90

Ms

90

0.359784

- g