Evaluation of Habitat Response to *In Situ* Burning as a Method of Oil Removal

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Abstract

Two greenhouse studies were conducted to determine the effects of oiling and oiling plus burning on *Spartina alterniflora*, *Panicum hemitomon* and *Sagittaria lancifolia* plants. Plant-sediment cores were collected from marsh habitats, transported back to the greenhouse, and equilibrated for 30 days. Main treatments included controls, oiling and oiling plus burning. South Louisiana Crude was applied to the oil and oil plus burn treatment replications at a rate of 2 L m^{-2}. Treatments designated for burning were burn and shoot regeneration, biomass production, and plant height. These treatments were monitored over the evaluation periods.

In the first greenhouse burn experiment, oiling and oiling plus burning had a significant effect on *Spartina alterniflora* and *Panicum hemitomon* shoot regrowth. For the oiling only treatment, shoot regeneration for the *Spartina alterniflora* cores increased approximately 33% over the 54 day sampling period. Shoot regeneration in the *Panicum hemitomon* cores decreased about 85% over the same time period for the oil treatment. Oiling plus burning had drastic effects on the *Spartina alterniflora* and *Panicum hemitomon* plants. No new shoots were recorded for either species over the 54 day monitoring period after the burn. Oiling plus burning killed the plants in the cores.

For the second greenhouse burn experiment, the oiling and oiling plus burn treatments did not have such drastic effects on *Sagittaria lancifolia* plants. For the oiling only treatment of saturated *Sagittaria lancifolia* cores the mean live shoot value increased 46% over the 26 day monitoring period. For the oiling plus burning (for saturated cores) the mean live shoot value increased from 0 (due to burn) to 22 live shoots per replication. Shoot regeneration in oil only cores with two inches of floodwater decreased from 14 (at day 0) to 12 live shoots per core at day 26. For the oil plus burn treatment (cores with two inches of floodwater) the live shoot mean increased from 0 (at day 0) to 21 live shoots per core at 26 days.

With the aid of gas chromatography mass spectroscopy (GC/MS) techniques the following was achieved: hydrocarbon identification, quantification of two oil sources (South Louisiana Crude and Arabian Crude), and identification of oil residues extracted from salt marsh sediment samples for the oil and oil plus burn treatments. The two oil sources displayed different polynuclear aromatic hydrocarbon fingerprints. The number and concentrations of polynuclear aromatic hydrocarbons decreased after oiling and oiling plus burning.

1.0 Introduction

1.1 Background

Coastal wetlands bordering the northern Gulf of Mexico account for 58% of all the coastal wetlands in the United States (Alexander *et al*. 1986). These coastal areas support a rich, diverse wildlife that is heavily dependent upon production of estuarine flora and fauna. In many areas of this region, considerable quantities of petroleum are transported, received, and stored on a regular basis. Intense activity associated with oil and gas production has the potential for reducing productivity of the area through oil spills. The potentially adverse effects of oil spills on marsh vegetation could have widespread repercussions for the entire estuarine ecosystem's food web. Studies by Webb *et al*. (1981) and Hershner and Moore (1977) suggest that the effects of oil on vegetation and subsequent recovery depend on many factors including oil type and concentration, the extent of coverage and the timing of the oil spill. These effects may also be species specific (Hershner and Moore, 1977; Webb *et al*. 1981, 1985).

1.2 Effects of Oil Spills on Vegetation

Data in the literature show great variations in responses of macrophytes to oil hydrocarbons (Crapp, 1971; American Petroleum Institute, 1985; Mendelssohn *et al*. 1995). This reflects several factors including the differences in species susceptibility to oil compounds, types of oil used, experimental conditions, and stage of growth for a given species during its life cycle. The toxicity of crude oil is primarily due to the lower boiling point of volatile aromatic hydrocarbons. Oil type and the degree of weathering largely determine the toxicity levels (Crapp, 1971).

Exposure to oil may adversely affect marsh vegetation (Cowell, 1969; Holt *et al*. 1978; de la Cruz *et al*. 1981, Pezeshki and DeLaune, 1993). An oil spill resulted in severe reduction of growth in a salt marsh in Texas (Holt *et al.* 1978). Exposure to crude oil at 1.5 L m^{-2} resulted in death of *Juncus roemerianus* with recovery reported within three years (de la Cruz *et al.* 1981). Petroleum hydrocarbons at 2 L m^{-2} adversely affected gas exchange functions of *Juncus roemerianus* and *S. alterniflora* under laboratory conditions (Pezeshki and DeLaune, 1993). Net photosynthesis decreased in both species shortly after treatment initiation and remained within 71 to 94% of control in *J. roemerianus* and within 53 to 80% of control in *S. alterniflora*. However, there were no lethal effects observed.

Under field conditions, plants would likely recover once residual oil is removed by rainfall or tidal action. Growth responses, including growth of new shoots and overall plant health, show adverse effects of oil application in several species. However, these effects are likely to be short-term (Sjotun and Lein, 1993). In previous studies (DeLaune *et al*. 1979, 1984), experimental application of oil to a Louisiana salt marsh caused no reduction in biomass production as measured by above-ground biomass at the end of the second growing season following oil addition. Application of 0.25 L m^{-2} of crude oil to *S. alterniflora* salt marsh caused little damage to the existing stocks or to the regeneration of new plants. DeLaune *et al*. (1979) observed no significant changes in regeneration of new shoots and above-ground biomass of *S. alterniflora* four and 16 months after oil addition at 1, 2, 3, 4 and 8 L m^{-2} to marsh under field conditions. Various studies have indicated that biomass in *S. alterniflora* is not sensitive to crude oil application as much as 32 L m^{-2} (DeLaune *et al*. 1979, 1984; Smith *et al*. 1981, 1984).

1.3 Effects of Oil Spills on Microbial Biomass

In general, microbial biomass increases initially after an oil spill in marsh sediments. Studies have documented changes in microbial populations in wetlands in response to oil impacts (e.g. Kator and Herwig, 1977; Hood *et al*. 1975). These responses were generally increases in total microbial populations and increases in the ratio of hydrocarbon degraders to total heterotrophs. Little is understood about how changes in microbial numbers affect the turnover of oil components and the length of time for remediation of wetland systems.

The rate and extent of microbial degradation of petroleum hydrocarbons is largely determined by environmental conditions. These conditions include temperature (Bartholomew and Pfaender, 1983), salinity (Bourquin and Pryzybyszewski, 1977), Eh (Hambrick *et al*. 1980; Pardue *et al*. 1988), pH (DeLaune *et al*. 1981), and the oxygen and nutrient status of the environment (Cooney, 1984). Biodegradation of petroleum hydrocarbons is primarily an aerobic process, requiring the presence of molecular oxygen (high Eh). Several novel microbial processes have been identified that degrade oil components under anaerobic conditions [e.g. degradation of BTEX that degrades oil components under anaerobic conditions and degradation of BTEX compounds (Hutchins *et al*. 1991) and naphthalene under denitrifying conditions (Milhelcic and Luthy, 1988a, b]. It is likely that other anaerobic processes have yet to be determined, however, aerobic processes act on a broader spectrum of compounds and are more rapid and complete (e.g. mineralization to CO_2 and H_2O).

1.4 Vegetative Responses to Burning

Fires in wetland habitats occur naturally (Wilbur and Christensen, 1983; Davison and Bratton, 1988), and as a marsh management tool they are used extensively in North America (Kirby *et al*. 1988). Periodic or annual burning is also used as a marsh management tool in Louisiana. In areas of intense management such as state refuges, as much as 80% of the marsh is burned annually compared to an estimated 25 to 30% in other areas (Feijtel *et al*. 1985). After a fire, there is an increase in species richness in marsh habitats (Davison and Bratton, 1988). Burning changes the relative importance of species in *S. cynosuroides* and in *Juncus* marshes (Hackney and de la Cruz, 1983). In other marsh systems, no change in species composition was found due to fire (Vogl, 1973; Van Arman and Goodrick, 1979). A shift in relative importance of species was reported due to fire in Florida marshes (Schmalzer *et al*. 1991).

The post-fire recovery of productivity is dependant on many factors including species at the time of burning during the growing season. A significant increase in regenerating culms, plant gas exchange, and above-ground production was found in annually burned *S. pectinata* as compared to biennially burned vegetation in a natural tallgrass in Kansas (Johnson and Knapp, 1993). Similar findings are reported in coastal species *S. bakeri* marsh in Florida (Schmalzer *et al*. 1991). Live biomass in burned *S. bakeri* marsh did not recover to preburn levels in one year (Schmalzer *et al*. 1991). Total biomass in *Cladium jamaicense* was only 38% of unburned stand within 18 months after burning (Steward and Ornes, 1975). On the other hand, productivity in a *S. cynosuroides* marsh was enhanced by burning (Hackney and de la Cruz, 1983). In other marsh habitats, there are reports of little change in productivity or reduced production due to burning (Smith and Kadlec, 1985a,b; Turner, 1987). The recovery appears to be rapid in marshes that die back annually. For example, burned *Panicum hemitomon* marshes produced greater live biomass within six months of burning as compared to unburned stands (Vog, 1973; van Arman and Goodrick, 1979).

Burning of grasses stimulates new growth of above-ground biomass, but the effects on root biomass in most cases are unknown. The effects on root biomass may be significant to marsh ecology because roots make up 90 to 95% of most organic peat soils. Although the root contribution to the soil organic matter is significant, little research has addressed the below-ground biomass responses to burning. Plant health, growth, and productivity are important as the main sources of organic matter for peat accumulation, which is in turn necessary for maintaining marsh surfaces intertidal (DeLaune *et al*. 1983). Marsh surfaces developed in sediment deficient habitats remain intertidal primarily due to plant growth, organic detritus accumulation, and limited mineral sediment deposition (DeLaune *et al*. 1983). Marsh burning reduces the organic source, which may indirectly affect marsh aggradation in areas experiencing aggradation deficits. Burning during dry periods can cause damage to plant root systems, which in turn may accelerate marsh deterioration in unstable coastal areas and lead to pond formation (Hoffpauer, 1968). Although burning may be an acceptable practice in stable coastal marsh regions, in areas where large aggradation deficits exist, marsh burning may reduce the source of organics below the critical level needed for maintaining a viable marsh.

1.5 Burning as a Method of Oil Removal

Burning has been used as a method of removing volatile oil components after an oil spill. This practice may employ burning agents (Freiberger and Byers, 1971). Various igniters including Knotax and primers such as gasoline and kerosene may be used along with combustion promoters such as wicking agents, thermal insulators and volatility modifiers (Energetex Engineering, 1979). A successful burning operation may leave a thin, viscous film between 0.5 to 1.2 mm thick on the marsh surface (American Petroleum Institute, 1985). The technique at best is controversial. It has been considered inefficient in certain habitats (Ford, 1970; Der and Ghormley, 1975; Logan *et al*. 1975), while it is regarded as one of the most effective cleanup techniques available in other habitats (Vandermeulen and Ross, 1977). For instance, in an oil affected *Spartina* marsh in Texas, burning resulted in partial oil removal from vegetation with some heavily oiled vegetation and residue remaining on unburned portions of stems. Within six months burned unoiled and lightly oiled vegetation had recovered rapidly while heavily oiled unburned marshes showed only moderate recovery (Holt *et al*. 1978).

1.6 Rationale and Significance

The susceptibility of individual wetland habitats to an oil spill and the proper cleanup method for habitat recovery have been topics of interest to various agencies. Potential impacts of burning as a method of oil removal include disturbance and death of biota from the direct effects of burning as well as the potentially toxic effects of residual compounds. These compounds include unburned oil and burned oil products that may penetrate the lower sediment where degradation is slow and potential for rerelease and continuous adverse effects on biota is high. These residual materials may have lethal or sublethal effects on various organisms. Marsh burning also results in temporary loss of cover, loss of detrital materials important to the food web, and loss of other functions such as feeding and resting areas for birds and other wildlife species. In marsh habitats, burning results in loss of vegetation and other organisms through direct heat effects. Uncontrolled burning may have adverse effects on adjacent marshes not directly affected by oil. This method also affects most of those species sensitive to burning, which may result in slow recovery or elimination of such species. However, the effects of

surface burns on oiled marsh biota have not been studied in detail.

While the existing work does address some aspects of oil effects on vegetation, it does not address the combined oiling and burning effects. We have recently completed two oil related research projects (Pezeshki and DeLaune, 1993; DeLaune *et al*. 1994) that addressed macrophyte responses to oiling but did not address oiling and burning. In addition very little is known about the behavior and fate of hydrocarbon compounds (or residual compounds left by burning) in various marsh systems. Using a multidisciplinary approach we attempted to address not only the effects of such stressors on biota but also the movement and degradation of hydrocarbon compounds (with or without burning) in various habitats. Several questions need to be answered including: what are the specific effects of oil spills on biota in each marsh type (salt and fresh)? What are the consequences of burning for marsh biota in various habitats? What percentages of carbohydrate components are removed by burning? What happens to the specific residual carbohydrate compounds that are byproducts of burning? What are the degradation rates of these compounds?

While the proposed project will not be able to answer all of these questions, we will attempt to answer as many as possible while providing some insights into various aspects of oil spills and burning. There are immediate benefits and direct application of this proposed research to Louisiana's coastal/interior wetlands. Data will allow evaluation of burning as a method of oil removal in specific habitats in Louisiana's vast wetland systems. The proposed research will address several areas of concern as listed in the RFP for OSRADP. In particular, the environmental consequences and effectiveness of *in situ* burning in a salt marsh (second year) and fresh marsh (third year) will be described. The proposed research is expected to contribute to our understanding of: (1) U.S. Gulf Coast marsh habitats' sensitivity to oil spills followed by *in situ* burning, and (2) spill timing in relation to the various life cycles for the species involved. The recovery of various habitats including vegetation and the post-treatment lethal/sublethal effects of burned and unburned oil residual components will be investigated. Based on this information, the feasibility of burning as a method of oil removal in various marsh habitats will be evaluated and quantified. This will allow us to make recommendations about the use of this technique for dealing with oil spills in various marsh habitats in U.S. Gulf Coast areas.

1.7 Hypotheses

- Burning of oiled marsh can be used as a remediation technique in selected marsh habitats of the U.S. Gulf Coast.

- Burning impact is short-term (one to two years). Marsh recovery following an oil spill is enhanced by the oil removal effects of burning.

1.8 Objectives

The proposed study will allow quantification of oil spill and burning impacts on several species representing a wide range of coastal habitats including salt marsh, brackish and freshwater. Greenhouse studies will be undertaken in Year One, and a salt water habitat field study will be performed in Year Two. Specific objectives are to evaluate:

- The effects of oiling and burning on flora in selected marsh habitats.
- The mechanisms of such impacts on marsh macrophytes.
- The impact of oiling and burning on flora recovery and dynamics during the post-treatment period.
- The percentage removal of oil compounds by fire and the degradation of various residual compounds that are byproducts of burning.

2.0 Methodology

2.1 Methods

The study will be conducted in three phases. Phase I of the study was conducted in a greenhouse and laboratory (Year One). Phase II will be conducted in the field and will complement and reconcile the proposed laboratory experiments performed in Year One. Work plans and time schedules for Phase I and Phase II are shown in Table 2.1 and 2.2.

2.1.1 Laboratory and Greenhouse Studies (Phase I - Year One)

South Louisiana "sweet" Crude (SLC) which is enriched with light aromatic hydrocarbons, paraffins and olefins, was used in the greenhouse studies. SLC is moderately toxic to various organisms and is degraded by indigenous microflora. Sediment cores 30 cm deep and 15 cm in diameter containing *Spartina alterniflora/S. patens* (representing salt marsh/brackish habitat) and *Panicum hemitomon/Sagittaria lancifolia* (representing freshwater habitat) were collected from Louisiana marshes. The cores were transferred to a greenhouse for the study. Replicated cores were randomly assigned to main treatments of: (1) oiling; (2) oiling plus burning; (3) no oiling or burning, plants clipped at marsh surface; and (4) a control (no oiling or burning).

Cores containing plants were placed in large containers 75 cm deep and partially filled with water from the field site where plants were collected. Oil was added to these containers at 2 L m^{-2}. The water level was raised slowly to mimic high tide conditions until it reached 25 cm above the soil surface of each pot. After eight hours, the water was released slowly by removing rubber stoppers and allowing the water level to fall to the soil surface as would happen during a low tide. Plants were thus coated with oil in a way that mimicked the rise and fall of tides in a real world oil spill. The pots designated for burning were then subjected to burning by ignition. The plants were monitored continuously for a period of post-treatment evaluations. Sediment/plant cores were kept waterlogged and/or a two to three inch floodwater layer maintained. The study compensated for evaporation by adding fresh water to the pots daily to maintain constant salinity levels. Each month the soil was flushed and water from the respective field sites was used to fill the pots.

The study was conducted in a greenhouse over the evaluation period allowing observation and measurements of plant responses to oiling, oiling plus burning, and control treatments. The experimental design for the greenhouse was a randomized block design offering a factorial treatment arrangement with four replications.

2.1.2 Field Studies (Phase II - Year Two)

Based on the ongoing greenhouse experiments, a study area will be established in a salt marsh of Barataria Basin, Louisiana (Phase II - 1995/1996). An additional *in situ* burn study will be conducted in a fresh marsh during Phase III (1996/1997) if additional funding is received.

Each plot will be 2m x 2m with enclosures made of aluminum sheets installed to a depth of 15 cm into the sediment to minimize oil leaks to adjacent areas. South Louisiana Crude will be slowly applied at 2 L m^{-2} to the surface of the marsh in each plot. Seventy-two hours after

Table 2.1 Work plan and time schedule of greenhouse experiments conducted during year 1.

	1994									1995				
	M	J	J	A	S	O	N	D	J	F	M	A	M	J
Greenhouse Studies														
Preparing planting stocks	X	X				X	X			X	X			
oiling/burning experiment			X									X		
plant health			X	X	X	X	X	X	X	X	X	X		
regeneration			X	X	X	X	X	X	X	X	X	X		
biomass			X	X	X	X	X	X	X	X				
degradation of hydrocarbons							X	X	X	X				
data analyses, report writing												X	X	X
preparation for the 2nd year (site selection & permit)						X	X	X	X	X	X	X	X	X

Table 2.2 Work plan and time schedule of proposed field burn study to be conducted in year 2.

	1995						1996					
	J	A	S	O	N	D	J	F	M	A	M	J
FIELD STUDIES (salt marsh)												
- site selection	X											
- obtain permit	X											
- plot layout	X											
- oiling/burning	X			X								
- plant growth	X	X	X	X	X	X		X	X			X
- regeneration	X	X	X	X	X	X		X	X			X
- biomass	X	X	X	X	X	X		X	X			X
- carbon/nitrogen flux	X	X	X	X	X	X		X	X			X
- degradation of hydrocarbons	X	X	X	X	X	X		X	X			X
- data analyses, report writing									X	X	X	X

completion of treatment, the designated plots will be burned following the procedure described in detail by Breuel (1981) and American Petroleum Institute (1985 Manual). Any remaining floating oil will be collected for proper disposal, and the retainers will be removed to allow normal water exchange between each plot and the surrounding area.

The experimental design will be a randomized block field design offering a factorial treatment arrangement with four replications. Two burn times (summer and fall), two oiling levels (oiled and unoiled), and two burning levels (burned and unburned) will be used. Data analysis will be conducted using Statistical Analysis System (SAS). The package is available to the researchers through the Louisiana State University Network Computer Systems.

Proposed measurements include those outlined (see Section 6.9.3). Field measurements will be taken one week, two weeks, one month, two months, four months, and eight months after each burn cycle (see Work Plan/Time Schedule).

2.1.2.1 Site Selection and Burn Permit

Permission to conduct the proposed research has been requested from the Coastal Management Division, the Louisiana Department of Environmental Quality, and other state agencies. Preliminary discussions with Mr. Chris Roberie (LA Department of Environmental Quality) indicate that exemptions to the rules under Special Request Provisions, "Section 1109.c.11.b of State Rule 33. III. 1109" can be justified for the research proposed and may be granted. Once a field site has been selected, a Louisiana Coastal Use Permit Application (ENG Form 4345) will be submitted with the required maps to the Coastal Management Division of the Louisiana Department of Natural Resources.

The principal investigators have acquired the burn permit form. Mr. Greg Ducote (Natural Resource Specialist, Oil Spill Coordinator's Office, Office of the Governor) supplied the form, explained the burn permitting process, and described the information to include on the form. We are presently selecting burn sites. A preliminary meeting with Mike Windom (Burn Refuge Biologist Program Manager, Wildlife and Fisheries-New Orleans) on 12/19/94 was held to see if state land could be used. A summary of our proposed field research was sent to Mike Windom (12/21/94), and we are waiting to hear if state land can be used for the *in situ* burn study. A copy of our proposed field study was also sent to Heather Finley, Oil Spill Coordinator's Office.

2.1.3 Proposed Measurements for Phases I, II and III

Various measurements outlined in this proposal were and will be conducted during Phases I, II and III as applicable. These methodologies and the necessary instrumentations are available at the Wetland Biogeochemistry Institute.

2.1.3.1 Plant Growth and Regeneration

Culm density (vegetative and reproductive) and the density of culms in flower will be recorded for each plot/treatment throughout the study.

To assess changes in carbon flux and net photosynthesis from plant/soil systems, light and dark chambers will be placed over each sub-plot in the field for determination of CO_2 and other biogeochemical gas fluxes (Smith *et al*. 1981). Light chambers constructed from 3 mm clear Plexiglass with 0.366 m^2 in a cross-sectioned area and an internal volume of 281 liters will

be used for measurements. Chambers similar in dimension, insulated with Styrofoam (2 cm thick), and covered with a reflective space blanket will be used for dark CO_2 flux measurements (respiration). Method and calculations will be performed according to Smith *et al*. (1981).

Measurements will also be conducted using portable infra-red gas analyzers (ADC, model A120 and PP systems, Model CIRAS-1). These techniques have been previously used for evaluation of plant responses both in the laboratory and in the field (Pezeshki and DeLaune, 1988; Pezeshki *et al*. 1989). Implementation of these methods will provide useful information on seasonal patterns of plant gas exchange for each habitat response to the proposed treatments.

2.1.3.2 Biomass

Changes in above-ground and below-ground biomass will be determined in Phase I, II and III experiments. In the greenhouse, replicated pots were harvested in order to assess the above-ground and below-ground biomass components. In the field, the above-ground biomass will be measured by cutting the vegetation at sediment level using a 0.25 m^2 quadrat in randomly selected sub-plots. The subplots will be carefully marked in each area to avoid resampling. The above-ground materials will be cut at sediment level and sealed in plastic bags. In the laboratory, biomass was separated into live and dead fractions and dried to a constant weight. In addition, the live materials were separated into stem and leaf components. In determining below-ground production in the field, replicated surface cores will be taken at each plot simultaneously with the above-ground sampling. The below-ground samples will be processed as described by Schubauer and Hopkinson (1984) and Hopkinson and Dunn (1984). Sections (10 cm) from 15 cm diameter cores will be placed in a solution of sodium metaphosphate, shaken, and washed through a sieve to separate organic size fractions. The organic material will then be separated into live and dead fractions. After determining live and dead components, production will be calculated according to Schubauer and Hopkinson (1984).

2.1.3.3 Plant Community Structure

The plant composition, structure and density will be determined in the field study plots. In addition, plots will be photographed for a visual record of change. The procedure is described in detail by (Kadlec and Wentz, 1974; Smith and Kadlec, 1985 a, b).

2.1.3.4 Degradation and Compositional Changes in Hydrocarbon Components

Following marsh burning, two degradation mechanisms will be quantified: (1) loss of oil components during burning, and (2) potential for microbial degradation by wetland microbiota following burning. To assess loss mechanisms from burning, soil cores (10 cm x 15 cm deep) will be taken from each plot immediately following treatment to determine the crude oil fraction remaining in the sediment. Cores will be extruded and vertically sectioned into 4 cm increments according to procedures outlined by DeLaune *et al*. (1983). The core section will be dried at 28 ^oC and ground to pass through a 25-mesh sieve. Soil will be extracted using supercritical fluid extraction with a suitable modifier. The extract will be fractionated on activated alumina. Hydrocarbon fractions will be analyzed by GC-MS using a modification of EPA Method 8720. A mass balance approach will be used in all studies.

To assess loss from microbial degradation, identical cores will be removed from each plot at one, four and eight weeks following treatment. Cores will be extracted and analyzed as

described above. Loss of oil during the period following burning will primarily be a result of microbial degradation plus abiotic processes such as volatilization. Loss rates will be correlated with measurements of microbial biomass, as described above.

2.1.3.5 Oil Extraction and GC-MS Techniques

Sediment samples (about 4 g) with and without oil (South Louisiana Crude and Arabian Crude), burned and unburned, will be extracted for hydrocarbons using a modified extraction procedure similar to Koques *et al*. (1994). In the past study year, sediment/oil samples were transferred to Teflon centrifuge tubes for hydrocarbon extraction. The extracting solution was a 1:1 mixture of hexane and acetone. Twenty ml of the extracting solution was added to the Teflon tubes, and the tubes were then shaken for 12 hours. After shaking, the Teflon tubes were centrifuged at 10,000 rpm for 13 minutes at laboratory temperature. The hexane/acetone solvent was decanted at the top, and anhydrous sodium sulfate was added to remove trace amounts of water. The solvent mixture was evaporated to 10 ml using ultra high pure nitrogen gas. The solvent/hydrocarbon mixture was then diluted 100 times to permit GC-MS analysis. From the diluted sample, 1 ml was added to GC-MS reduced volume vials, and 0.04 ml of internal standard was added to each vial before GC-MS instrumental analysis.

Hydrocarbon analyses were performed on a gas chromatograph (Hewlett Packard 5890 Series II plus) equipped with an automatic sampler and a HP-5 high resolution capillary column (30 m, 0.25 ：m film thickness, 0.25 mm i.d.). The capillary column was directly interfaced to a quadrupole mass spectrometer (Hewlett Packard 5972 Mass Selective Detector). The carrier gas (ultra high pure helium) flow rate was 1.0 ml/min. Injector temperature was 300 ºC. Column temperature was programmed from 50 ºC to 310 ºC at 8 ºC/min rate with an initial 3.0 minute delay and a 15.0 minute hold at the end of the run. The interface to the mass selective detector was maintained at 28 ºC. Sample and standard injections were made using a Hewlett Packard 7673 automatic liquid sampler into a splitless injection port.

A Hewlett Packard Vectra 486/66 XM computer system and Hewlett Packard 61034C software for the MS chem station (DOS series) were used to collect and analyze data. Hydrocarbon peaks were identified using the G1033A NIST PBM Library software.

3.0 Results and Discussion

3.1 Plant Species

Plant species used in the two greenhouse burn studies were collected from freshwater, brackish, and salt marsh habitats. *Spartina alterniflora*, *Panicum hemitomon* and *Sagittaria lancifolia* were the species selected for the greenhouse burn experiments.

Spartina alterniflora Lois. (Figure 3.1) is a perennial grass that grows from extensive rhizomes and forms dense stands over broad areas of the marsh. *Spartina alterniflora* plants grow in brackish and salt marshes. Culms are thick and grow two to four feet tall with wide tapering leaves. *Spartina alterniflora* is a major contributor of detritus to aquatic food chains and is also called oystergrass or smooth cordgrass.

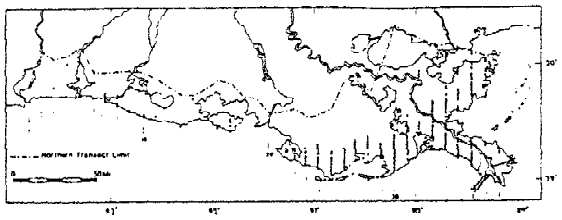
Panicum hemitomon (Figure 3.2) is a perennial grass commonly found in fresh marsh habitats. The plant can produce vast dense stands and cane-like culms two to four feet tall. The plant has extensive creeping rhizomes. *Panicum hemitomon* is probably the major contributor of organic material to Louisiana coastal marshes.

Sagittaria lancifolia (bulltongue) is a perennial herb found growing in fresh and brackish marshes (Figure 3.3). Plants produce lanceolate blades two to three feet tall and white flowers arranged in whorls. *Sagittaria lancifolia* plants grow in dense stands, and the species is a major contributor to marsh building and aquatic food chains.

3.2 Greenhouse Design

To conduct the two greenhouse burn experiments, *Spartina alterniflora*, *Panicum hemitomon* and *Sagittaria lancifolia* plant-sediment cores were collected from Louisiana marshes. The plant-soil cores were collected in polyvinyl chloride (PVC) cylinders (30 cm length by 15 cm i.d.) sealed with a PVC end cap. Plant cores were collected from selected marsh field sites and transported back to the greenhouse for a 30 day equilibration time before the burn experiments. Replicated cores of each species were assigned to four main treatments: (1) oiling; (2) oiling and burning; (3) no oiling or burning, plants clipped at the sediment surface; and (4) control (no oiling, burning, or clipping). Oil treatments received 2 L m^{-2} of South Louisiana Crude and designated treatments were burned after oil application. After burning, the treatment cores were returned to the greenhouse and the plants were monitored over the evaluation periods (see Methods Section). The first greenhouse burn experiment was conducted in the summer of 1994 using *Spartina alterniflora* and *Panicum hemitomon* cores in which the sediment was saturated only with water. Gasoline (5 cc) was used to ignite the oil for the oil plus burn treatments. Based on the data collected from the first burn study, a second greenhouse study was conducted in the spring of 1995 using *Sagittaria lancifolia* cores. Prior to burning, a two to three inch floodwater layer was established in the *Sagittaria lancifolia* cores, and a propane torch was used to ignite the South Louisiana Crude oil.

Spartina alterniflora and *Panicum hemitomon* plant responses were monitored for about 60 days after the initial burn. *Sagittaria lancifolia* plants were evaluated for about 30 days after the second burn experiment was initiated. Evaluation of *Sagittaria lancifolia* was cut short due to project termination and final report due dates.



Spartina alterniflora Lois.

Gramineae

Oyster grass
Smooth cordgrass
Seacane

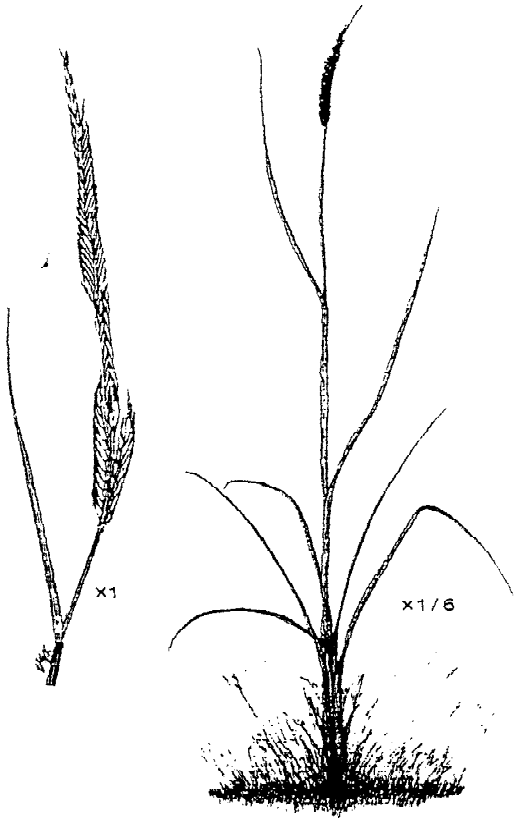


Figure 3.1 *Description of Spartina alterniflora grass. (from Common Vascular Plants of the Louisiana Marsh, R.H. Chabreck and R.E. Condrey, Sea Grant Publication No. LSU-T-79-003, Center for Wetland Resources)*

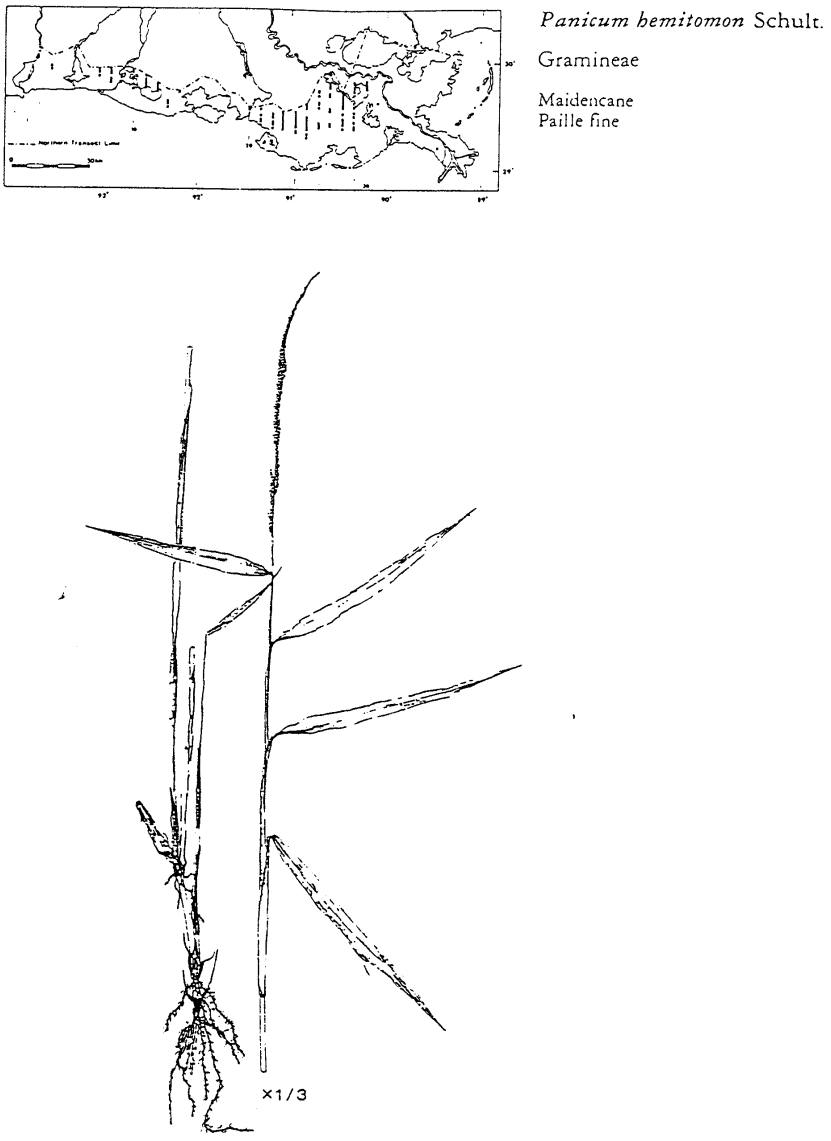
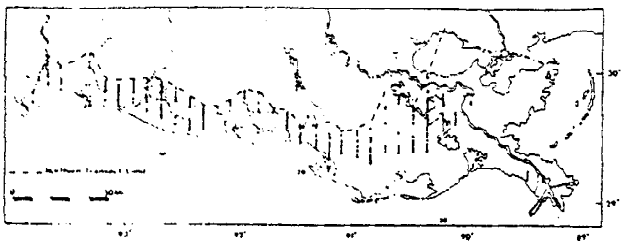


Figure 3.2 *Description of Panicum hemitomon grass. (from Common Vascular Plants of the Louisiana Marsh, R.H. Chabreck and R.E. Condrey, Sea Grant Publication No. LSU-T-79-003, Center for Wetland Resources)*



Sagittaria lancifolia]
(Sagittaria falcata)
Alismataceae
Bulltongue

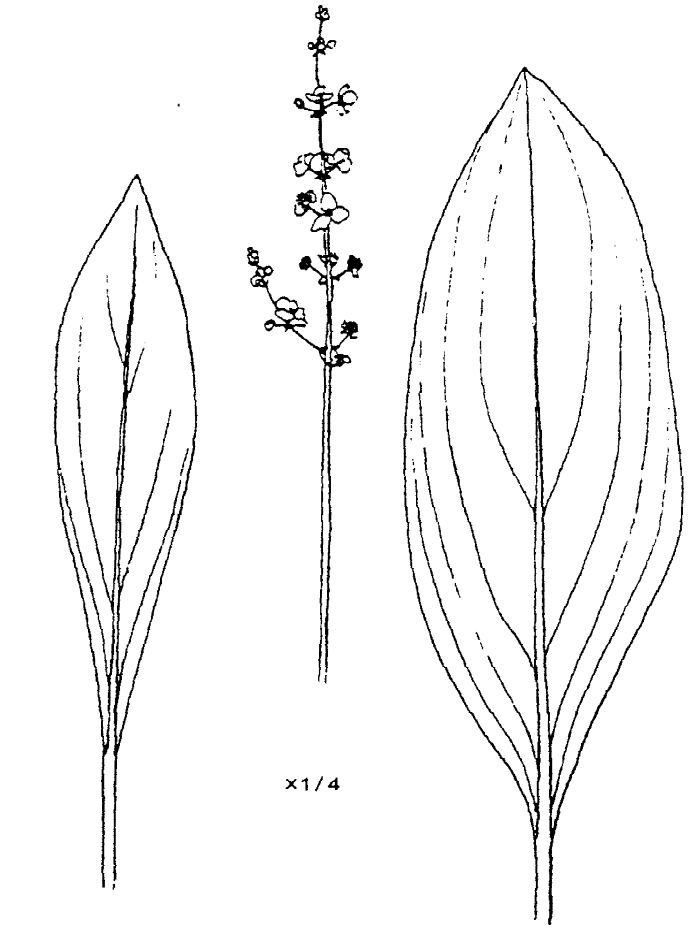


Figure 3.3 *Description of Sagittaria lancifolia herb.*
(from Common Vascular Plants of the Louisiana Marsh, R.H. Chabreck and R.E. Condrey, Sea Grant Publication No. LSU-T-79-003, Center for Wetland Resources)

3.3 Results of First Greenhouse Burn

A photographic sequence of the first greenhouse burn experiment with *Spartina alterniflora* and *Panicum hemitomon* plant species is shown in Figures 3.4 through 3.8.

Figure 3.4 *Spartina alterniflora* and *Panicum hemitomon* collected from field marsh sites and equilibrated for 30 days in the greenhouse.

Figure 3.5 Application of South Louisiana Crude oil (2 L m^{-2}) to oil and oil plus burn treatments.

Figure 3.6 Ignition of applied oil using 5 cc of gasoline and burning of SLC.

Figure 3.7 Burning of *Spartina alterniflora* and *Panicum hemitomon* oil plus burn treatments.

Figure 3.8 Clipping of plant cores and greenhouse setup design for post-burn evaluation.

3.3.1 Spartina alterniflora

3.3.1.1 Shoot Regeneration

Shoot regeneration capacity of the *Spartina alterniflora* treatment is given by individual replication in Table 3.1, and mean live shoot values (six replications) is graphed in Figure 3.9. Treatments applied were: (1) oiling, no clipping, (2) oiling and burning, and (3) control—no oil, burning, or clipping. Also given in Table 3.1 is the number of dead shoots counted on each sampling date.

The mean live shoot values for the control *Spartina alterniflora* cores increased from 11 live shoots three days after the burn to a mean value of 23 live shoots recorded 54 days after the burn. This represents approximately a 100% increase in new shoots over the study period for the control cores.

Shoot regeneration in the oil treatment was greatly reduced compared to the control treatment. The mean live shoot value was about nine at day three. At the end of the greenhouse study (54 days), the mean live shoot value had only increased to about 12 (Figure 3.9). This represents only a 33% increase in live shoots over the 54 days. The South Louisiana Crude had a significant effect on the shoot regeneration capacity of *Spartina alterniflora* plants that can be seen in the number of dead shoots recorded for the oil treatment replications (Table 3.1). From day 18, dead shoots were recorded in the cores, and after 54 days a mean value of three dead shoots per pot was calculated. Only two dead shoots were recorded in the *Spartina alterniflora* control treatment cores over the entire sampling period (Table 3.1).

The oil plus burn treatment had a drastic effect on the *Spartina alterniflora* plants (Table 3.1, Figure 3.9). Both Table 3.1 and Figure 3.9 show that burning South Louisiana Crude in the saturated cores killed all *Spartina alterniflora* plants. No new shoots were recorded over the entire 54 day data collection period. The lack of plant survival was mainly attributed to the absence of a floodwater layer and the use of gasoline as an ignitor.

3.3.1.2 Biomass Production

Total above-ground biomass data collected from the *Spartina alterniflora* treatments at the conclusion of the greenhouse study are given in Table 3.2. The effects of oiling and oiling plus burning can be observed in the measured dry weights of the four treatments investigated.

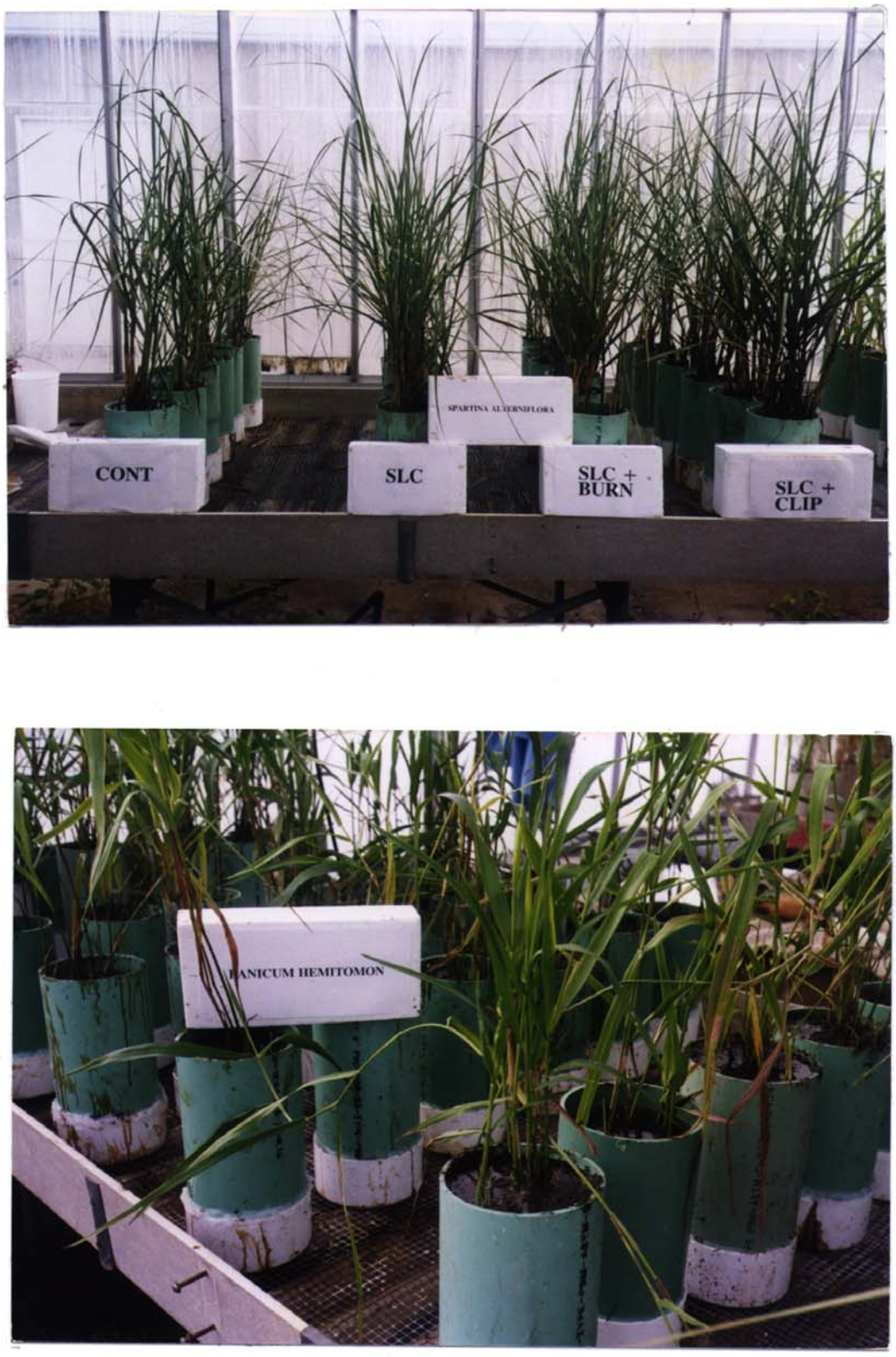


Figure 3.4 *Spartina alterniflora and Panicum hemitomon collected from field sites for greenhouse experiments.*

Figure 3.5 *Application of South Louisiana Crude to oil and oil plus burn treatments.*

Figure 3.6 *Ignition of applied oil using 5 cc of gasoline and burning of South Louisiana Crude.*

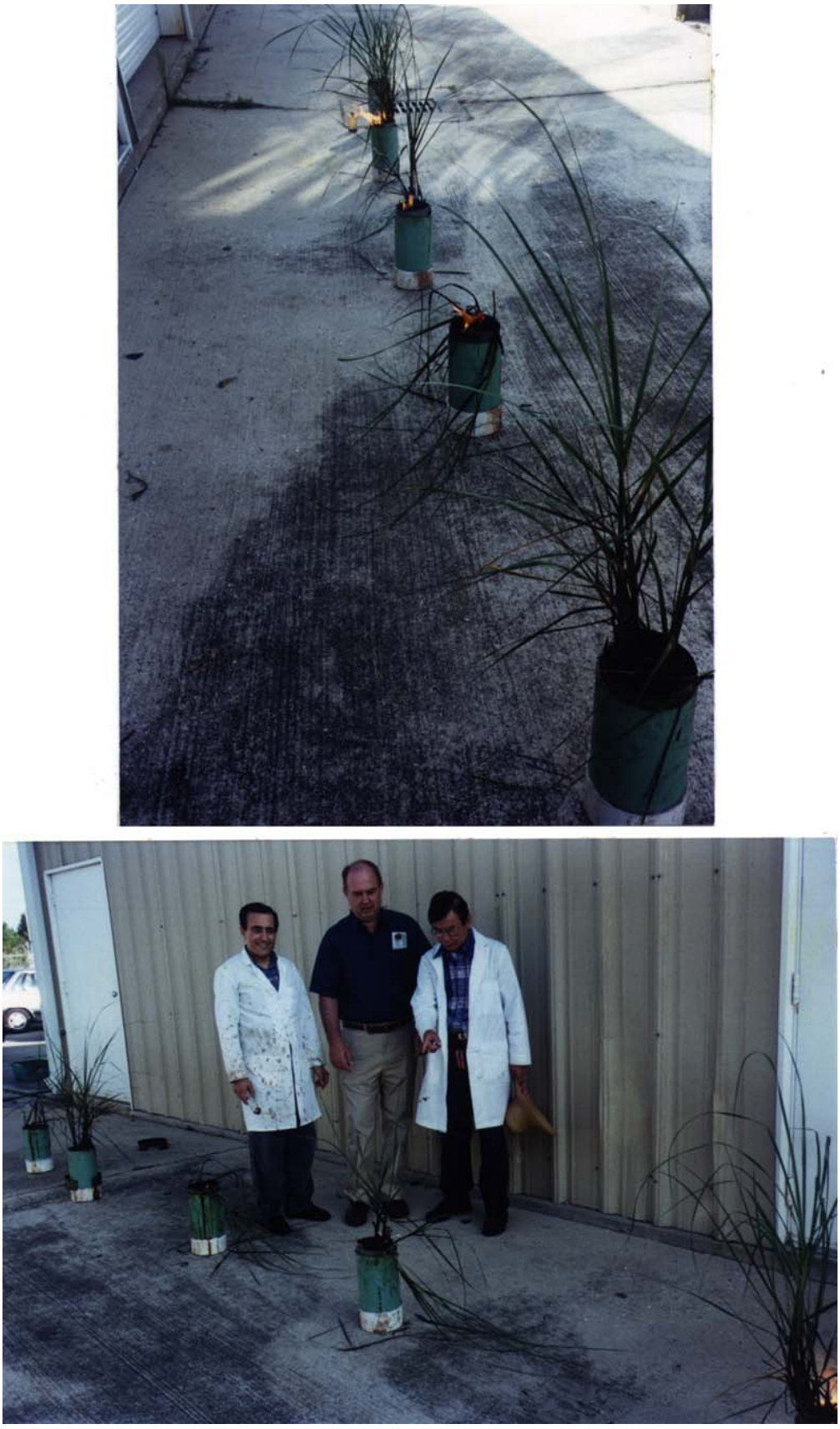


Figure 3.7 *Burning of Spartina alterniflora and panicum hemitomon treatments.*

Figure 3.8 *Clipping of plant cores and greenhouse design for post-burn evaluation.*

Table 3.1 Shoot regeneration capacity of *Spartina alterniflora* cores (saturated) for control (no oiling or burning), SLC-T_1 (oiling only) and SLC + Burn - T_2 (oiling and burn) treatments.

Species	Treatment	Rep#		Number of Shoots							
			Date: Days:	06/23 03	06/29 09	07/08 18	07/15 25	07/22 32	07/29 41	08/05 48	08/13 54
Spartina alterniflora	Control	1		8	14	18	21	25	26	26	26
		2		11	14	16	18	23	26	27	27
		3		11	19	21	20	19	23	24	22
		4		15	20	23	22	20	23	27	30
		5		8	13	15	17.	17	19	18	19 (+2d)
		6		14	16	16	16	16	18	18	16
	SLC (T_1)	1		7	6	7	6 (+1d)	4 (+2d)	5 (+3d)	8 (+3d)	8 (+2d)
		2		6	5	5	2 (+3d)	2 (+3d)	2 (+3d)	2 (+3d)	1 (+4d)
		3		8	8	5 (+2d)	5 (+4d)	5 (+2d)	4 (+3d)	5 (+3d)	4 (+3d)
		4		9	7	5 (+2d)	7 (+3d)	7 (+1d)	5 (+3d)	6 (+4d)	9 (+3d)
		5		9	11	10 (+2d)	14 (+2d)	15 (+2d)	16 (+3d)	14 (+4d)	18 (+3d)
		6		13	17	15	17(+3d (+3d)	16 (+3d)	20 (+3d)	21 (+3d)	23 (+1d)
	SLC + BURN (T_2)	1		0	0	0	0	0	0	0	0
		2		0	0	0	0	0	0	0	0
		3		0	0	0	0	0	0	0	0
		4		0	0	0	0	0	0	0	0
		5		0	0	0	0	0	0	0	0
		6		0	0	0	0	0	0	0	0

* SLC - South Louisiana Crude

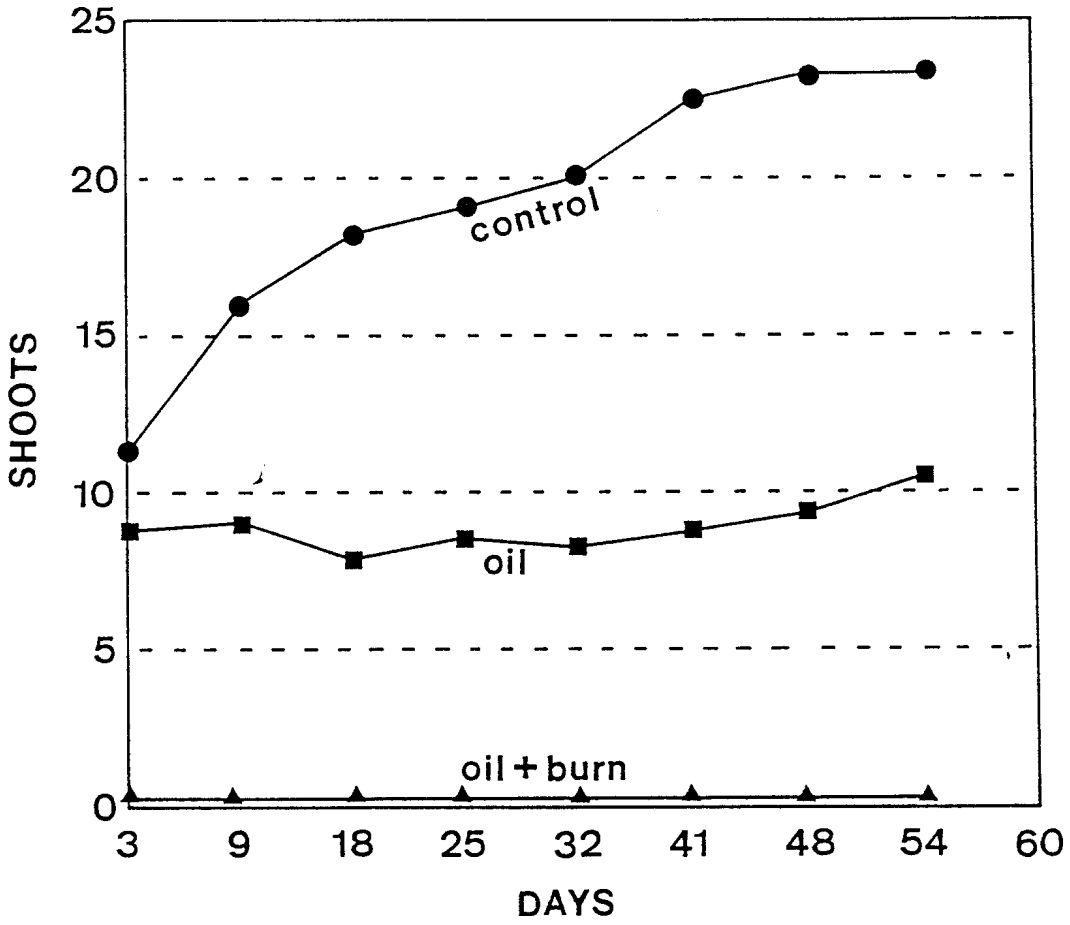


Figure 3.9 *Number of shoots of Spartina alterniflora, after burn, in saturated cores. Each observation is a mean of six values.*

The greatest dry matter yield was from the control *Spartina alterniflora* treatment (no clip). The mean dry weight of the three replications was 81.69 grams per control core. This compares to reduced biomass means of 40.00 and 36.72 grams for T_1 (oiling, no clip) and T_4 (oil plus clipped) treatments, respectively. For treatment #2 (oil plus burn) zero biomass was produced after the burn, which indicates that the plants were killed during the burning sequence.

3.3.2 Panicum hemitomon

3.3.2.1 Shoot Regeneration

New shoot growth for the *Panicum hemitomon* treatments is shown in Table 3.3 for individual cores. Mean live shoot values (six replications) are graphed in Figure 3.10. Treatments were the same as those applied to the *Spartina alterniflora* cores. Also recorded in Table 3.3 are the number of dead shoots observed on each sampling date.

The mean live shoot value for the control *Panicum hemitomon* cores increased from 20 live shoots on day three after the burn to a value of 53 live shoots recorded at 54 days. This represents an increase of approximately 165% over the experimental evaluation period (Figure 3.10). No dead shoots were recorded from the *Panicum hemitomon* control cores over the eight sampling periods.

Shoot regeneration for the oil only treatment (T_1) was drastically reduced over the study time. The mean live shoot value at day three was about seven shoots (Figure 3.10) and over the next 51 days slowly declined to less than one live shoot per core recorded at day 54 (Table 5). Over the same time frame, the number of dead shoots observed for the oil treatment increased and averaged 4.5 dead shoots per core. This shows that oiling had a significant effect on the health of *Panicum hemitomon* plants.

Oiling plus burning killed all *Panicum hemitomon* plants in the treatment replications (Table 3.3, Figure 3.10). The lack of survival was mainly attributed to the use of gasoline as an ignitor and the absence of a floodwater layer to absorb heat generated by the burn.

3.3.2.2 Biomass Production

Above-ground biomass collected and measured for the *Panicum hemitomon* treatment at the conclusion of the greenhouse study is tabulated in Table 3.4. The effects of oiling and oiling plus burning can be seen in the total dry weight means. The largest amount of dry matter produced was measured for the control replications. The mean dry weight of the three control replications was 107.88 grams per core. This compares to reduced biomass means of 9.85, 7.51, 3.3 and 0.0 grams for the T_3 (clipped), T_1 (oil only), T_4 (oil plus clipped) and T_2 (oil plus burn) treatments respectively.

Table 3.2 Biomass data for *Spartina alterniflora* treatments. No biomass remained or was regenerated after the oiling plus burning treatment (T_2 - SLC + Burn).

Species	Treatment	Rep #	Dry weight (gms)	Notes
Spartina alterniflora	Control	1	106.840	Reps # 3,4, 5 were left in the g.h.
		2	106.737	
		6	31.499	
	T_1 (SLC)	7	43.535	
		8	26.370	
		9	31.851	
		10	34.117	
		11	38.148	
		12	66.018	
	T_2 (SLC + Burn)	13	0.0	
		14	0.0	
		15	0.0	
		16	0.0	
		17	0.0	
		18	0.0	
	T_4 (SLC + CLIPPED)	19	69.017	
		20	36.210	
		21	24.819	
		22	23.496	
		23	44.380	
		24	22.402	

Table 3.3 Shoot regeneration capacity of *Panicum hemitomon* cores (saturated) for control (no oiling or burning), SLC-T_1 (oiling only) and SLC + Burn - T_2 (oiling and burn) treatments.

Species	Treatment	Rep#		Number of Shoots							
			Date: Days:	06/23 03	06/29 09	07/08 18	07/15 25	07/22 32	07/29 41	08/05 48	08/13 54
Panicum hemitomon	Control	1		18	19	19	29	31	29	31	35
		2		15	22	22	26	27	27	29	30
		3		24	26	45	52	58	56	66	63
		4		25	45	54	60	62	73	76	77
		5		14	19	27	40	51	54	53	56
		6		24	28	38	47	39	43	54	58
	SLC (T_1)	1		11	9	6 (+2d)	5 (+2d)	6 (+1d)	2 (+3d)	4 (+2d)	2 (+6d)
		2		7	5	0 (+4d)	0 (+4d)	0 (+3d)	0 (+3d)	0 (+4d)	0 (+5d)
		3		8	5	0 (+4d)	0 (+4d)	0 (+3d)	0 (+3d)	0 (+5d)	0 (+6d)
		4		5	5	0 (+4d)	0 (+3d)	0 (+3d)	1 (+3d)	2 (+3d)	2 (+4d)
		5		3	4	0 (+2d)	3	3	3 (+1d)	0 (+3d)	0 (+3d)
		6		5	3	0 (+3d)	0 (+3d)	0 (+3d)	0 (+3d)	0 (+3d)	0
	SLC + BURN (T_2)	1		0	0	0	0	0	0	0	0
		2		0	0	0	0	0	0	0	0
		3		0	0	0	0	0	0	0	0
		4		0	0	0	0	0	0	0	0
		5		0	0	0	0	0	0	0	0
		6		0	0	0	0	0	0	0	0

* SLC - South Louisiana Crude

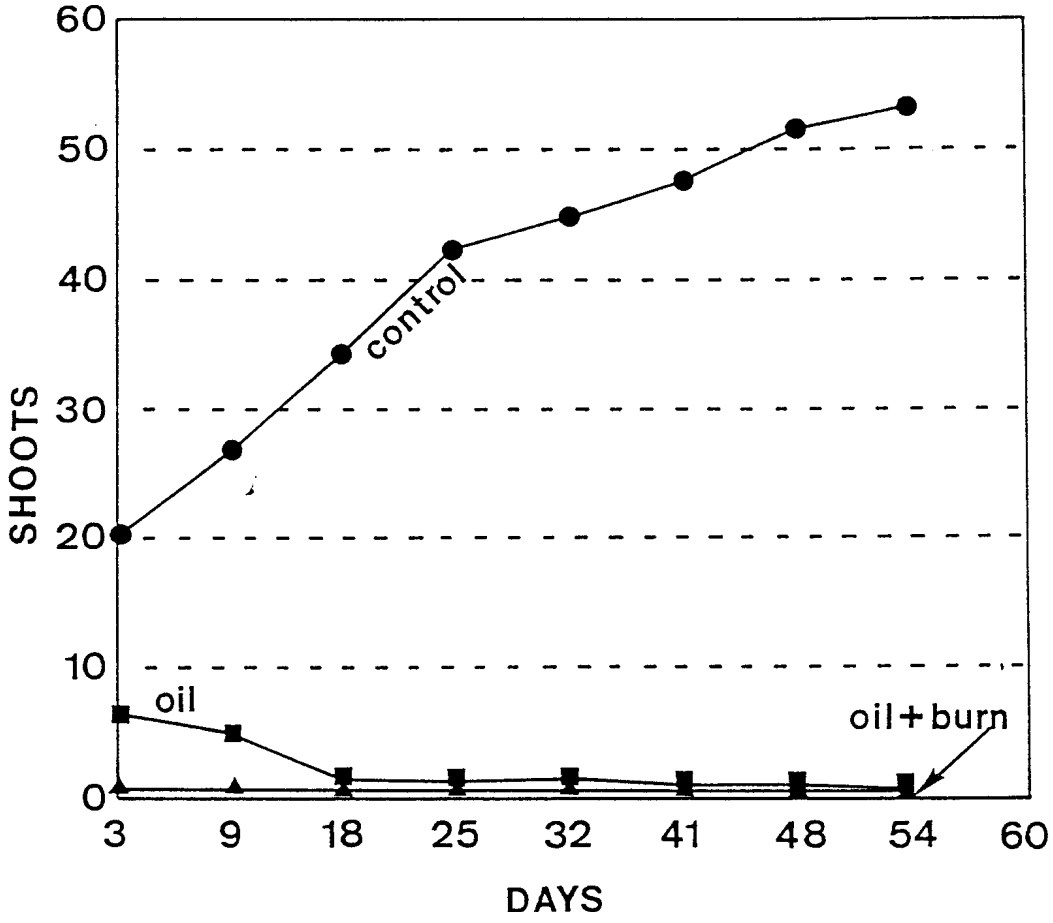


Figure 3.10 *Number of shoots of Panicum hemitomon, after burn, in saturated cores. Each observation is a mean of six values.*

Table 3.4 Biomass data for *Panicum hemitomon* treatments. No biomass remained or was regenerated after the oiling plus burning treatment (T_2 - SLC + Burn).

Species	Treatment	Rep#	Dry Weight (gms)	Notes
Panicum hemitomon	Control	2	64.705	Reps.# 1,4, 5 were left in the g.h.
		3	129.272	
		6	129.651	
	T_1 (SLC)	7	31.417	
		8	no shoots	
		9	no shoots	
		10	0.190	
		11	5.919	
	T_2 (SLC+BURN)	13	0.0	
		14	0.0	
		15	0.0	
		16	0.0	
		17	0.0	
		18	0.0	
	T_3 (clipped)	19	3.717	
		20	3.544	
		21	24.460	
		22	6.398	
		23	7.660	
		24	13.350	
	(SLC + CLIPPED)	21	7.600	
		22	no shoots	
		23	no shoots	
		24	8.885	
		25	no shoots	

3.4 Results of Second Greenhouse Burn

A photographic sequence of the second greenhouse burn experimental procedures using *Sagittaria lancifolia* plants is shown in Figure 3.11 through Figure 3.15.

Figure 3.11 Oiling and burning of *Sagittaria lancifolia* cores collected from a freshwater marsh and equilibrated for 30 days in the greenhouse.

Figure 3.12 Oiling and burning of *Sagittaria lancifolia* treatments (saturated cores and two inches of floodwater) using a propane torch to ignite the oil sources.

Figure 3.13 Greenhouse design of *Sagittaria lancifolia* treatments for post-burn measurement and evaluation.

Figure 3.14 New shoot regeneration of the oil plus burn treatments three days after burn.

Figure 3.15 Shoot regeneration of Sagittaria lancifolia plants 26 days after burn.

3.4.1 Sagittaria lancifolia

Based on the drastic effects of oiling plus burning on saturated cores of *Spartina alterniflora* and *Panicum hemitomon,* the number of treatments for the *Sagittaria lancifolia* greenhouse burn experiment was increased. Control treatments (1 and 2), oiling and oiling plus burning, were conducted on *Sagittaria lancifolia* cores that were saturated with water. Control tests were also conducted on a second set of *Sagittaria lancifolia* cores that had a two inch floodwater layer permanently established above the sediment surface. Gasoline was not used on the *Sagittaria lancifolia* cores to ignite the applied oil. A propane torch was used to ignite the oil source for the burn treatments.

3.4.1.1 Shoot Regeneration

Shoot regeneration of the *Sagittaria lancifolia* treatments (for saturated cores) is given in Table 3.5 and graphed in Figure 3.16. Treatments were: Control-1 (no oiling, burning, or clipping), Control-2 (clipping, no oiling, or burning), SLC-T_1 (oiling only) and oiling plus burning (T_2). The mean live shoot value per core for the Control-1 treatment increased from 16.5 shoots (at 0 days) to 19.5 shoots measured after 26 days. This represents an 18% increase in live shoots over the study period. The number of dead shoots in the Control-1 treatment increased over time and averaged 5.5 dead shoots per core on the final evaluation date (Table 3.5).

Control-2 new shoot growth increased rapidly from day 0 (0 shoots) to a mean value of 17 live shoots per core on day 26 (Figure 3.16). Dead shoots averaged about one per core on day 26, which was far less than what was observed for the Control-1 treatment.

For the oiling only treatment of *Sagittaria lancifolia* cores, the mean live shoot value was about 13 per replication at day 0 and increased to 19 per core at day 26. The mean dead shoot value at day 26 was seven shoots per core (Figure 3.16).

The oiling plus burn treatment showed a large response in new shoot regrowth. At day 0, new shoots averaged 0 and steadily increased to a mean live shoot value per replication of 22 at day 26. Three days after the burn, new growth was observed with a live shoot mean of four per core. From day three to day 26, a 45% increase in live shoots was recorded. No dead shoots were observed over the entire sampling period for the burn treatment (Table 3.5). As seen in Figure 3.16, the number of live shoots per core for the oil plus burn treatment was higher compared to the controls at day 26.

Figure 3.11 *Oiling and burning of Sagittaria lancifolia cores collected from a freshwater marsh.*

Figure 3.12 *Oiling and burning of Sagittaria lancifolia treatments.*

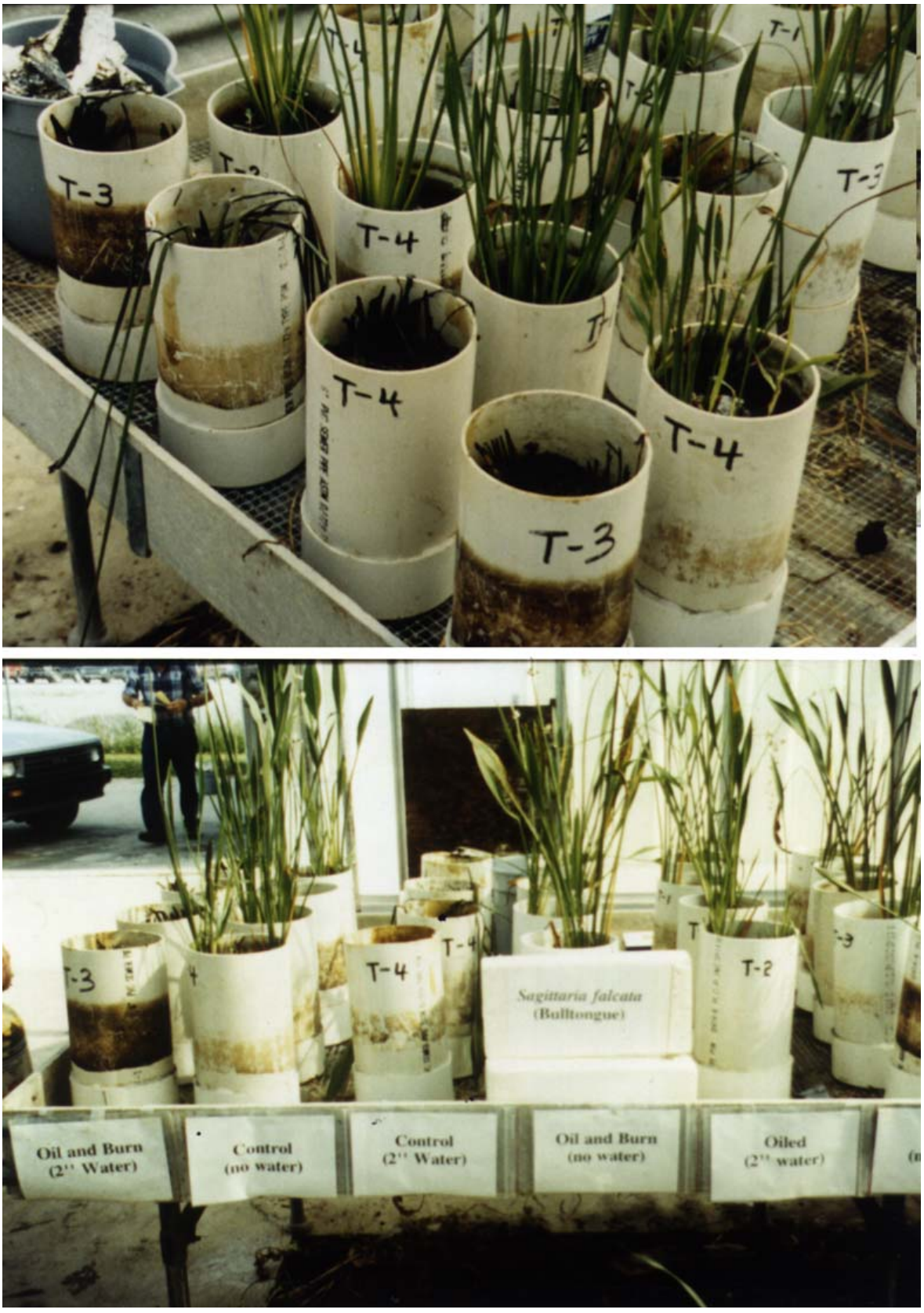


Figure 3.13 *Greenhouse design of Sagittaria lancifolia treatments for post-burn measurement and evaluation.*

Figure 3.14 *New shoot regeneration of the oil plus burn treatment 3 days after burn.*

Figure 3.15 *Shoot regeneration of Sagittaria lancifolia plants 26 days after burn.*

Table 3.5 Shoot regeneration capacity of *Sagittaria lancifolia* cores (saturated) for Control-1 (no oiling or burning or clipping), Control-2 (no oiling or burning but clipped), SLC-T_1 (oiling only) and SLC + Burn - T_2 (oiling and burn) treatments.

Species	Treatment	Rep#		Number of Shoots					
			Date Days	04/07 0	04/10 3	04/14 7	04/21 14	04/28 21	05/05 26
Sagittaria lancifolia (saturated cores)	Control-1	1		10	10	11	13	10(+5d)	13(+5d)
		2		29	30	34	26	33(+5d)	35(+6d)
		3		11	11	14	15	12(+3d)	15(+5d)
		4		16	16	17	15(+2d)	12(+3d)	15(+6d)
	Control-2 (Clip)	1		0	19	33	37	35(+3d)	35(+3d)
		2		0	6	8	12	14	15
		3		0	5	5	7	10	11(+1d)
		4		0	1	3	4	6	6
	SLC (T_1)	1		12	13	20	20(+1d)	20(+3d)	19(+5d)
		2		12	12	15	15(+2d)	15(+4d)	18(+7d)
		3		11	11	15	17(+1d)	17(+3d)	17(+8d)
		4		18	18	17(+1d)	15(+2d)	21(+3d)	21(+7d)
	SLC + Burn (T_2)	1		0	4	10	14	17	17
		2		0	6	11	19	22	27
		3		0	2	5	12	16	18
		4		0	4	13	17	24	27

* SLC - South Louisiana Crude

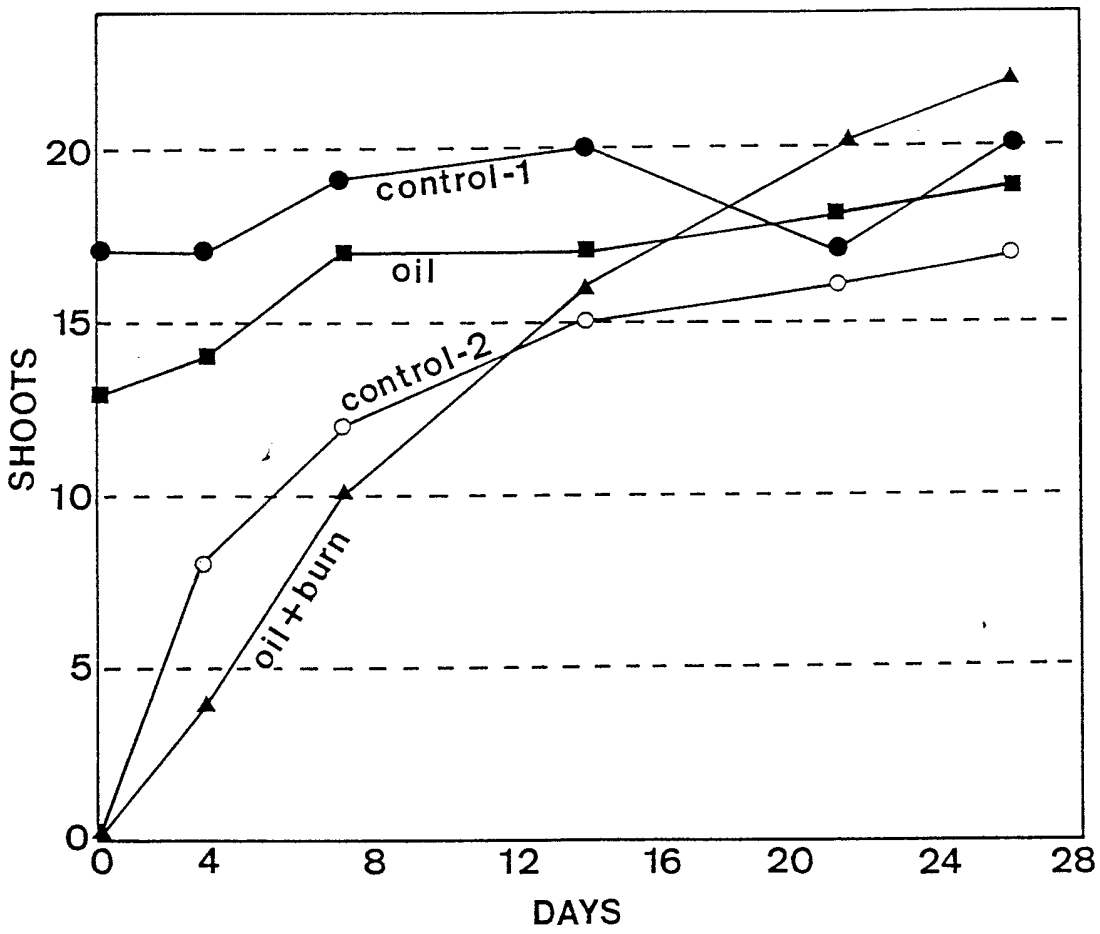


Figure 3.16 *Number of shoots of Sagittaria lancifolia, after burn, in saturated cores. Each observation is a mean of four values.*

Shoot regeneration for the *Sagittaria lancifolia* cores with a two inch floodwater layer is shown in Figure 3.17 and Table 3.6. Control-1 (no oiling, burning, or clipping) cores showed a mean increase in live shoots from 17 per replication (day 0) to 21 per core recorded on day 26. This represents a 23.5% increase in new shoots over the evaluation period. Dead shoots were counted on the last two sampling dates and averaged about four dead shoots per core at day 26 (Table 3.6).

Control-2 (clipped) treatment, with a two inch floodwater layer, also showed new shoot regrowth. After clipping, and on the third day, four new shoots per pot were measured. After 26 days, 13 live shoots per replication were recorded.

Shoot regeneration in the oil only treatment decreased over time (Figure 3.17). At 0 days, a live shoot mean of 14 per core was recorded. After 26 days the live shoot mean decreased to 12 live shoots and seven dead shoots per treatment replication. More dead shoots were recorded for this treatment than were observed for the remaining three treatments of *Sagittaria lancifolia*.

The greatest rate increase in new shoot regeneration occurred in the oiling plus burning treatment. Three days after the burn, a live shoot mean of five was recorded; at 26 days 21 live shoots per core were measured. This represents a 320% increase in live shoot production over a 23 day period.

3.4.1.2 Biomass Production

Above-ground biomass production over the 26 day greenhouse experiment for the *Sagittaria lancifolia* treatments is shown in Table 3.7 (saturated cores) and Table 3.8 (two inches of floodwater). All treatments of *Sagittaria lancifolia* displayed an increase in biomass produced compared to *Spartina alterniflora* and *Panicum hemitomon* results where no new biomass was measured from the oil plus burn treatment.

Dry weight measured from the saturated *Sagittaria lancifolia* core treatments averaged (four replications) 5.45, 3.82, 6.33 and 3.36 grams for the Control-1, Control-2, T_1 (oil only) and T_2 (oil + burn) treatments, respectively.

The mean (four replications) dry weight measured at 26 days for the *Sagittaria lancifolia* treatments with two inches of floodwater, Control-1, Control-2, T_1 (oiling only) and T_2 (oiling plus burning) was 4.99, 3.44, 6.47 and 4.62 grams per core, respectively.

3.4.1.3 Plant Height

In addition to shoot regeneration and biomass production measurements, plant growth was measured and recorded over the 26 days for all *Sagittaria lancifolia* saturated and flooded treatments. The results of this investigation are graphed in Figures 3.18 and 3.19 and are tabulated in Tables 3.9 and 3.10.

The mean (four replications) maximum height increase over the 26 day sampling period for the saturated *Sagittaria lancifolia* cores from Control-1 (no oiling, burning, or clipping) and Control-2 (clipped, no oiling or burning) was 40 and 51 cm, respectively. For the oil treatment (saturated cores) the mean height increase was 43 cm over 26 days. The greatest height increase was observed in the oil plus burn treatments (Table 3.9). The average maximum height increase was 58 cm over the 26 day monitoring period (Figure 3.18).

The same general trend in plant growth response was also observed for the *Sagittaria lancifolia* treatments that had a permanent floodwater layer established (two inches) above the

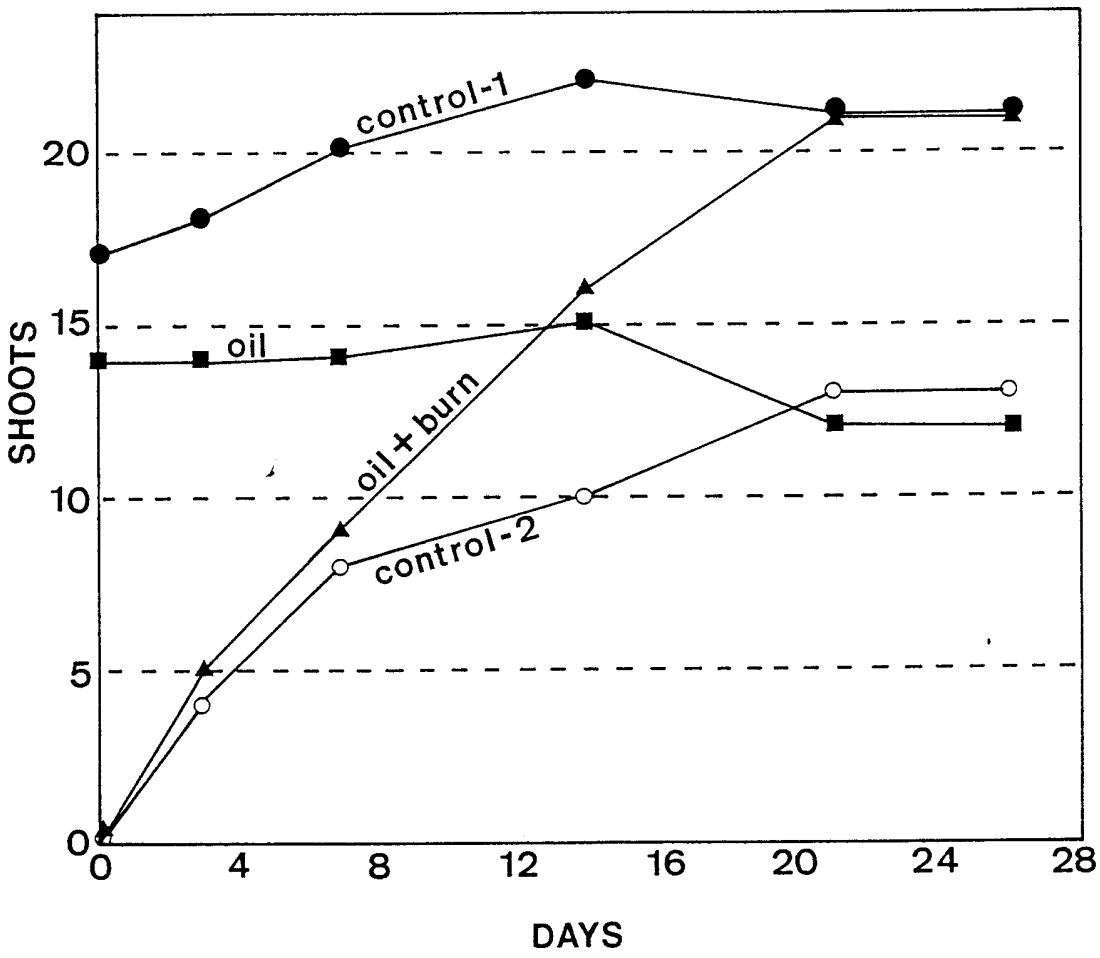


Figure 3.17 *Number of shoots of Sagittaria lancifolia, after burn, with two inches of floodwater in cores. Each observation is a mean of four values.*

Table 3.6 Shoot regeneration capacity of *Sagittaria lancifolia* cores (2 inches of floodwater) for Control-1 (no oiling or burning or clipping), Control-2 (no oiling or burning but clipped), SLC-T_1 (oiling only) and SLC + Burn - T_2 (oiling and burn) treatments.

Species	Treatment	Rep#	Number of Shoots						
			Date Days	04/07 0	04/10 3	04/14 7	04/21 14	04/28 21	05/05 26
Sagittaria lancifolia (2 inches of flood-water)	Control-1	1 2 3 4		17 31 9 12	18 31 9 12	20 33 10 16	24 33 11 18	20(+4d) 32 13 17(+3d)	21(+5d) 33 13(+3d) 17(+7d)
	Control-2 (clip)	1 2 3 4		0 0 0 0	1 0 13 1	4 2 20 6	4 3 27 7	7 4 30(+1d) 10	7 4 29(+1d) 12
	SLC (T_1)	1 2 3 4		16 10 14 15	16 10 14 15	15(+1d) 11 16 14	14(+2d) 10(+1d) 19 16	11(+5d) 7(+8d) 19(+3d) 12(+8d)	12(+6d) 8(+9d) 16(+6d) 12(+7d)
	SLC + Burn (T_2)	1 2 3 4		0 0 0 0	6 6 5 3	13 12 7 5	24 20 10 9	29 25 15 13	32 25 14(+1d) 13

* SLC - South Louisiana Crude

Table 3.7 Biomass data for *Sagittaria lancifolia* treatments with saturated cores.

Species	Treatment	Rep #	Dry weight (gms)
Sagittaria lancifolia	[1]C-1	1	[2] 6.40
		2	7.59
		3	3.09
		4	4.72
	C-2 (clipped)	1	9.02
		2	2.86
		3	0.64
		4	2.75
	T_1 (SLC)	1	4.08
		2	6.00
		3	6.04
		4	9.23
	T_2 (SLC + Burn)	1	2.77
		2	2.70
		3	3.89
		4	4.09

[1] C-1 (no clip/no oil), C-2 (clipped/no oil), T_1 - SLC (oil/no clip), T_2 - SLC + Burn (Oil and Burn)

[2] plants harvested 26 days after burn

SLC - South Louisiana Crude

Table 3.8 Biomass data for *Sagittaria lancifolia* treatments with two inches of floodwater in cores.

Species	Treatment	Rep #	Dry weight (gms)
Sagittaria lancifolia (2 inches of water)	[1] C-1	1	[2] 6.36
		2	2.19
		3	5.40
		4	6.02
	C-2 (clipped)	1	4.24
		2	1.27
		3	6.54
		4	1.71
	T_1 (SLC)	1	4.85
		2	8.44
		3	5.90
		4	6.69
	T_2 (SLC + Burn)	1	3.66
		2	4.94
		3	5.35
		4	4.54

[1] C-1 (no clip/no oil), C-2 (clipped/no oil),

T_1 - SLC (oil/no clip), T_2 - SLC + Burn (oil and burn)

[2] plant harvested 26 days after burn

SLC - South Louisiana Crude

sediment surface. The tabulated and graphed results are displayed in Table 3.10 and Figure 3.19. Over the 26 day monitoring period the mean (four replications) maximum height increase for Control-1 and Control-2 was 43 and 63 cm respectively. For the oil only treatment an increase of 31 cm was recorded over the 26 days and for the oil (South Louisiana Crude) plus burn treatment (with two inches of floodwater) the mean maximum height increase was 68 cm. Of the four treatments, the greatest mean height increase was measured in the oiling plus burning treatment cores (Table 3.10, Figure 3.19).

3.5 Identification of Oil Hydrocarbon Components

To evaluate the effects of oiling and oiling plus burning on the distribution of oil hydrocarbon components two oil sources were used: South Louisiana Crude and Arabian Crude. The two crude oil sources were applied (2 L m^{-2}) to the surface of sediment samples collected from a *Spartina alterniflora* marsh. Treatments consisted of oiling and oiling plus burning. After burning, the hydrocarbons and residual oil components were extracted from the salt marsh sediment (see 2.0 Methodology) for identification and comparison to the crude oil sources. The hydrocarbons of particular interest were the polynuclear aromatic hydrocarbons because of their toxic effect on organisms (Roques *et al*. 1994).

3.5.1 South Louisiana Crude

Chromatograms and peak identification of the South Louisiana Crude and hydrocarbons extracted from the salt marsh sediment samples for the oiling and oiling plus burning treatments are shown in Figures 3.20 to 3.22 and Tables 3.11 to 3.13.

Internal standards were used to calibrate the GC/MS instrument and are indicated in the tables as the first six compounds listed. Site specific deuterium labelled polynuclear aromatic hydrocarbons were used as calibration standards. The labelled compounds included 1,4 dichlorobenzene-d4, naphthalene-d8, acenaphthene-d10, phenathrene-d10, chrysene-d12, and perylene-d12. The deuterium labelled standards along with their respective column retention times and measured concentrations are tabulated in Tables 3.11 to 3.13.

The saturated hydrocarbons and polynuclear aromatic hydrocarbons identified in the South Louisiana Crude are graphed in Figure 3.20 by retention time and relative abundance. In general, the lighter hydrocarbons had shorter retention times in the columns. Fifteen polynuclear aromatic hydrocarbon components were identified in the South Louisiana Crude (Table 3.11). The four polynuclear aromatic hydrocarbons with the highest concentrations were naphthalene (6.3 micrograms), phenathrene (2.3 micrograms) chrysene (1.0 micrograms) and fluorene (1.0 micrograms). The remaining polynuclear aromatic hydrocarbons (acenaphthylene, fluoranthene, pyrene, benzo (A) anthracene, benzo (B) fluoranthene, benzo (A) pyrene, and dibenzo (A,H) anthracenes were found in much smaller concentrations (0.2 to 0.8 micrograms). Anthracene was the only polynuclear aromatic hydrocarbon not detected in the South Louisiana Crude (Table 3.11). Based upon the chromatogram, identification, and quantification of the polynuclear aromatic hydrocarbons, the South Louisiana Crude has a distinctive fingerprint that can be identified by GC/MS techniques.

Hydrocarbon component identification of South Louisiana Crude extracted from salt marsh sediment samples is displayed in Table 3.12 and Figure 3.21. After extraction from the sediment, only phenathrene was found in a concentration greater than 1.0 microgram with a

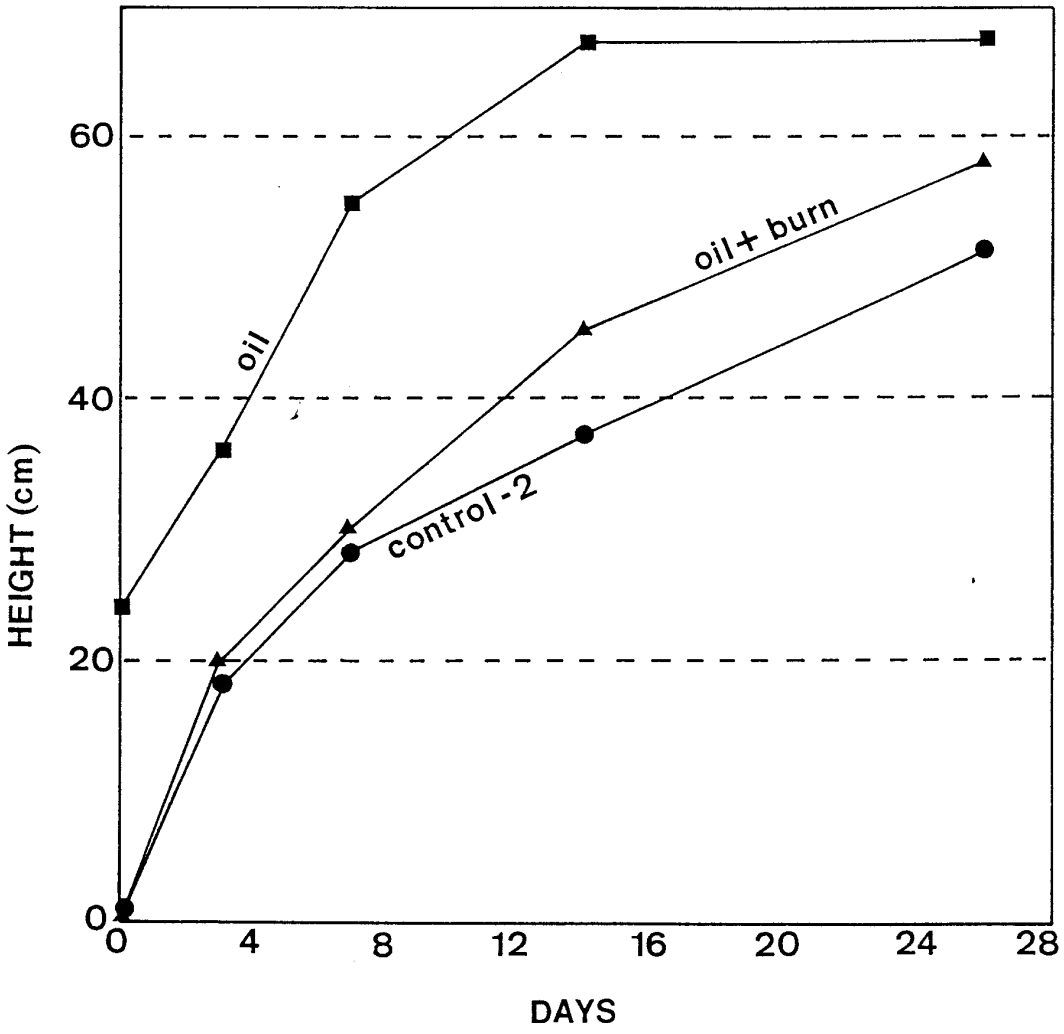


Figure 3.18 *Maximum height of Sagittaria lancifolia shoots, after burn, in saturated cores. Each observation is a mean of four values.*

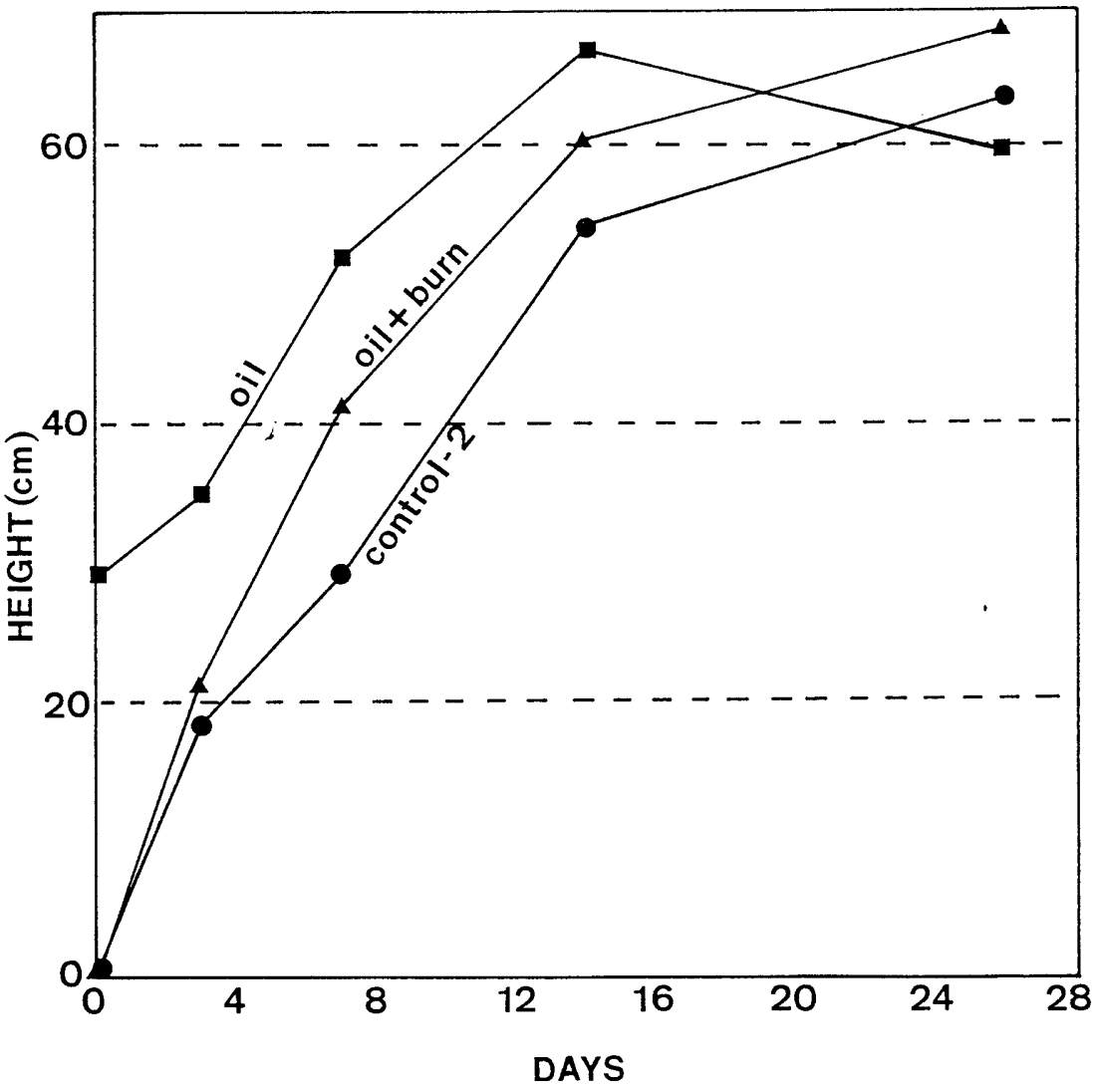


Figure 3.19 *Maximum height of Sagittaria lancifolia shoots, after burn, in cores with two inches of floodwater. Each observation is a mean of four values.*

Table 3.9 Maximum height of *Sagittaria lancifolia* plants (saturated cores) for Control-1, Control-2, SLC-T_1 and SLC + Burn - T_2 treatments.

Species	Treatment	Rep#		Height (cm)				
			Date: Days:	04/07 0	04/10 3	04/14 7	04/21 14	05/05 26
Sagittaria lancifolia (saturated cores)	Control-1	1		29	37	75	100	96
		2		24	30	53	71	69
		3		28	33	40	42	45
		4		36	41	47	64	64
	Control-2 (clip)	1		0	20	31	40	50
		2		0	19	25	36	44
		3		0	14	22	31	36
		4		0	18	34	42	72
	SLC (T_1)	1		29	41	53	63	45
		2		21	38	57	71	61
		3		19	29	61	80	64
		4		26	36	49	55	96
	SLC + Burn (T_2)	1		0	14	25	41	49
		2		0	21	23	55	55
		3		0	15	33	42	72
		4		0	28	37	42	54

*SLC - South Louisiana Crude

Table 3.10 Maximum height of *Sagittaria lancifolia* plants (2 inches of floodwater) for Control-1, Control-2, SLC-T_1 and SLC + Burn - T_2 treatments.

Species	Treatment	Rep #		Height (cm)				
			Date Days	04/07 0	04/10 3	04/14 7	04/21 14	05/05/95 26
Sagittaria lancifolia (2 inches of flood-water)	Control - 1	1 2 3 4		23 30 25 17	28 31 30 20	39 43 39 23	58 75 50 67	56 78 69 66
	Control - 2	1 2 3 4		0 0 0 0	21 17 16 19	39 28 20 30	86 42 43 45	95 54 57 46
	SLC (T_1)	1 2 3 4		29 35 24 27	41 37 33 29	63 61 43 39	80 79 52 57	51 79 57 55
	SLC + Burn (T_2)	1 2 3 4		0 0 0 0	20 16 25 23	31 35 59 38	39 63 75 61	47 76 80 69

* SLC - South Louisiana Crude

measured concentration of 2.8 micrograms. The concentrations of nine polynuclear aromatic hydrocarbons were below 1.0 microgram, and six compounds were not found in the oil extracted from the sediment samples (Table 3.12). Some of the original polynuclear aromatic hydrocarbons originally found in the South Louisiana Crude were apparently absorbed by the sediment, and the concentrations extracted were too small to be detected on the GC-MS instrument.

After burning, the distribution and concentrations of extracted residual polynuclear aromatic hydrocarbons changed significantly compared to the oil source and the oil treatment extractions. After burning and extraction, no polynuclear aromatic hydrocarbon was found in a concentration greater than 0.8 microgram, and eight compounds were not found in high enough concentrations to be detected (Table 3.13). Compared to the original source oil (see Table 3.11), naphthalene, fluorene, phenathrene and chrysene concentrations extracted from the burn treatment (see Table 3.13) decreased significantly. Burning reduced the concentrations of many of the polynuclear aromatic hydrocarbons originally identified in the source oil.

3.5.2 Arabian Crude

Mass chromatograms, peak quantification, and identification of the Arabian Crude and total petroleum hydrocarbon components extracted from the salt marsh sediment samples for the oil and oil plus burn treatments are displayed in Figures 3.23 to 3.25 and tabulated in Tables 3.14 to 3.16.

The polynuclear aromatic hydrocarbons identified in the Arabian Crude are shown in Figure 3.23 and Table 3.14. The first six compounds listed are internal standards used to calibrate the instrument (deuterium labelled).

Naphthalene (3.0 micrograms) and phenathrene (1.2 micrograms) are the two polynuclear aromatic hydrocarbons identified with the highest concentrations. Acenaphthene, anthracene, fluoroanthene and indeno (1,2,3-cd) pyrene aromatics were not found in the Arabian Crude. The remaining compounds identified had concentrations equal to or less than 0.6 micrograms (see Table 3.14).

Comparing the South Louisiana Crude (Table 3.11) to the Arabian Crude (Table 3.14) showed that the South Louisiana Crude had higher concentrations of naphthalene (6.3 micrograms), fluorene (1.0 micrograms), phenathrene (2.3 micrograms) and chrysene (1.0 microgram). In addition, acenapthylene, fluoranthene and indeno (1,2,3-cd) pyrene were absent from the Arabian Crude but were present in the South Louisiana Crude. Based upon the GC-MS analyses the two source oils (South Louisiana Crude and Arabian Crude) had different chromatograms or fingerprints, which is important for source identification.

The hydrocarbon concentrations and distribution of the Arabian Crude extracted from the marsh sediments are shown in Figure 3.24 and Table 3.15. Compared to the source material, the concentration of naphthalene in sediment extracts decreased from 3.0 to 0.2 micrograms. The concentration of phenathrene decreased slightly in the extracted sediment samples (1.1 micrograms) compared to the sources (1.2 micrograms) and six hydrocarbon compounds were not found in the sediment extracts compared to only four compounds not found in the Arabian Crude (Table 3.14). The remaining compounds identified had concentrations equal to or less than 0.5 micrograms.

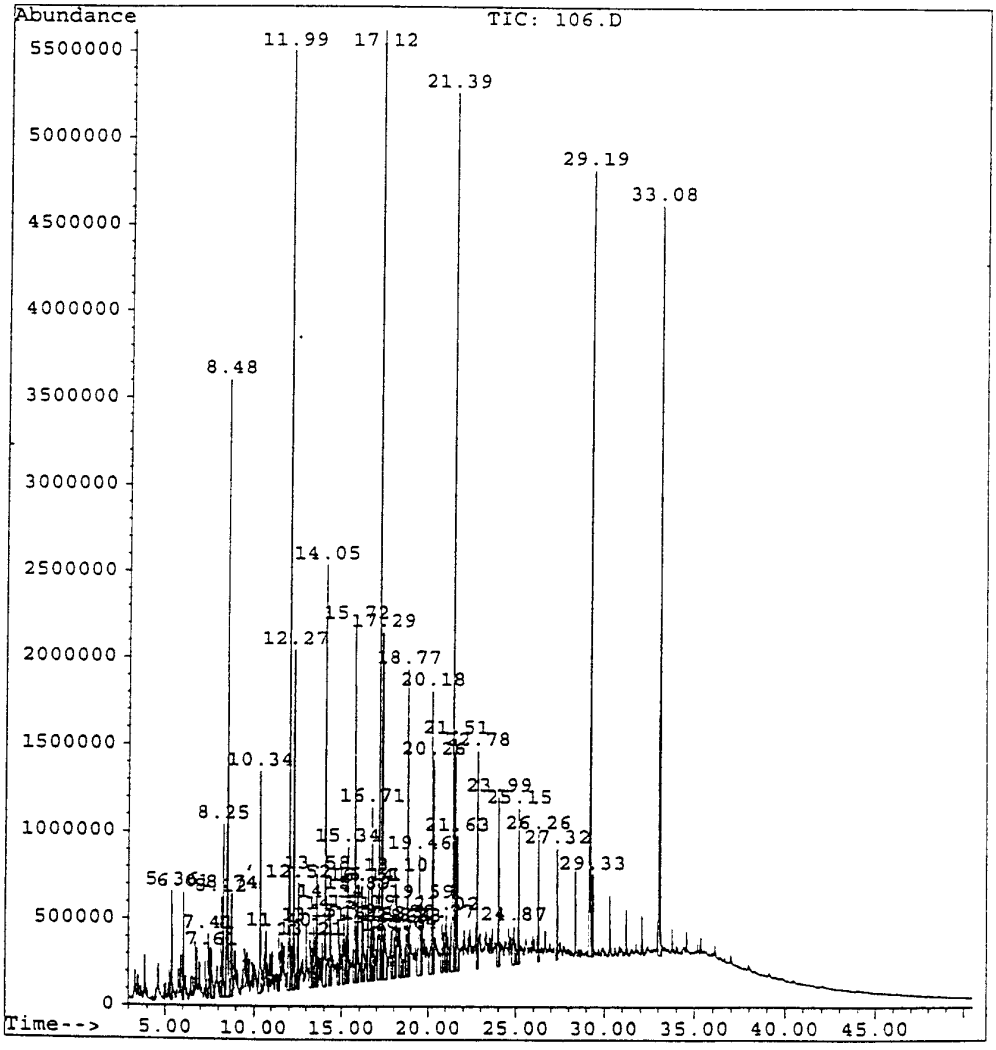


Figure 3.20 *Chromatogram of South Louisiana Crude oil used as source material.*

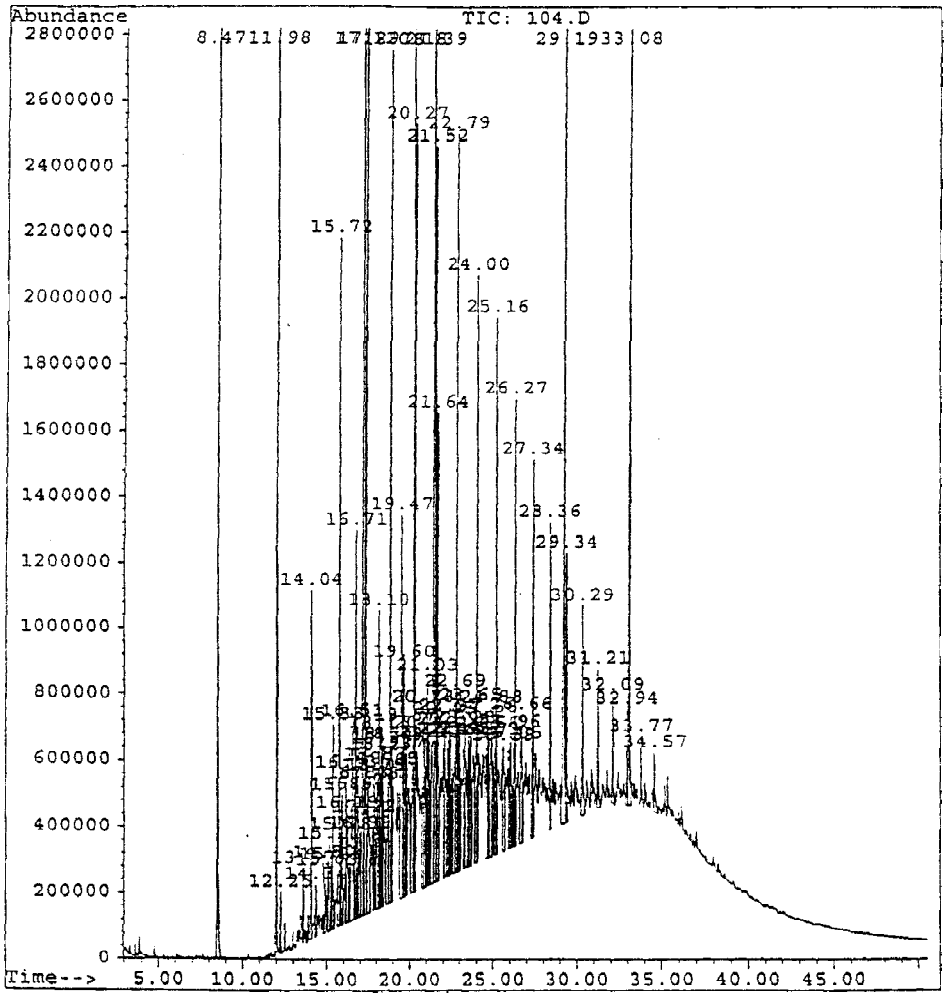


Figure 3.21 *Chromatogram of South Louisiana Crude oil extracted from salt marsh sediment.*

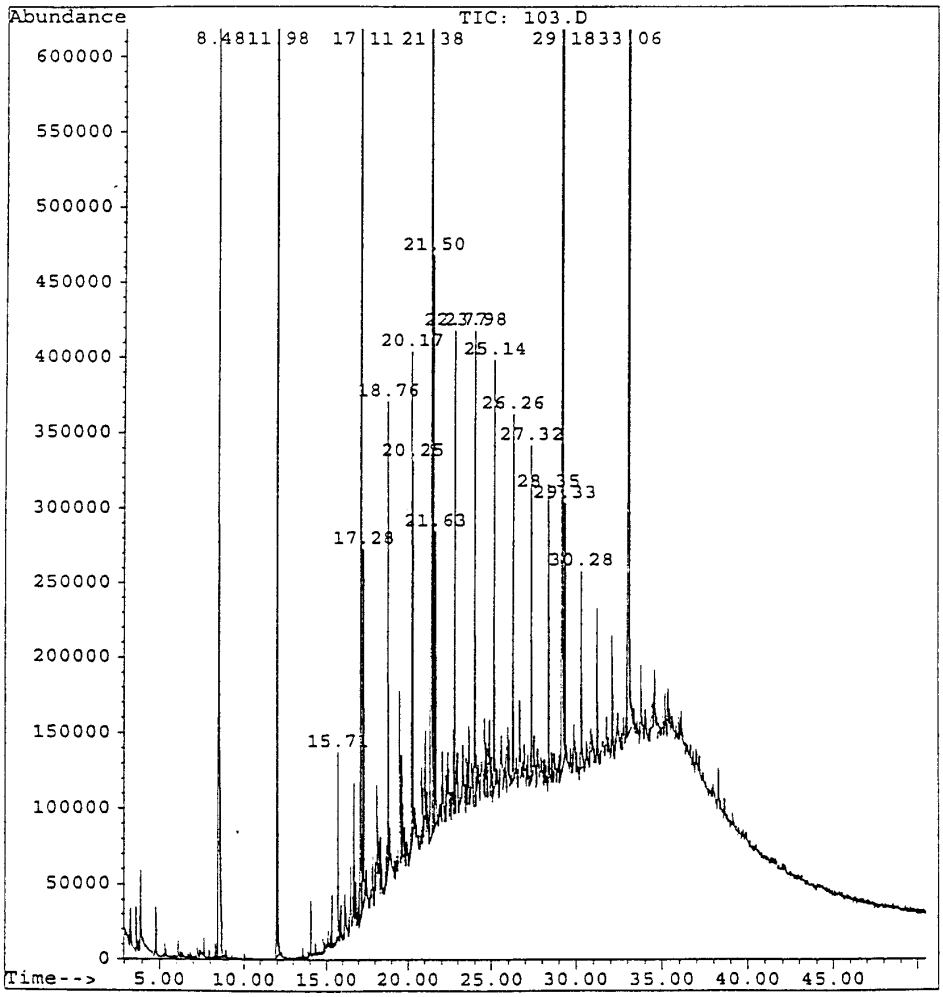


Figure 3.22 *Chromatogram of South Louisiana Crude oil residues extracted from salt marsh sediment after burn.*

Table 3.11 Concentration and identification of polynuclear aromatic hydrocarbons extracted from the South Louisiana Crude oil source.

#	Compound	Ret Time	Concentration	Peak Type
1	1,4-Dichlorobenzene-d4	8.48	135.3 ug	
2	Naphthalene-d8	11.99	144.5 ug	
3	Acenaphthene-d10	17.12	150.4 ug	
4	Phenanthrene-d10	21.39	140.1 ug	
5	Chrysene-d12	29.19	153.9 ug	
6	Perylene-d12	33.08	155.4 ug	
7	Naphthalene	12.03	6.3 ug	
8	Acenaphthylene	16.71	0.2 ug	#
9	Acenaphthene	17.16	0.8 ug	#
10	Fluorene	18.66	1.0 ug	#
11	Phenanthrene	21.43	2.3 ug	#
12	Anthracene	***** NOT FOUND *****		
13	Fluoranthene	24.90	0.3 ug	#
14	Pyrene	25.52	0.4 ug	#
15	Benzo (A) anthracene	29.10	0.4 ug	#
16	Chrysene	29.22	1.0 ug	#
17	Benzo (B) fluoranthene	32.13	0.3 ug	#
18	Benzo (A) pyrene	32.13	0.3 ug	#
19	Benzo (K) fluoranthene	32.86	0.3 ug	#
20	Indeno (1,2,3-cd) pyrene	35.45	0.2 ug	#
21	Dibenzo (A,H) anthracene	35.52	0.2 ug	#
22	Benzo (G,H,I) perylene	35.97	0.3 ug	

END OF REPORT
Qualifiers Not Satisfied

Table 3.12 Concentration and identification of South Louisiana Crude oil polynuclear aromatic hydrocarbons extracted from salt marsh sediment.

#	Compound	Ret Time	Concentration	Peak Type
1	1,4-Dichlorobenzene-d4	8.47	120.9 ug	
2	Naphthalene-d8	11.98	124.6 ug	
3	Acenaphthene-d10	17.11	139.3 ug	
4	Phenanthrene-d10	21.39	131.9 ug	
5	Chrysene-d12	29.19	136.4 ug	
6	Perylene-d12	33.08	129.9 ug	
7	Naphthalene	12.02	0.2 ug	#
8	Acenaphthylene	16.71	0.2 ug	#
9	Acenaphthene	17.16	0.7 ug	#
10	Fluorene	18.66	0.9 ug	#
11	Phenanthrene	21.44	2.8 ug	#
12	Anthracene	***** NOT FOUND *****		
13	Fluoranthene	***** NOT FOUND *****		
14	Pyrene	25.53	0.2 ug	#
15	Benzo (A) anthracene	29.22	0.9 ug	#
16	Chrysene	29.22	0.9 ug	#
17	Benzo (B) fluoranthene	32.08	0.1 ug	#
18	Benzo (A) pyrene	32.08	0.1 ug	#
19	Benzo (K) fluoranthene	***** NOT FOUND *****		
20	Indeno (1,2,3-cd) pyrene	***** NOT FOUND *****		
21	Dibenzo (A,H) anthracene	***** NOT FOUND *****		
22	Benzo (G,H,I) perylene	***** NOT FOUND *****		

END OF REPORT
Qualifiers Not Satisfied

Table 3.13 Concentration and identification of South Louisiana Crude oil polynuclear aromatic hydrocarbons extracted from salt marsh sediment after burn.

#	Compound	Ret Time	Concentration	Peak Type
1	1,4-Dichlorobenzene-d4	8.48	93.8 ug	
2	Naphthalene-d8	11.98	96.6 ug	
3	Acenaphthene-d10	17.11	102.5 ug	
4	Phenanthrene-d10	21.38	102.1 ug	
5	Chrysene-d12	29.18	115.9 ug	
6	Perylene-d12	33.07	115.9 ug	
7	Naphthalene	***** NOT FOUND *****		
8	Acenaphthylene	***** NOT FOUND *****		
9	Acenaphthene	***** NOT FOUND *****		
10	Fluorene	18.66	0.1 ug	#
11	Phenanthrene	21.42	0.8 ug	#
12	Anthracene	***** NOT FOUND *****		
13	Fluoranthene	24.90	0.1 ug	#
14	Pyrene	25.52	0.1 ug	#
15	Benzo (A) anthracene	29.17	0.5 ug	#
16	Chrysene	29.21	0.2 ug	#
17	Benzo (B) fluoranthene	***** NOT FOUND *****		
18	Benzo (A) pyrene	32.07	0.1 ug	#
19	Benzo (K) fluoranthene	***** NOT FOUND *****		
20	Indeno (1,2,3-cd) pyrene	35.45	0.0 ug	#
21	Dibenzo (A,H) anthracene	***** NOT FOUND *****		
22	Benzo (G,H,I) perylene	***** NOT FOUND *****		

END OF REPORT
Qualifiers Not Satisfied

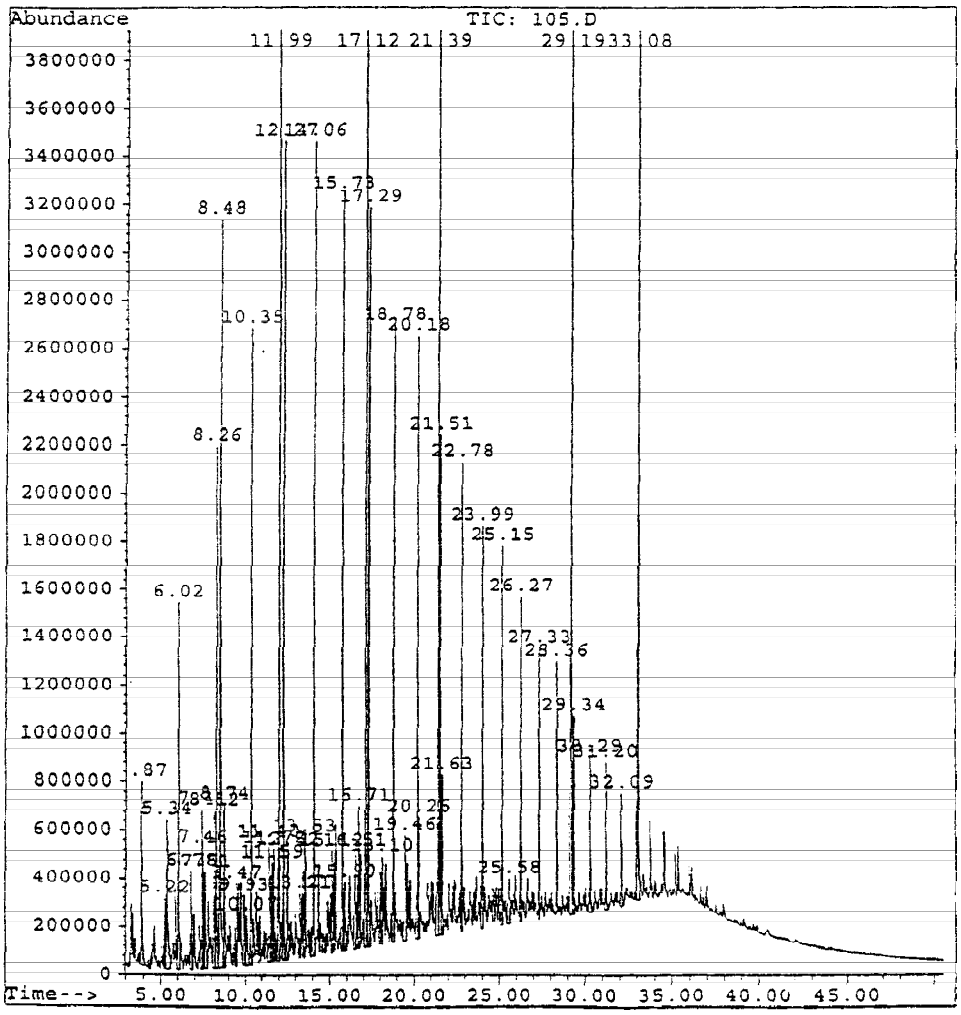


Figure 3.23 *Chromatogram of Arabian Crude oil used as source material.*

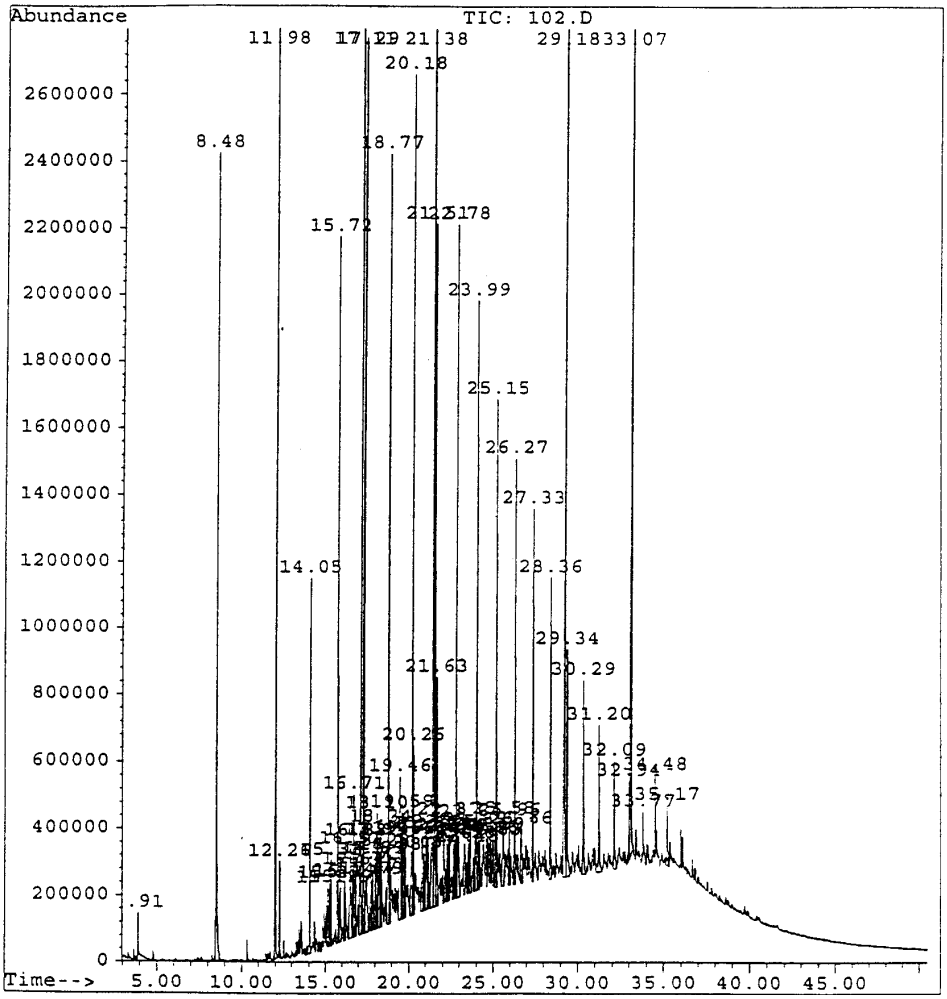


Figure 3.24 *Chromatogram of Arabian Crude oil extracted from salt marsh sediment.*

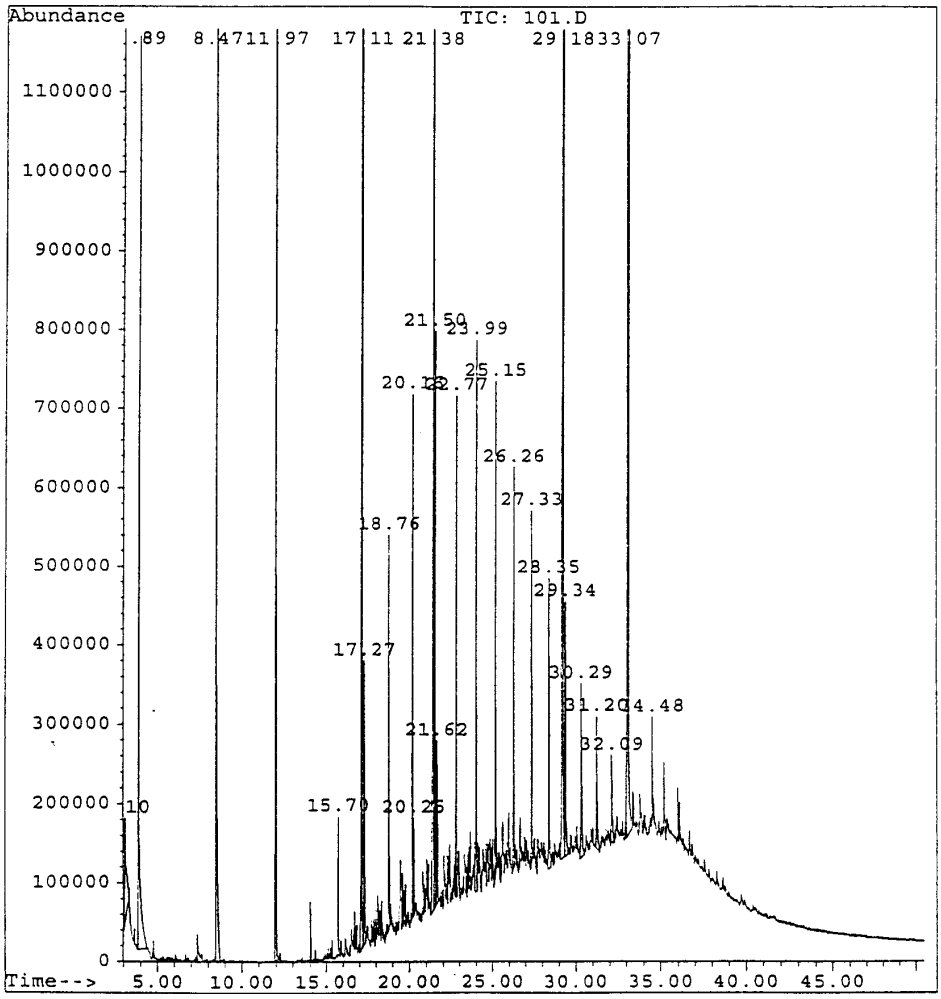


Figure 3.25 *Chromatogram of Arabian Crude oil residue extracted from salt marsh sediment after burn.*

After the burn, all of the polynuclear aromatic hydrocarbons extracted from the sediment samples showed much less than the respective concentrations found in the Arabian Crude and oil extracted from the sediments. In addition, the number of compounds not found increased to nine for the burn treatment compared to six for the sediment extracted samples (oil only). Only four hydrocarbons were not found in the Arabian Crude. Phenathrene extracted from the sediment samples after the burn was the only polynuclear aromatic hydrocarbon with the highest concentration of 0.6 micrograms. All remaining hydrocarbons had concentrations equal to or less than 0.4 micrograms. It appears that burning reduced many of the concentrations of polynuclear aromatic hydrocarbons that were originally found in the Arabian Crude.

3.6 Field Site Selection and Burn Permit Application

Two of the main objectives of Phase I (Year One) were: (1) the selection of a salt marsh site where an *in situ* simulated oil burn could be conducted and monitored, and (2) the acquisition of a coastal burn use permit to conduct the proposed field burns during Phase II (Year Two) of the research project.

After visiting several salt marshes located in the coastal regions of Louisiana, one site was proposed for our second year field burns. The *Spartina alterniflora* salt marsh selected is located within the Pointe Au Chien State Wildlife Management Area in Terrebonne Parish. The site was chosen because the public has limited access to it and because a small road and waterway border the marsh site. Use of the adjacent road and adjoining waterway will enable the investigators to efficiently construct plot enclosures and conduct and monitor the burn experiments with minimal environmental damage.

A coastal use permit to conduct the proposed *in situ* field burns (Phase II) has been applied for through the Coastal Management Division of the Louisiana Department of Natural Resources. The coastal use permit (ENG FORM 4345) has been completed and sent to the Coastal Management Division (March 1, 1995). The Louisiana coastal burn use permit application was sent out for public notice and comment on March 27, 1995. On April 5, 1995 preliminary written approval was received from the Corps of Engineers (New Orleans) and on April 10, 1995 written approval was received from the Department of Health and Hospitals. The principal investigators are currently waiting final approval of the submitted coastal burn use permit.

Table 3.14 Concentration and identification of polynuclear aromatic hydrocarbons extracted from the Arabian Crude oil source.

#	Compound	Ret Time	Concentration	Peak Type
1	1,4-Dichlorobenzene-d4	8.48	126.1 ug	
2	Naphthalene-d8	11.99	132.3 ug	
3	Acenaphthene-d10	17.12	138.4 ug	
4	Phenanthrene-d10	21.39	133.8 ug	
5	Chrysene-d12	29.19	154.3 ug	
6	Perylene-d12	33.08	158.4 ug	
7	Naphthalene	12.03	3.0 ug	#
8	Acenaphthylene	16.71	0.2 ug	#
9	Acenaphthene	***** NOT FOUND *****		
10	Fluorene	18.66	0.5 ug	#
11	Phenanthrene	21.43	1.2 ug	#
12	Anthracene	***** NOT FOUND *****		
13	Fluoranthene	***** NOT FOUND *****		
14	Pyrene	25.52	0.1 ug	#
15	Benzo (A) anthracene	29.19	0.6 ug	#
16	Chrysene	29.19	0.6 ug	#
17	Benzo (B) fluoranthene	32.08	0.0 ug	#
18	Benzo (A) pyrene	32.08	0.0 ug	#
19	Benzo (K) fluoranthene	33.09	0.5 ug	#
20	Indeno (1,2,3-cd) pyrene	***** NOT FOUND *****		
21	Dibenzo (A,H) anthracene	35.53	0.1 ug	#
22	Benzo (G,H,I) perylene	35.98	0.1 ug	#

END OF REPORT
Qualifiers Not Satisfied

Table 3.15 Concentration and identification of Arabian oil polynuclear aromatic hydrocarbons extracted from salt marsh sediment.

#	Compound	Ret Time	Concentration	Peak Type
1	1,4-Dichlorobenzene-d4	8.48	92.6 ug	
2	Naphthalene-d8	11.98	98.6 ug	
3	Acenaphthene-d10	17.11	110.1 ug	
4	Phenanthrene-d10	21.38	109.0 ug	
5	Chrysene-d12	29.18	119.6 ug	
6	Perylene-d12	33.07	117.5 ug	
7	Naphthalene	12.02	0.2 ug	#
8	Acenaphthylene	16.71	0.1 ug	#
9	Acenaphthene	***** NOT FOUND *****		
10	Fluorene	18.66	0.4 ug	#
11	Phenanthrene	21.43	1.1 ug	#
12	Anthracene	***** NOT FOUND *****		
13	Fluoranthene	***** NOT FOUND *****		
14	Pyrene	25.52	0.1 ug	#
15	Benzo (A) anthracene	29.21	0.5 ug	#
16	Chrysene	29.21	0.5 ug	#
17	Benzo (B) fluoranthene	32.09	0.1 ug	#
18	Benzo (A) pyrene	32.09	0.0 ug	#
19	Benzo (K) fluoranthene	33.07	0.4 ug	#
20	Indeno (1,2,3-cd) pyrene	***** NOT FOUND *****		
21	Dibenzo (A,H) anthracene	***** NOT FOUND *****		
22	Benzo (G,H,I) perylene	***** NOT FOUND *****		

END OF REPORT
Qualifiers Not Satisfied

Table 3.16 Concentration and identification of Arabian oil polynuclear aromatic hydrocarbons extracted from salt marsh sediment after burn.

#	Compound	Ret Time	Concentration	Peak Type
1	1,4-Dichlorobenzene-d4	8.47	97.1 ug	
2	Naphthalene-d8	11.97	101.3 ug	
3	Acenaphthene-d10	17.11	112.9 ug	
4	Phenanthrene-d10	21.38	107.9 ug	
5	Chrysene-d12	29.18	110.5 ug	
6	Perylene-d12	33.07	108.4 ug	
7	Naphthalene	12.01	0.1 ug	#
8	Acenaphthylene	***** NOT FOUND *****		
9	Acenaphthene	***** NOT FOUND *****		
10	Fluorene	18.65	0.2 ug	#
11	Phenanthrene	21.42	0.6 ug	#
12	Anthracene	***** NOT FOUND *****		
13	Fluoranthene	***** NOT FOUND *****		
14	Pyrene	25.52	0.1 ug	#
15	Benzo (A) anthracene	29.18	0.4 ug	#
16	Chrysene	29.18	0.4 ug	#
17	Benzo (B) fluoranthene	***** NOT FOUND *****		
18	Benzo (A) pyrene	***** NOT FOUND *****		
19	Benzo (K) fluoranthene	33.07	0.4 ug	#
20	Indeno (1,2,3-cd) pyrene	***** NOT FOUND *****		
21	Dibenzo (A,H) anthracene	***** NOT FOUND *****		
22	Benzo (G,H,I) perylene	***** NOT FOUND *****		

END OF REPORT
Qualifiers Not Satisfied

4.0 Conclusions and Recommendations

The first greenhouse burn study conducted during the summer of 1994 used *Spartina alterniflora* and *Panicum hemitomon* plant-sediment cores collected from salt marsh and fresh marsh habitats of coastal Louisiana. A greenhouse study was initiated to determine the effects of oiling and oiling plus burning on the collected marsh species. South Louisiana Crude oil was applied at a rate of 2 L m^{-2} to replicated cores (saturated) of *Spartina alterniflora* and *Panicum hemitomon.* The burn treatments were ignited with the aid of gasoline (5 cc). Shoot regeneration and biomass production were monitored for 54 days after the burn.

For both the *Spartina alterniflora* and *Panicum hemitomon* control cores (no oiling, burning, or clipping) the number of live shoots increased over the 54 day sampling period. The number of live shoots in the replicated control cores increased approximately 100% and 165% for the *Spartina alterniflora* and *Panicum hemitomon* treatments, respectively. For the oiling only treatment, a slight increase (33%) in live shoot regeneration was observed for the *Spartina alterniflora* cores over the study period. Oil had a much greater effect on *Panicum hemitomon* plants. On day three about seven live shoots per replication were recorded, and at 54 days the number of live shoots per core had decreased to about one per core. Compared to the control cores, oiling had a significant effect on *Spartina alterniflora* and *Panicum hemitomon* plants. Oiling plus burning had a drastic effect on shoot regrowth of the two plant species. The burn procedure killed all *Spartina alterniflora* and *Panicum hemitomon* plants in the oil plus burn treatment cores. No new shoot regrowth was observed in any of the cores for either plant species over the entire sampling period.

The complete kill of *Spartina alterniflora* and *Panicum hemitomon* plants in the saturated cores for the oil plus burn treatments was caused mainly by the lack of a floodwater layer (two to four inches) above the marsh surface in the cores. Ordinarily the floodwater layer can insulate the plant roots from heat. The application of 5 cc of gasoline to each oil plus burn replication may also have had a negative effect on the survival of plants.

Based on the data collected from the first greenhouse burn experiment, a second study was initiated. In the spring of 1995, plant-sediment cores of *Sagittaria lancifolia* were collected from a freshwater marsh for the second burn experiment. Treatments increased and included two sets of controls and oiling only and oiling plus burning treatments. All treatments were applied to one set of *Sagittaria lancifolia* in which only the sediment cores were saturated along with a second set of cores that had a two inch floodwater layer permanently established above the sediment surface.

For the Control-1 (no oiling, burning, or clipping) and Control-2 (clipped, no oiling or burning), the number of new shoots recorded over the 26 day monitoring period increased from a mean of 16.5 to 19.5 and from 0 to 17 live shoots per core, respectively. For the oiling only treatment of the saturated cores the mean live shoot value increased about 46% over the 26 days. The oiling plus burning treatment showed a large response in new shoot regrowth. Over the 26 days, the mean live shoot value increased from 0 (due to burn) to 22 live shoots per core at the conclusion of the experiment.

Measurements collected from the second set of *Sagittaria lancifolia* cores with two inches of floodwater also showed good shoot regrowth after the oil burn. Shoot regeneration in the oil only treatment decreased slightly over time. At 0 days 14 live shoots per core were recorded, and after 26 days the live shoot mean had decreased to 12 per core. The greatest rate of increase was observed for the oil plus burn treatment cores with two inches of floodwater. Three days after the burn, a live shoot mean of five per core was observed, and at 26 days 21 live

shoots per core were measured, which represents a 320% increase over 23 days. Shoot regeneration in the control cores with two inches of floodwater also increased over the sampling period.

After burning, maximum plant height for the saturated and two inch floodwater layer core sets steadily increased over the 26 days and approached the same maximum height as the *Sagittaria lancifolia* plants in the control treatments. The survival of *Sagittaria lancifolia* plants after the oil burn was mainly attributed to the establishment of a floodwater layer and the use of a propane torch instead of gasoline.

Hydrocarbon identification and quantification of two oil sources (South Louisiana Crude and Arabian Crude) and identification of oil residues extracted from salt marsh sediments for the oiling and oiling plus burning treatments were successful. Differences in polynuclear aromatic hydrocarbons and concentrations were noted for the two oil sources. The South Louisiana Crude was higher in naphthalene, fluorene, phenathrene and chrysene compared to the polynuclear aromatic hydrocarbon distribution of the Arabian Crude. Burning significantly reduced the number of polynuclear aromatic hydrocarbons that could be identified, and reduced the concentrations of many other hydrocarbons compared to the source material and hydrocarbons extracted from the sediment (oil only treatment).

A *Spartina alterniflora* salt marsh habitat located within the Pointe Au Chien State Wildlife Management Area has been selected for the *in situ* field burns proposed for the second year of the project. A coastal use permit has been completed and returned to the Coastal Management Division of the Louisiana Department of Natural Resources for processing and approval. Written conditional approvals from the Corps of Engineers and from the Department of Health and Hospitals have been received. The principal investigators are currently waiting for final approval of the coastal burn use permit.

Based on the data collected from the two greenhouse burn experiments, using *Spartina alterniflora, Panicum hemitomon* and *Sagittaria lancifolia* species, the investigators recommend that a floodwater layer be established above the marsh surface before burning. In addition, use of a propane torch to ignite the oil is recommended instead of gasoline. Both recommendations should greatly increase the plants' survival rates after an oil burn.

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