Dispersants as Oil Spill Countermeasures for the Remediation and Restoration of Sensitive Coastal Habitats

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Submitted by

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Abstract

Dispersants have received considerable attention for offshore, deep-sea oil spills. However, dispersants may also have potential for minimizing the impact of oil spills in nearshore and estuarine water, where spilled oil may eventually be transported to sensitive coastal habitats, such as coastal salt marshes, and cause considerable damage. The potential impacts might be reduced if the oil could be dispersed before it makes contact with the shoreline. This approach to oil spill response, however, is impaired by an absence of experimental evidence documenting the effect and efficacy of dispersed oil on sensitive coastal habitats, in particular, tidal salt marshes. The objectives of this research were to (1) evaluate the effect and efficacy of different dispersants in reducing the impact of oil on coastal salt marshes, (2) quantify the effect of oil type and concentration in modifying dispersant action, and (3) determine if the effect and efficacy of dispersants are influenced by marsh substrate type and plant growing season.

The effect and efficacy of dispersants applied to South Louisiana crude (SLC) oil and No. 2 fuel oil in different spill scenarios in the nearshore environment were simulated for different marsh types and seasons. Short- and long-term oil responses of the salt marsh plant, *Spartina alterniflora*, oil content in the soil, and soil microbial populations were analyzed in different spill scenarios. Without dispersant application, both SLC oil and No. 2 fuel oil at a concentration of 150 ppm considerably impacted *S. alterniflora* in the short term. During a two-month period, the plants showed a significantly higher leaf mortality rate and dead stem density and significantly lower community photosynthetic rate, live stem density, and live aboveground biomass compared to the control. However, application of dispersants, both JD-2000 and Corexit 9500, significantly relieved the short-, mid- and long-term impacts of the oil on the plants in both organic and sandy salt marsh soils. In addition, dispersant application in both the active summer plant growing season and in the inactive fall plant growing season greatly relieved the impacts of SLC oil and No. 2 fuel oil on the salt marsh plant, *S. alterniflora*. Thus, regardless of marsh substrate type and application season, the dispersants greatly reduced the adverse effects of low concentrations of oil (150 ppm of SLC or No. 2 fuel oil) on this species of marsh plant.

The application of dispersants not only relieved the impact of the 150-ppm oil concentration, but also reduced impact at a much higher oil concentration of 750 ppm. The high concentration of oil without dispersants resulted in much greater impacts on the marsh plants compared to the low concentration of oil. Leaf mortality was as high as 90% after treatment with 750 ppm of No. 2 fuel oil in the absence of dispersants. However, application of the dispersants to the high oil concentration significantly reduced the impact of the oil on the plants. Corexit 9500 was not as effective as JD-2000 for reducing the impact of the high concentration of SLC oil in the short-term, although it still significantly relieved the impact of the oil compared to SLC oil alone.

The application of dispersants did not adversely affect soil microbes. Populations of heterotrophic microbes and oil degrading microbes in the soil were generally unaffected by dispersant application. The residual total targeted alkanes and polycyclic aromatic hydrocarbons (PAHs) in the 150-ppm low oil concentration treatment were completely degraded one year after treatment-initiation regardless of dispersant application. However, for the 750-ppm high oil concentration treatment, total targeted alkanes, PAHs, and total petroleum hydrocarbons (TPH) were lower for dispersed oil treatments than undispersed oil treatments, especially when n-alkanes and PAHs were

enriched by No. 2 fuel. This suggested that application of dispersants enhanced oil degradation in the soil.

This research indicated that the application of dispersants in nearshore environments before oil comes in contact with coastal marshes has considerable potential for protecting sensitive coastal habitats. The results of this study provided information for future research on a larger scale with, for example, wave tanks and/or actual field investigations of oil dispersants.

Key Words: Dispersants, Oil spill, *Spartina*, Salt marshes, Habitat protection, Nearshore

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Dispersants as Oil Spill Countermeasures for the Remediation and Restoration of Sensitive Coastal Habitats

1.0 Introduction

Oil spilled in nearshore and estuarine water may eventually be transported by currents, tides and winds into adjacent sensitive coastal habitats. Cleanup of stranded oil in these habitats, such as coastal salt marshes containing dense vegetation, is difficult and problematic, and often does more damage to these highly sensitive habitats than the oil itself. Nonetheless, it is often essential to remove the oil that strands and impacts sensitive coastal habitats, such as coastal marshes, even though conventional cleanup is expensive and problematic (Lin *et al*. 1999b). Therefore, it is important to develop cost-effective and less-intrusive procedures to prevent, restore, and clean up oil-contaminated marshes.

Dispersants have received considerable attention as countermeasures for offshore and deep open-sea oil spills; the National Research Council (NRC, 1989) summarized the effectiveness and possible impact of dispersed oil in the deep sea. However, dispersants may also have significant potential for reducing the impact of oil spills in nearshore and estuarine water, where the spilled oil may be transported to sensitive coastal habitats, such as salt marshes, and cause considerable damage. The potential impacts might be reduced if the oil could be dispersed before it makes contact with the shoreline. This approach to oil spill response, however, is impaired by an absence of experimental evidence documenting the effect and efficacy of dispersed oil on sensitive coastal habitats, in particular, tidal salt marshes. More than 15 years after the NRC's summary of open-water dispersant effectiveness, the Council (NRC 2005) suggested in 2005 that there were significant gaps in the knowledge needed to make sound decisions regarding the use of dispersants in nearshore or shallow areas with restricted flushing rates.

Dispersants were first used during Torrey Canyon spill in 1967 to remediate a major oil spill. The use of dispersants in oil-spill cleanup has attracted considerable attention since the Exxon Valdez oil spill in 1989 (Cunningham *et al*. 1991; Venosa *et al*. 1999). Dispersants are chemicals that contain surfactants, or compounds that act to break hydrophobic liquid substances such as oil into tiny droplets in the water column, thus helping to clear the oil from the water surface. Dispersant use has been recommended for oil slicks in the sea before coastal habitats are impacted (Page *et al*. 2000). Therefore, studies of the effects of dispersants have primarily focused on marine organisms, such as fishes and shrimp, and the larvae of fishes, crabs, and corals (Singer *et al*. 1994; Rhoton *et al*. 1999; Gulec and Holdway 2000; Epstein *et al*. 2000; Wolfe *et al*. 2001, NRC 2005). Most of these studies utilized acute toxicity tests. However, it has been suggested that decisions to use oil spill response chemicals should not be based solely on aquatic toxicity (George and Clark 2000).

Oil spilled in nearshore environments usually moves into sensitive coastal habitats with currents, tides, and winds. One strategy may be to apply dispersants to oil slicks to disperse the oil into the water column before the oil reaches the coast, thus preventing oil from stranding and coating sensitive habitats, such as coastal salt marshes. Only a few studies have been conducted on the effect of dispersants on coastal wetland plants, and these results were conflicting. Studies (Baker *et al*. 1984, Lane *et al*. 1987, Little and Scales 1987) reported that the application of dispersants BP1100WD, Corexit 9527, and BP Enersperse 1037 to oil, such as weathered Nigerian crude, fuel oil and mousse, were more destructive to salt marsh plants, such as *Spartina anglica*, *Salicornia* spp., and *Aster* spp. than oil alone. In contrast, Smith *et al*. (1984) and DeLaune *et al*. (1984) demonstrated that dispersants applied to Louisiana crude oil-contaminated *Spartina alterniflora* had short-term benefits to plant photosynthesis. The more recent studies (Teas *et al*. 1993; Pezeshki *et al*. 1995; DeLaune *et al*. 1998, Duke *et al*. 2000) indicated that application of Corexit 9580 and Corexit 9527 accelerated the recovery of *Spartina alterniflora*, *Sagittaria lancifolia*, and mangrove trees from oiling.

Lin and Mendelssohn (2003) demonstrated that toxicity of dispersed oil applied to the soil on marsh plants was much lower than on marine organisms. The LC_{50} for *Sagittaria lancifolia* was about 24,000 ppm of JD-2000 dispersed diesel for a three-week exposure compared to the LC_{50} for *Marnidia beryllina* of 3.59 ppm of JD-2000-dispersed No. 2 fuel for 96 hours (EPA 2001). These results suggested that some dispersants might be used for coastal habitat protection. The principal biological benefit of dispersant use is to prevent oil from stranding in sensitive habitats (NRC 1989), such as coastal marshes, thus reducing oil impacts.

Marsh sediment characteristics, such as soil texture and soil organic matter content (OMC), may affect oil adsorption to the sediment. Previous studies (Lin and Mendelssohn 1996 and 1998) indicated that sediment with higher OMC adsorbed more oil in the soil. In addition, oil penetrated sediment with coarser texture more than with finer texture (Lin *et al*. 1999). As a result, sediment characteristics may affect oil retention in the soil, thus impacting the organisms in the soil. Furthermore, oil impact on marsh vegetation may differ with plant growth season. Oil adsorbed into the soil had a greater impact during the more active plant growing season (late spring and summer) than it did during the inactive growth season (fall and winter), thus affecting habitat recovery after oiling. However, oil-coated plant leaves accelerated leaf senescence more in the fall than in the late spring and summer (Lin 1996). Therefore, the effect and efficacy of dispersants in reducing the impact of oil on marsh vegetation might vary with environmental conditions. However, effects of growing season and sediment type on the toxicity and remediation of dispersed oil have received little scientific attention.

Dispersants used today are more effective and less toxic than those used previously. For example, the more recently marketed dispersant, Corexit 9500, contains the same surfactants present in Corexit 9527 but has an improved solvent delivery system. Also, the dispersant JD-2000 marketed in 2001 is effective in both salt and fresh water (EPA 2001). Both Corexit 9500 and JD-2000 are high-performance, biodegradable oil spill dispersants listed in the National Contingency Plan (EPA 2001). Efficacy of dispersants may vary with different spill scenarios. Dispersants generally are more effective for salt water than fresh water, for less viscous oil than more viscous oil, and for warmer temperatures than cooler ones (NCR 1989; Guyomarch *et al*. 1999; EPA 2001). However, little information is available on the fate and effect of dispersed oil in coastal habitats, especially for these new-generation dispersants, and even less information is available concerning the extent to which these dispersants protect coastal habitats and promote habitat restoration after oil spills.

2.0 Objectives

The overall goal of this research was to investigate the potential of using dispersants as an oil spill countermeasure in nearshore environments before oil slicks reach sensitive coastal marshes by tides, currents, and winds. Specifically, the objectives of this study were to (1) evaluate the effect and efficacy of different dispersants in reducing the impact of oil on coastal salt marshes, (2) quantify the effect of oil type and concentration in modifying dispersant action, and (3) determine if the effect and efficacy of dispersants are influenced by marsh substrate type and plant growing season.

3.0 Materials and Methods

3.1 Materials and experimental procedures

Intact soil sods (28 cm in diameter and 20 cm in depth) of both organic and sandy substrates were extracted from coastal salt marshes dominated by *Spartina alterniflora*. As suggested by the CRRC's Science Advisory Panel, we tested different sizes of marsh sods to determine if the size used in the experiment was large enough for plant survival and growth. We found that survival rates of salt marsh plants in sods with dimensions of 28 cm by 20 cm, 20 cm by 20 cm and 10 cm by 10 cm in diameter and depth, respectively, were about 100%, and the growth of new stems resulted in plant stem densities that more than doubled in three months for all sod sizes. We used 28 cm by 20 cm sods in the present experiments as a precaution. Using intact, un-disturbed marsh sods ensure that results will be more similar to the field. We used salt marsh sods dominated by *S. alterniflora* because salt marshes are generally more vulnerable to nearshore spills due to their location within the coastal zone. Furthermore, the salt marsh plant, *Spartina alterniflora*, is a dominant species along the Northern Gulf of Mexico and Atlantic coasts.

The following treatments simulate the application of dispersants on oil spilled in nearshore, shallow water, and estuarine environments before the oil contacts adjacent marshes. Generally, mixing forces such as currents, tides, and winds are needed for dispersants to disperse oil in nearshore environments. The dispersed oil may then move into marshes with the rising tide or with onshore winds. With the ebbing tide, the dispersed oil may then move out of the marshes.

This research consisted of three separate experiments:
(1) Summer application of 150 ppm of SLC or No. 2 fuel oil, dispersed and undispersed.
(2) Summer application of 750 ppm of SLC or No. 2 fuel oil, dispersed and undispersed
(3) Fall application of 150 ppm of SLC or No. 2 fuel oil, dispersed and undispersed.

The actual amounts of oil applied were 6 g and 30 g of dispersed oil or undispersed oil in 40 L of salt water (25 ppt) for the 150-ppm and 750-ppm oil level per experimental unit, respectively. The application rates were equivalent to 98 g m^{-2} and 496 g m^{-2} based on marsh surface area for the 150-ppm and 750-ppm treatments, respectively.

The following factors were tested in each experiment:
(1) Dispersant type—no dispersant, the dispersant Corexit 9500, or the dispersant JD-2000.

(2) Oil type – SLC oil or No. 2 fuel oil. In addition, treatments receiving neither oil nor dispersants served as the overall control.
Thus, for each experiment, there were seven treatment levels: control, JD-2000 dispersed SLC oil, Corexit 9500 dispersed SLC oil, SLC oil alone, JD-2000 dispersed No. 2 fuel oil, Corexit 9500 dispersed No. 2 fuel oil, and No. 2 fuel oil alone. Each treatment level was replicated five times. The ratio of dispersant to oil was 1:20 based on the manufacture's recommendation of a 1:10 to 1:50 ratio. SCL oil and No. 2 fuel oil were artificially weathered by 20% (v/v). The oil and dispersants were premixed.

We placed an 80-cm high and 28-cm diameter watertight collar over the bucket holding the marsh sods to enable a tidal regime. We simulated nearshore dispersed oil or oil moving in and out of salt marshes by generating a tide with appropriate concentrations of oil or dispersed oil in the water so that the plant shoots were covered for a 30-minute period at high tide. A small submersible pump was used to create a gentle current in the surface water to provide energy for dispersant action during the 30-minute high tide. Subsequently, the water receded to 10 cm above the soil surface for the rest of the 12-hour high tide period. For the 12-hour low tide, the water table was lowered to 10 cm below the soil surface. The dispersed oil or oil in the water column moved out of the experimental unit from a hole on the wall of the collar 30 cm above the soil surface during high tide. For low tide, water drained through a hole on the wall of the bucket at 10 cm below the soil surface. Thereafter, we maintained the water table fluctuation between 0 and 10 cm below the soil surface throughout the experiment. The experiments were conducted in an open-ended greenhouse in which environmental conditions were held relatively constant compared to those in the field. Thus, the effect and efficacy of dispersants were examined with natural variability minimized. In addition, the open-end greenhouse let weather variables, such as temperature, humidity and day-length, change with the season; thus, the results were more realistic.

The experiment lasted for a full plant growth cycle (one year) to determine the short- and long-term effects of dispersed and undispersed oil on marsh plants, soil microbes, and oil content in the soil. The effect and toxicity of dispersed and undispersed oil were determined by analyzing plant leaf mortality, plant community photosynthetic rate, live plant stem density, dead plant stem density, plant live and dead aboveground biomass, heterotrophic microbial populations, petroleum degrader populations, total petroleum hydrocarbons (TPH), total targeted alkanes, and aromatic hydrocarbons (PAHs) in the soil.

3.2 Methods and QA/AC

Community photosynthesis

Plant community photosynthetic rates were measured by using a portable infrared photosynthesis system (CID CI-301PS) and a large transparent chamber. The soil surface was covered with a 5-cm water layer. The transparent chamber was placed over the experimental unit and made gas-tight by the gaskets and clamps. Measurements were conducted under full sunlight. The change in concentration of CO_2 in the chamber in a 60-second period was measured by an infrared photosynthesis system. Plant community photosynthetic rate was calculated based on the area of the soil surface, expressed as μmol $CO_2/m^2/s$. The infrared gas analyzer was calibrated and rechecked every 10 measurements with a standard gas of 300 ppm CO_2 to conduct Quality Assurance and Quality Control (QA/QC).

Plant stem density and plant leaf mortality rate

Plant stem density was determined by counting the number of live and dead stems in each experimental unit. Also, mortality rate was estimated for each experimental unit (Lin and Mendelssohn 1996).

Plant aboveground biomass

Aboveground biomass was harvested two and six months after treatment-initiation, and at the end of the experiment (12 months after the treatment began). Live and dead plant tissue was separated and dried at 65 °C to a constant weight (Lin *et al*. 2002) to determine the short-term, mid-term, and long-term effects of dispersed and undispersed oil on the salt marsh grass, *Spartina alterniflora*.

The TPH in the sediment

Total dichloromethane (DCM) extractable petroleum hydrocarbons (TPH) in the sediment were analyzed gravimetrically (Lin & Mendelssohn, 1996). Soil samples collected from a depth interval of 0 to 2 cm were mixed with anhydrous sodium sulfate and extracted with DCM. The DCM extracts were transferred to pre-weighed dishes, the DCM evaporated, and the un-evaporated oil remaining in the dishes was weighed to the nearest 0.0001 g. After extraction, each sediment sample was dried at 65 ^{0}C and weighed. Total hydrocarbon concentration in the sediment was calculated and expressed as mg TPH g^{-1} dry soil.

Residual normal alkanes (n-alkanes) and polycyclic aromatic hydrocarbons (PAHs) in the soil

Residual n-alkanes and PAHs in the soil were analyzed by gas chromatography-mass spectrometry (GC/MS). The soil was sampled from a depth interval of 0 to 2 cm. Five replicate samples from the same treatment were composited and mixed with anhydrous sodium sulfate, extracted with dichloromethane (DCM), and analyzed by GC/MS. The MS was operated in the Selective Ion Monitoring (SIM) Mode to characterize the composition of selected n-alkanes and PAHs. GC separations used a 30 meter, 0.25 mm i.d. column with a 5% phenyl-95% dimethylpolysiloxane (DB-5) stationary phase. The initial GC temperature was 50 °C for two minutes followed by temperature programming to 280 °C at a rate of 15 °C /minute. The temperature was held at 280 °C for an additional 12 minutes (Lin *et al*. 1999a).

Most-Probable-Number (MPN) procedures for microbial populations

Total heterotrophic and hydrocarbon-degrading microbial populations were estimated using the most-probable-number (MPN) procedure outlined by Wrenn and Venosa (1995), Haines *et al*. (1996), Binet *et al*. (2000), and SSSA (1994). Briefly, No. 2 fuel oil was the selective substrate for determination of total petroleum hydrocarbon degraders. No. 2 fuel oil (5 μL/well) was added to the 96-well microtiter plates after the wells were filled with growth medium, but before they were inoculated. Bushnell-Hass medium (Difco Products) was used as the growth medium for all hydrocarbon-degrader MPN procedures (180 μL B-H/well). Soil samples were diluted serially, ranging from 10^{-3} ~ to 10^{-9}. The plates were inoculated by adding 20 μL of each dilution to three wells. One row remained un-inoculated to serve as sterile controls. The total hydrocarbon-degrader MPN plates were incubated for two weeks at room temperature. Positive wells were scored and recorded.

Total heterotrophic microbial populations were enumerated in separate 96-well microtiter plates filled with Nutrient Broth (Difco) medium, using the same soil suspension and dilution method as above. After one week, the growth of microbial populations was determined. The MPN numbers were calculated for each category of microbes according to the Most-Probable-Number procedure (SSSA 1994) and expressed as number per gram soil dry weight.

3.3 Statistical Analysis

Statistical analyses were conducted with the Statistical Analysis System (SAS, version 9.1.3). Plant parameters, microbial populations, and TPH concentrations were analyzed with general linear models (PROC GLM). The least square means test was used to evaluate statistical differences between treatment-level combinations. Significant differences were reported at the 0.05 probability level, unless otherwise stated.

4.0 Results

Composition of n-alkanes and polycyclic aromatic hydrocarbons (PAHs) in weathered South Louisiana crude oil and No. 2 fuel oil

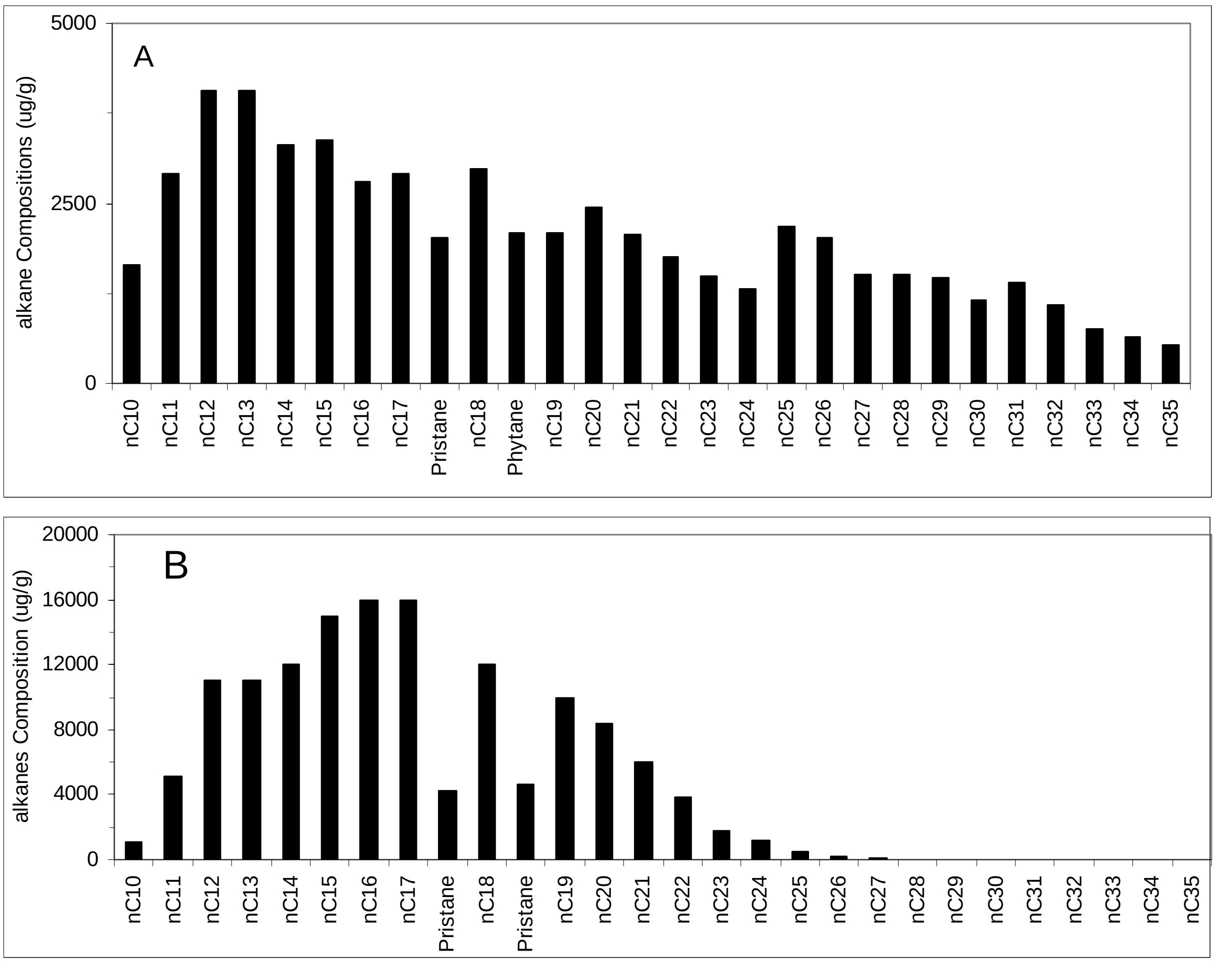


Fig. 1. The composition of alkanes in weathered South Louisiana crude oil (A) and No. 2 fuel oil (B) used in the study

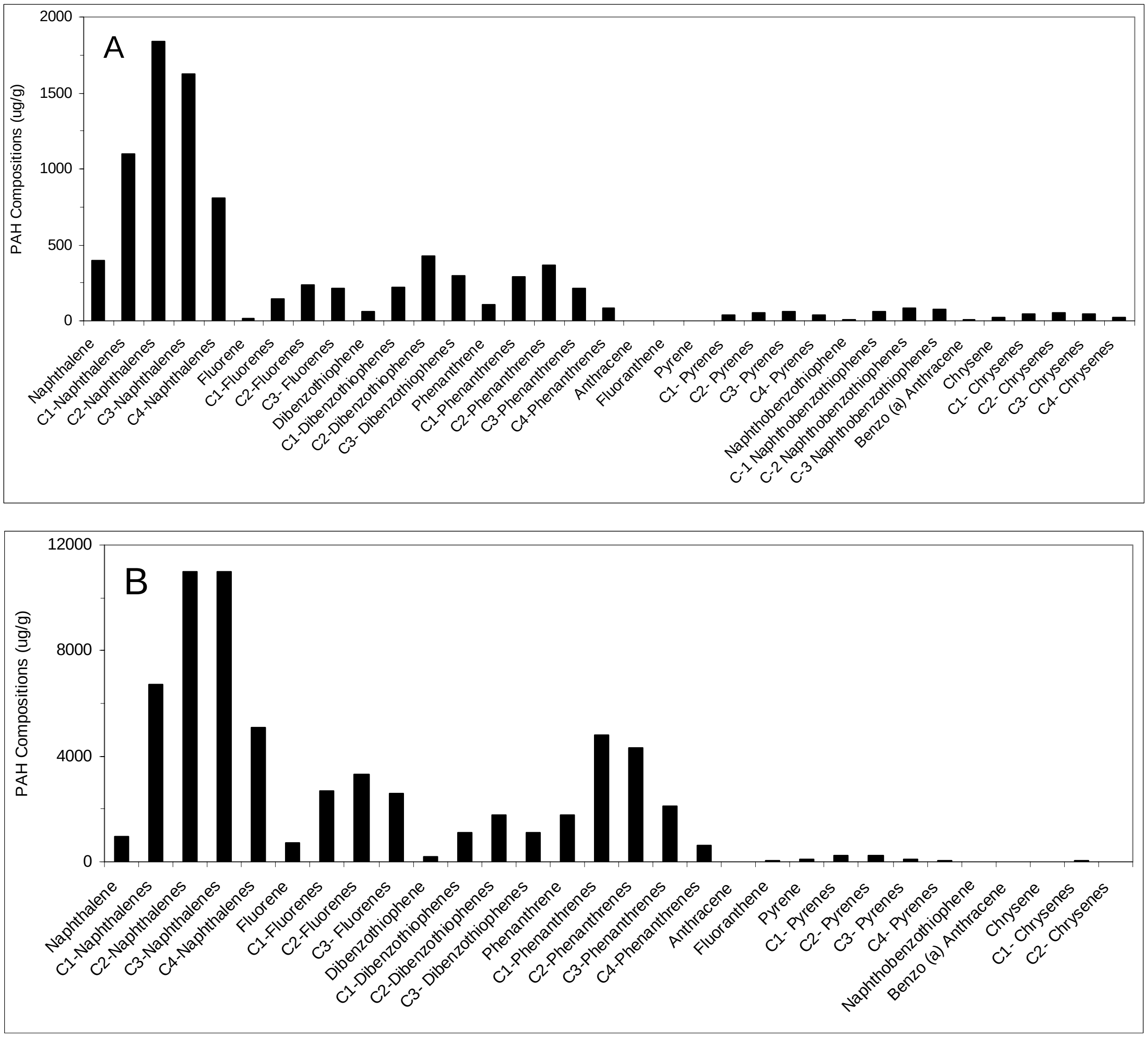


Fig. 2. The composition of polycyclic aromatic hydrocarbons (PAHs) in weathered South Louisiana crude oil (A) and No. 2 fuel oil (B)

The composition of n-alkenes and PAHs in SLC and No. 2 fuel oil used in the study were analyzed by GC/MS. No. 2 fuel oil is composed of more light alkanes than SLC oil (Fig. 1). No nC28 to nC35 alkanes were detected in No. 2 fuel oil (Fig. 1). The total targeted n-alkanes, summed from nC10 to nC35, comprised about 5.8% for SLC oil and 14% for No. 2 fuel oil. In addition, total targeted aromatic hydrocarbons comprised about 0.9% for SLC oil and 6.2% for No. 2 fuel oil (Fig. 2). The difference in composition between SLC and No. 2 fuel oil will affect their toxicity.

4.1. Effect and efficacy of dispersants applied to low concentrations (150 ppm) of South Louisiana crude oil (SLC) and No. 2 fuel oil during the summer, when plants are actively growing in the salt marsh

4.1.1 Effect of dispersants applied to the 150-ppm oil in the summer for the organic substrate salt marsh

4.1.1.1 Effect of dispersed and undispersed oil on leaf mortality of *Spartina alterniflora*

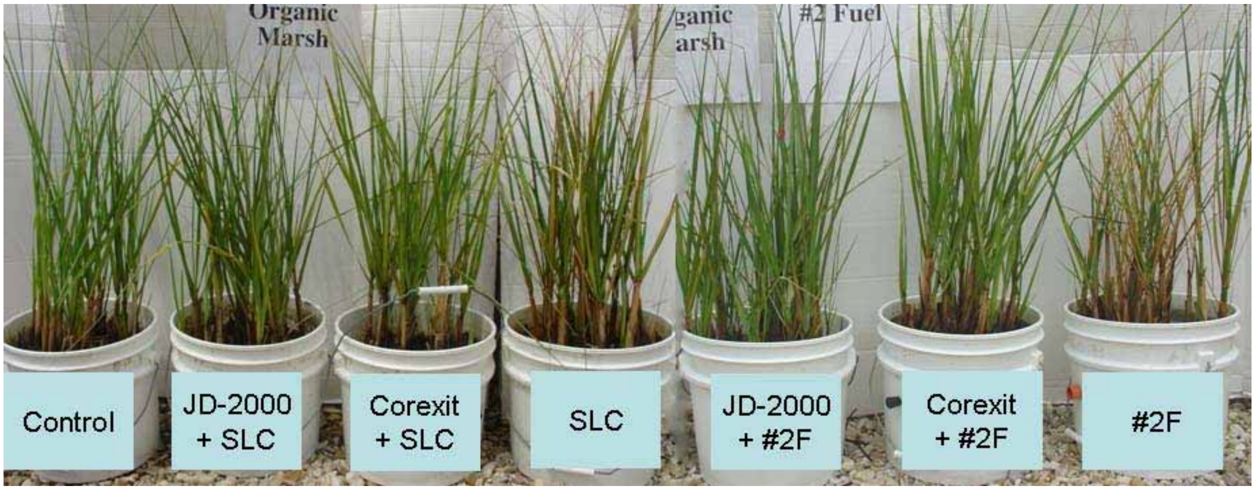


Fig. 3. The visual condition of *Spartina alterniflora* grown in an organic substrate two weeks after the initiation of the summer treatment with either dispersed or undispersed oil at a concentration of 150 ppm. SLC—South Louisiana crude oil, #2F—No. 2 fuel oil, Corexit—Corexit 9500.

The growth status of *Spartina alterniflora* was affected by summer treatments of oil (Fig. 3). Simulating dispersant application in nearshore environments, we found that the detrimental effect of the oil on the leaf mortality of this marsh species was greatly reduced. Plant shoots and leaves were exposed to SLC oil or No.2 fuel oil at a concentration of 150 ppm with or without the dispersant JD-2000 or Corexit 9500 during the simulated high tide. One week after treatment, leaf mortality rate of *S. alterniflora* was significantly ($p<0.0001$) higher in treatments receiving SLC oil or No. 2 fuel oil than the control (Fig. 4). In addition, the leaf mortality rate was significantly ($p<0.0001$) higher in the No. 2 fuel oil treatment than in the SLC oil treatment. Six weeks after treatment, the leaf mortality rate was still significantly higher in treatments receiving SLC oil or No. 2 fuel oil than the control (Fig. 4). However, with addition of dispersants JD-2000 or Corexit 9500, the impact of the oil on plant leaves was greatly relieved; plant leaf mortality rates in treatments receiving either the JD-2000 or Corexit 9500 dispersed oil were not significantly different from the control at both one and six weeks after treatment (Fig. 4).

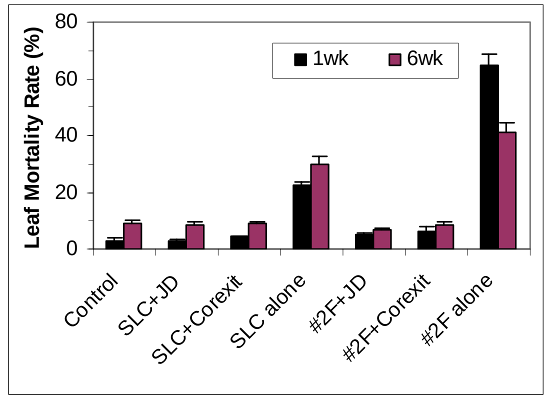


Fig. 4. Effect of dispersed and undispersed oil (150-ppm) on leaf mortality rate of *S. alterniflora* growing in an organic marsh substrate one and six weeks after treatment. Notation on the graph: JD—JD-2000, Corexit—Corexit 9500, SLC—South Louisiana crude oil, #2F—No. 2 fuel oil. All the following figures have the same notation.

Twenty-four hours after treatment, we sampled two plant culms from each experiment unit. The culms were rinsed with dichloromethane, and the amount of oil coating the leaves and stems analyzed. The concentration of TPH coating the leaves and stems was significantly higher in treatments receiving SLC oil or No. 2 fuel oil than the control (Fig. 5). However, the concentration of TPH coating the leaves and stems in treatments receiving the dispersed oil was not significantly different from the control.

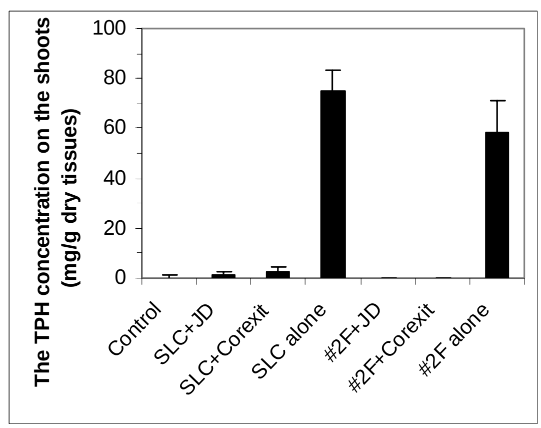


Fig. 5. Effect of dispersed and undispersed oil (150-ppm) on the TPH concentration of leaves and stems of *S. alterniflora* growing in an organic marsh substrate 24 hours after treatment.

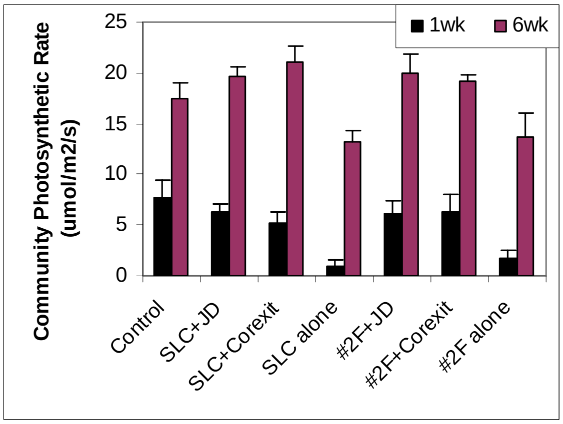


Fig. 6. Effect of dispersed and undispersed oil (150-ppm) on the community photosynthetic rate of *S. alterniflora* growing in an organic marsh substrate one and six weeks after treatment.

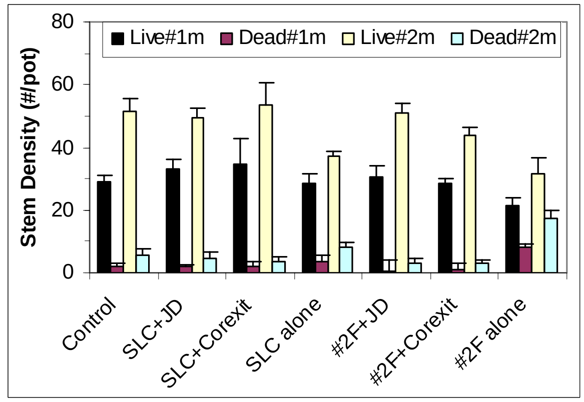


Fig. 7. Effect of dispersed and undispersed oil (150-ppm) on the stem density of *S. alterniflora* growing in an organic marsh substrate one and two months after treatment.

4.1.1.2. Effect of dispersed and undispersed oil on community photosynthetic rate of *Spartina alterniflora*

Community photosynthetic rate of *S. alterniflora*, an indicator of the overall plant health status over a given land surface area, was significantly ($p<0.005$) lower in treatments receiving 150 ppm of SLC oil or No. 2 fuel oil than the control one week after treatment (Fig. 6). However, after six weeks of treatment, the community photosynthetic rate was similar in treatments receiving 150 ppm of SLC oil or No. 2 fuel oil compared to the controls, indicating a recovery from the oil stress. Application of JD-2000 and Corexit 9500 to both SLC oil and No. 2 fuel oil significantly relieved the impact of the oil on community photosynthetic rate of *S. alterniflora*. Community photosynthetic rates one and six weeks after treatment with dispersed oil were not significantly different from the control.

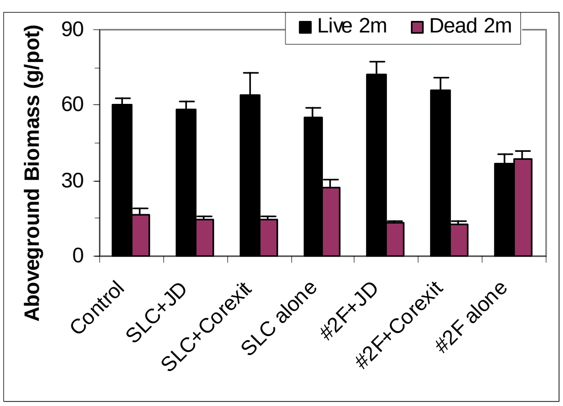


Fig. 8. Effect of dispersed and undispersed oil (150-ppm) on the aboveground biomass of *S. alterniflora* growing in an organic marsh substrate two months after treatment.

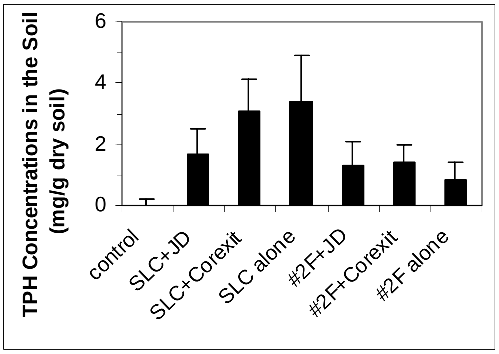


Fig. 9. Effect of dispersed and undispersed oil (150-ppm) on the TPH concentration in the soil of the organic substrate marsh one week after treatment.

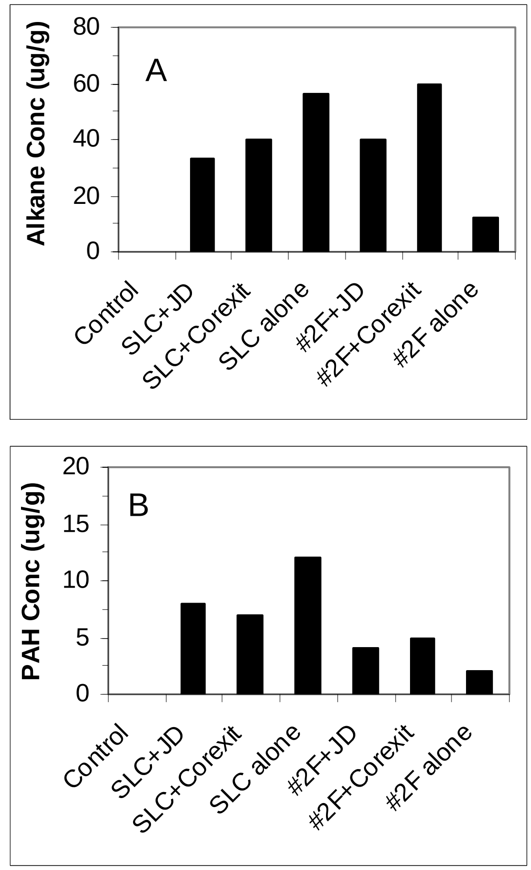


Fig. 10. Effect of dispersed and undispersed oil (150-ppm) on concentrations of alkanes (A) and PAHs (B) in the soil of the organic substrate marsh one week after treatment. Values are composited from five samples of the same treatment.

4.1.1.3. Effect of dispersed and undispersed oil on stem density of *Spartina alterniflora*

Live stem density of *S. alterniflora* was not significantly different among all treatments (Fig. 7) one month after treatment-initiation. However, two months after treatment, live stem density of *S. alterniflora* was significantly ($p<0.005$) lower in the treatment receiving No. 2 fuel oil than the control. Dead stem density of *S. alterniflora* was significantly ($p<0.005$) higher in the treatment receiving No. 2 fuel oil than the control one and two months after treatment. In the other treatments, including SLC oil alone, live and dead stem densities of *S. alterniflora* were not significantly different from the control one and two months after treatment.

4.1.1.4. Effect of dispersed and undispersed oil on aboveground biomass of *Spartina alterniflora*

Two months after treatment, plant aboveground biomass was harvested to determine the short-term effects of oil and dispersed oil on plants. Live aboveground biomass of *S. alterniflora* was significantly ($p<0.005$) lower in the treatment receiving No. 2 fuel oil than in the control. Live aboveground biomass of *S. alterniflora* was not significantly different in all other treatments compared to the control (Fig. 8). In addition, dead aboveground biomass of *S. alterniflora* was significantly ($p<0.005$) higher in the treatment receiving No. 2 fuel oil or SLC oil than the control. However, dispersant application, both JD-2000 and Corexit 9500, relieved the impact of the oil on *S. alterniflora*; live and dead aboveground biomass of *S. alterniflora* at treatments of

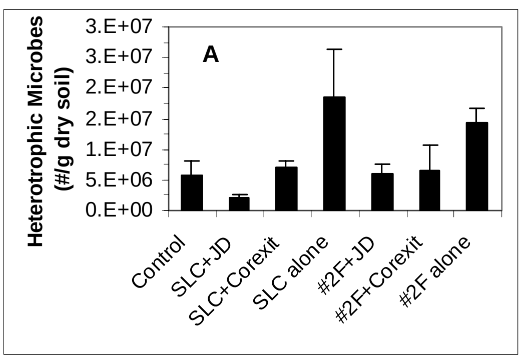
150 ppm of SLC oil or No. 2 fuel oil with application of either JD-2000 or Corexit 9500 were not significantly different from the control.

4.1.1.5. Effects of dispersants on concentrations of TPH, alkanes, and PAHs in the organic marsh soil

One week after treatment, concentrations of total petroleum hydrocarbons (TPH), total targeted alkanes and aromatic hydrocarbons in the marsh soil were analyzed to determine the effect of the treatments on oil adsorption into the marsh sediment. The concentration of TPH in the soil was significantly higher in treatments receiving SLC oil alone and the Corexit 9500 dispersed SLC than the control (Fig. 9). Large variation existed within treatments. In addition, concentrations of total targeted alkanes and PAHs were also higher in treatments receiving oil or dispersed oil than the control (Figs. 10A and 10B).

4.1.1.6. Effect of dispersed and undispersed oil on microbial populations in the marsh soil

Populations of heterotrophic microbes and petroleum hydrocarbon degraders in the soil were analyzed to determine the effect of the treatments on microbial numbers in the marsh soil. The heterotrophic microbial population was not significantly different among all treatments (Fig. 11A). Also, the population of the petroleum hydrocarbon degraders was not significantly different among all treatments (Fig 11B). Large variation existed within treatments.



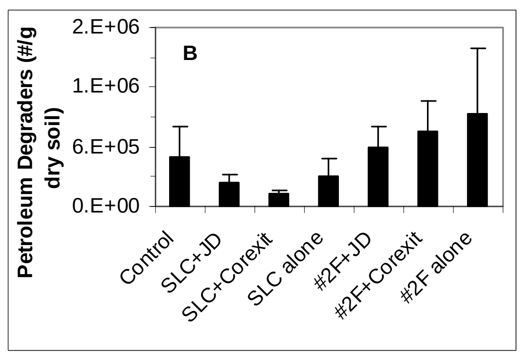


Fig. 11. Effect of dispersed and undispersed oil (150-ppm) on heterotrophic (A) and petroleum degrader (B) populations in the soil of the organic substrate marsh two months after treatment.

4.1.1.7. Effect of dispersed and undispersed oil on the regrowth of *Spartina alterniflora* after the first harvest of aboveground biomass

After the first harvest of aboveground biomass of *S. alterniflora*, two months after treatment initiation for evaluating the short-term effect of oil and dispersed oil, we allowed the plants to regrow from their belowground rhizomes for four months until the end of the plant growing season to evaluate the mid-term effect of oil or dispersed oil on the plants. Six months after treatment, community photosynthetic rate (Fig. 12A), stem density (Fig. 12B) and aboveground biomass (Fig. 12C) of *S. alterniflora* were not significantly different among all treatments, suggesting full recovery of plants from the initial coating impact of the oil. It should be noted that all shoots re-grew from their rhizomes and were alive.

4.1.1.8. Effect of dispersed and undispersed oil on the regrowth of *Spartina alterniflora* after the second harvest of aboveground biomass

After the second harvest of aboveground biomass of *Spartina alterniflora* at the end of the growing season for evaluating the mid-term effect of oil and dispersed oil, we allowed plants to regrow from their belowground rhizomes for another six months in the spring and early summer to evaluate the long-term effect of oil or dispersed oil on the plants. One year after treatment, stem density (Fig. 13A) and aboveground biomass (Fig. 13B) of the regrown *Spartina alterniflora* were not significantly different among all treatments. In addition, the concentration of TPH, the heterotrophic microbial populations, and petroleum degrading populations in the soil were not significantly different among all treatments (data not shown) one year after treatment. Furthermore, alkanes and PAHs in the soil for all treatments were undetectable one year after treatment.

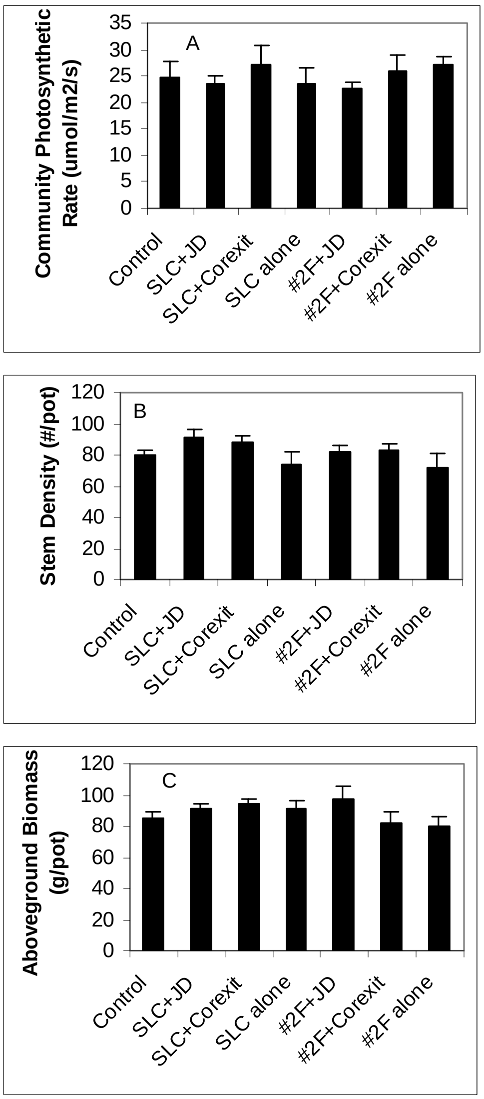


Fig. 12. Effect of dispersed and undispersed oil (150-ppm) on the community photosynthetic rate (A), stem density (B) and aboveground biomass (C) of *S. alterniflora* growing in an organic marsh substrate six months after treatment.

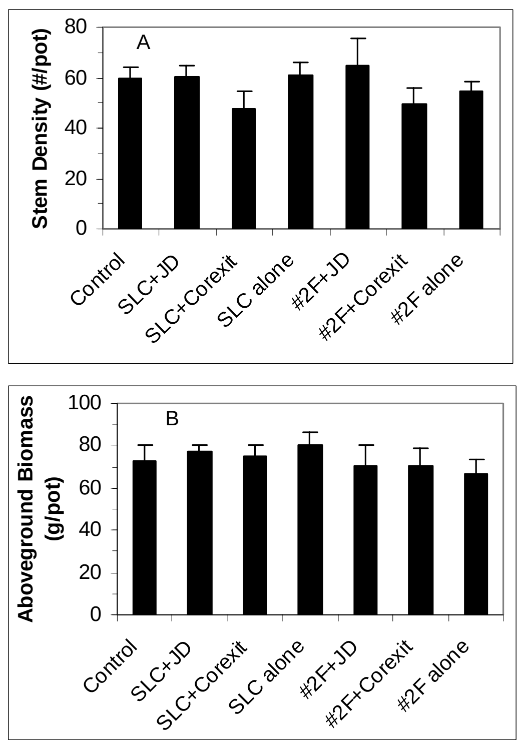


Fig. 13. Effect of dispersed and undispersed oil (150-ppm) on stem density (A) and aboveground biomass (B) of *S. alterniflora* growing in an organic marsh substrate 12 months after treatment.

4.1.2. Effect of dispersants applied to 150-ppm oil in the summer for the sandy salt marsh substrate

4.1.2.1. Effect of dispersed and undispersed oil on leaf mortality of *Spartina alterniflora* growing in a sandy marsh substrate

Oil treatments affected the growth of *Spartina alterniflora* in a sandy marsh substrate (Fig. 14). Simulating dispersant application in nearshore environments also greatly reduced the detrimental effect of the oil on plant leaf mortality of this species growing in a sandy marsh substrate.

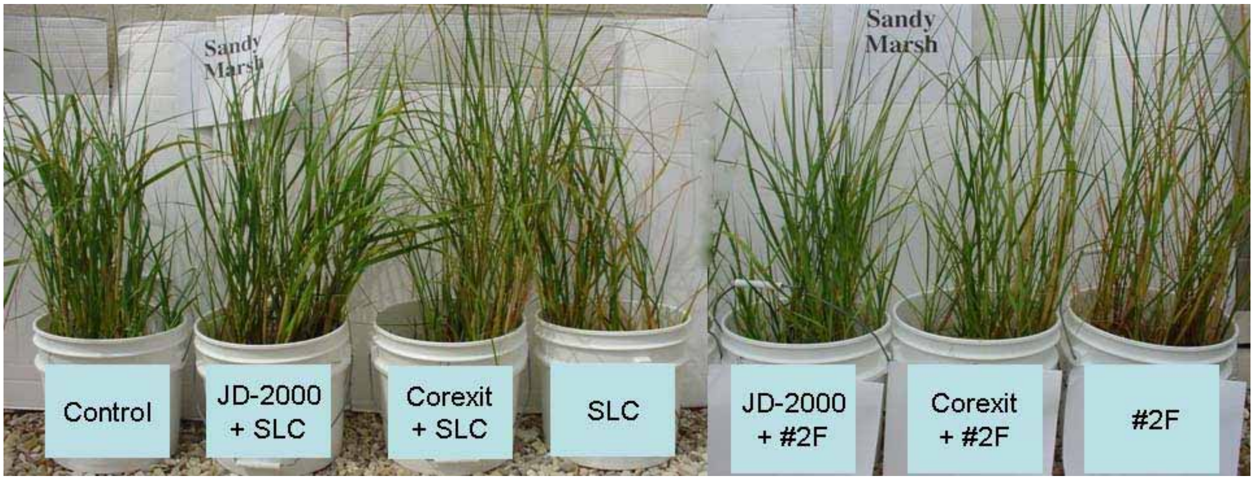


Fig. 14. The visual condition of *Spartina alterniflora* grown in a sandy marsh substrate two weeks after the initiation of the summer treatment with either dispersed or undispersed oil at a concentration of 150 ppm. SLC—South Louisiana crude oil, #2F—No. 2 fuel oil, Corexit—Corexit 9500.

Leaf mortality rate of *S. alterniflora* was significantly ($p<0.0001$) higher in treatments receiving SLC oil or No. 2 fuel oil than the control (Fig. 15) one and six weeks after treatment. However, with addition of dispersants JD-2000 or Corexit 9500, the impact of the oil on plant leaves was greatly relieved; plant leaf mortality rates in treatments receiving either the JD-2000 or Corexit 9500 dispersed oil were not significantly different from the control at both one and six weeks after treatment (Fig. 15).

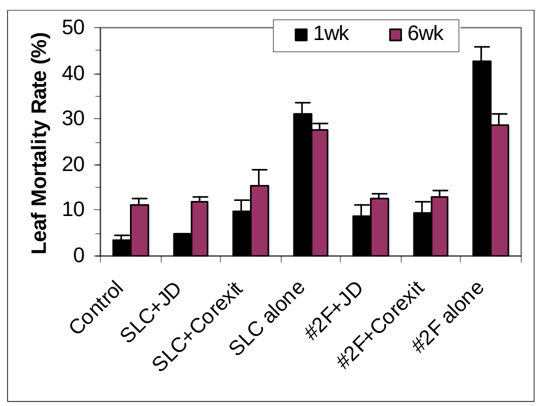


Fig. 15. Effect of dispersed and undispersed oil (150-ppm) on leaf mortality rate of *S. alterniflora* growing in the sandy marsh substrate one and six weeks after treatment.

4.1.2.2. Effect of dispersed and undispersed oil on community photosynthetic rate of *Spartina alterniflora*

Community photosynthetic rate of *S. alterniflora* growing in a sandy marsh substrate was significantly lower in treatments receiving 150 ppm of SLC oil or No. 2 fuel oil than the control one week after treatment (Fig. 16). However, community photosynthetic rates were not significantly lower in treatments receiving 150 ppm of SLC oil or No. 2 fuel oil than the control six weeks after treatment. Application of dispersants JD2000 and Corexit 9500 to both SLC and No. 2 fuel oil greatly relieved the impact of the oil on community photosynthetic rate of *S. alterniflora*. Community photosynthetic rates one and six weeks after treatment with dispersed oil were not significantly different from the control (Fig. 16).

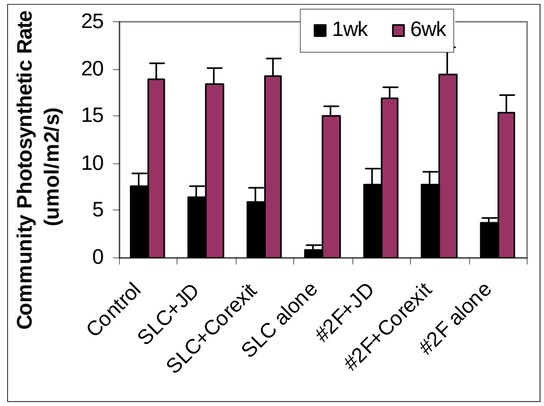


Fig. 16. Effect of dispersed and undispersed oil (150-ppm) on community photosynthetic rate of *S. alterniflora* growing in a sandy marsh substrate one and six weeks after treatment.

4.1.2.3. Effect of dispersed and undispersed oil on stem density of *Spartina alterniflora*

Live stem density of *S. alterniflora* was not significantly different among all treatments (Fig. 17) one and two months after treatment initiation. However, two months after treatment, dead stem density of *Spartina alterniflora* was significantly ($p<0.005$) higher in treatments receiving SLC and No. 2 fuel oil than the control.

4.1.2.4. Effect of dispersed and undispersed oil on aboveground biomass of *Spartina alterniflora*

Two months after treatment-initiation, live aboveground biomass of *S. alterniflora* was not significantly different among all treatments (Fig. 18). However, dead aboveground biomass of *S. alterniflora* was significantly higher in the treatment receiving No. 2 fuel oil than the control. But, dead aboveground biomass of *S. alterniflora* of all other treatments was not significantly different from the control.

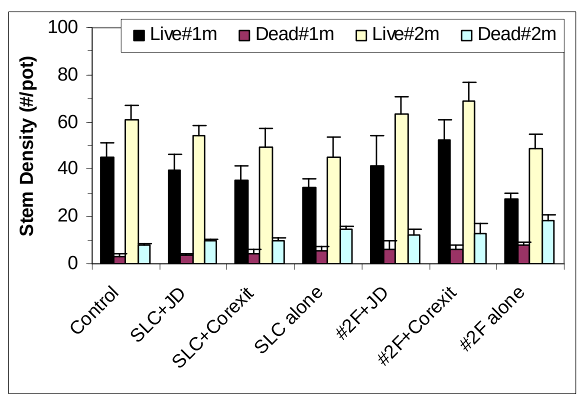


Fig. 17. Effect of dispersed and undispersed oil (150-ppm) on stem density of *S. alterniflora* growing in a sandy marsh substrate one and two months after treatment.

4.1.2.5. Effect of dispersants on concentrations of TPH, alkanes and PAHs in the sandy marsh soil

The concentration of TPH in the sandy marsh soil was significantly higher in treatments receiving dispersed or undispersed oil than in the control (Fig. 19). However, the concentration of TPH in the soil was not significantly different between dispersed oil and undispersed oil treatments. In addition, concentrations of total targeted alkanes (Fig. 20A) and PAHs (Fig. 20B) were higher in all oil treatments compared to the control.

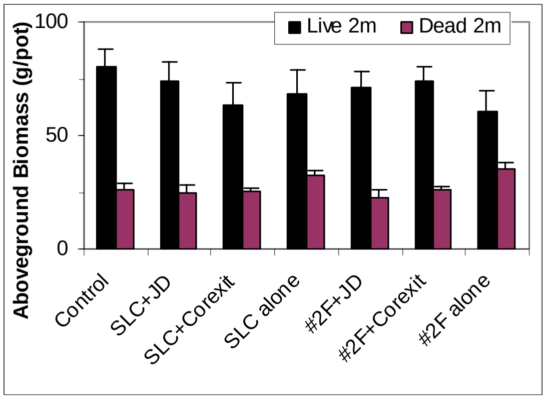


Fig. 18. Effect of dispersed and undispersed oil (150-ppm) on aboveground biomass of *S. alterniflora* growing in a sandy marsh substrate two months after treatment.

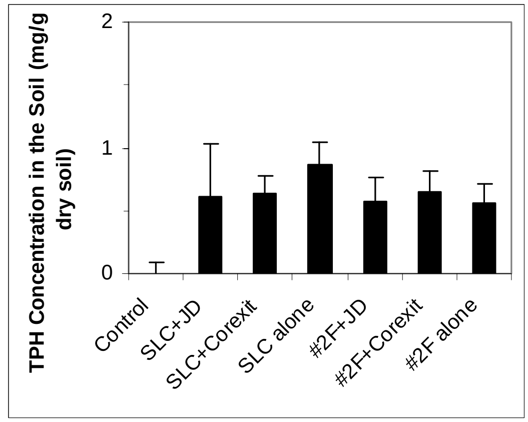


Fig. 19. Effect of dispersed and undispersed oil (150-ppm) on the TPH concentration in the soil of the sandy substrate marsh one week after treatment.

4.1.2.6. Effect of dispersed and undispersed oil on microbial populations in the marsh soil

Populations of heterotrophic microbes and petroleum hydrocarbon degraders in the soil were analyzed to determine the treatment effect on microbial numbers in the sandy marsh soil. Heterotrophic microbial populations were not significantly different among all treatments (Fig. 21A). Also, populations of petroleum hydrocarbon degraders were not significantly different among all treatments (Fig. 21B). Large variation existed within treatments.

4.1.2.7. Effect of dispersed and undispersed oil on the regrowth of *Spartina alterniflora* after the first harvest of aboveground biomass

After the first harvest of aboveground biomass of *Spartina alterniflora*, two months after treatment initiation for evaluating the short-term effect of oil and dispersed oil, we allowed the plants to re-grow from their belowground rhizomes for four months until the end of the plant growing season to evaluate the mid-term effect of oil or dispersed oil on the plants. Six months after treatment, community photosynthetic rate (Fig. 22A), stem density (Fig. 22B), and aboveground biomass (Fig. 22C) of *Spartina alterniflora* growing in the sandy marsh substrate were not significantly different among all treatments.

4.1.2.8. Effect of dispersed and undispersed oil on the regrowth of *Spartina alterniflora* after the second harvest of aboveground biomass

After the second harvest of aboveground biomass of *S. alterniflora* at the end of the growing season for evaluating the mid-term effect of oil and

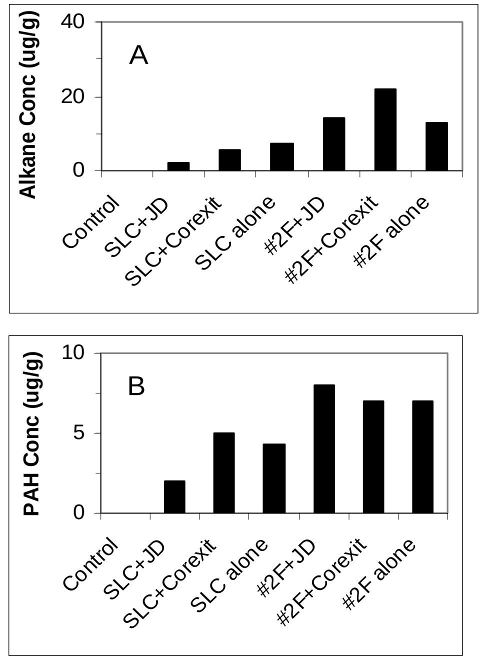


Fig. 20. Effect of dispersed and undispersed oil (150-ppm) on concentrations of alkanes (A) and PAHs (B) in the sandy marsh soil one week after treatment. Values are composited from five samples of the same treatment.

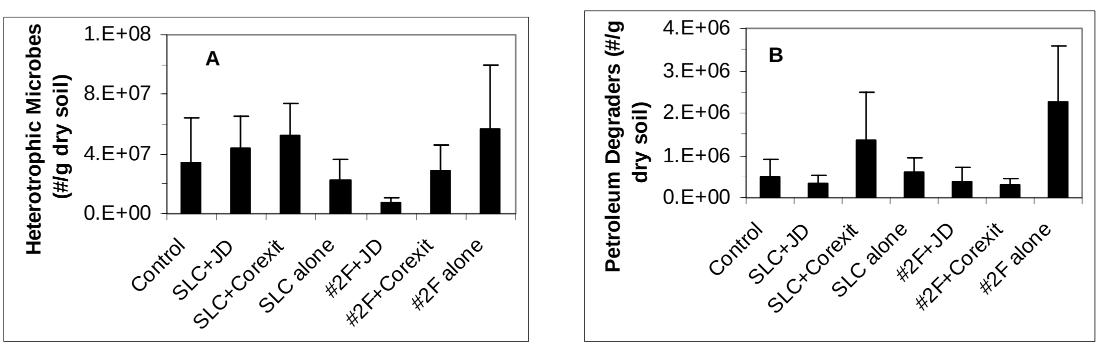


Fig. 21. Effect of dispersed and undispersed oil (150-ppm) on heterotrophic (A) and petroleum degrader (B) populations in the soil of the sandy substrate marsh two months after treatment.

dispersed oil, we allowed the plants to regrow from their belowground rhizomes for another six months in the spring and early summer to evaluate the long-term effect of oil or dispersed oil on the plants. One year after treatment, stem density (Fig. 23A) and aboveground biomass (Fig. 23B) of *S. alterniflora* regrowing in the sandy marsh substrate were not significantly different among all treatments, suggesting a full recovery of the plants from the initial impact of the oil. Alkanes and PAHs in the soil for all treatments were not detected one year after treatment.

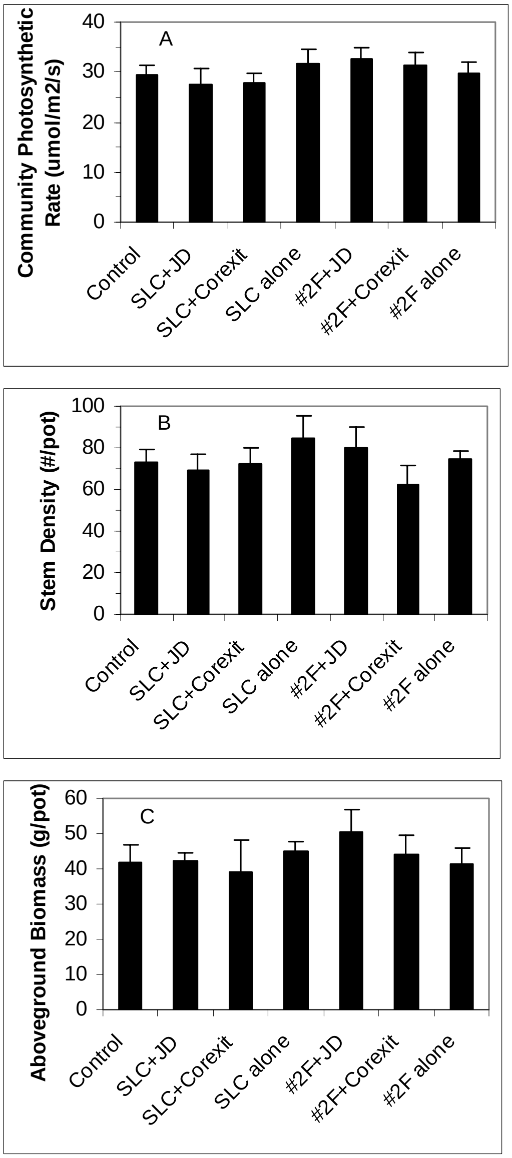


Fig. 22. Effect of dispersed and undispersed oil (150-ppm) on community photosynthetic rate (A), stem density (B) and aboveground biomass (C) of *S. alterniflora* regrowing in the sandy marsh substrate six months after treatment.

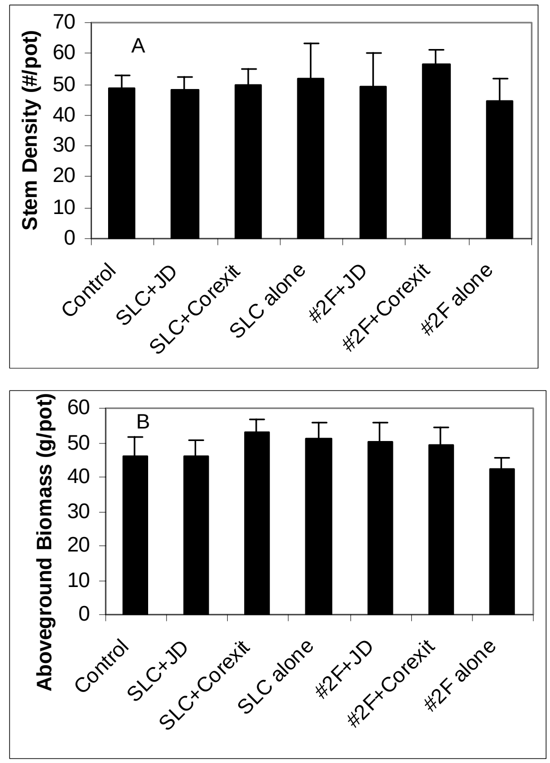


Fig. 23. Effect of dispersed and undispersed oil (150-ppm) on stem density (A) and aboveground biomass (B) of *S. alterniflora* regrowing in the sandy marsh substrate 12 months after treatment.

4.2. Effect and efficacy of dispersants applied to high concentrations (750 ppm) of SLC and No. 2 fuel oil during the summer, when plants are actively growing in the salt marsh

In this experiment, we simulated dispersed and undispersed oil at a concentration of 750 ppm in nearshore water moving in and out of adjacent coastal salt marshes. This study was similar to the low-concentration (150 ppm) experiment. The growth status of *Spartina alterniflora* was severely affected by treatments of the oil at a concentration of 750 ppm (Fig. 24).

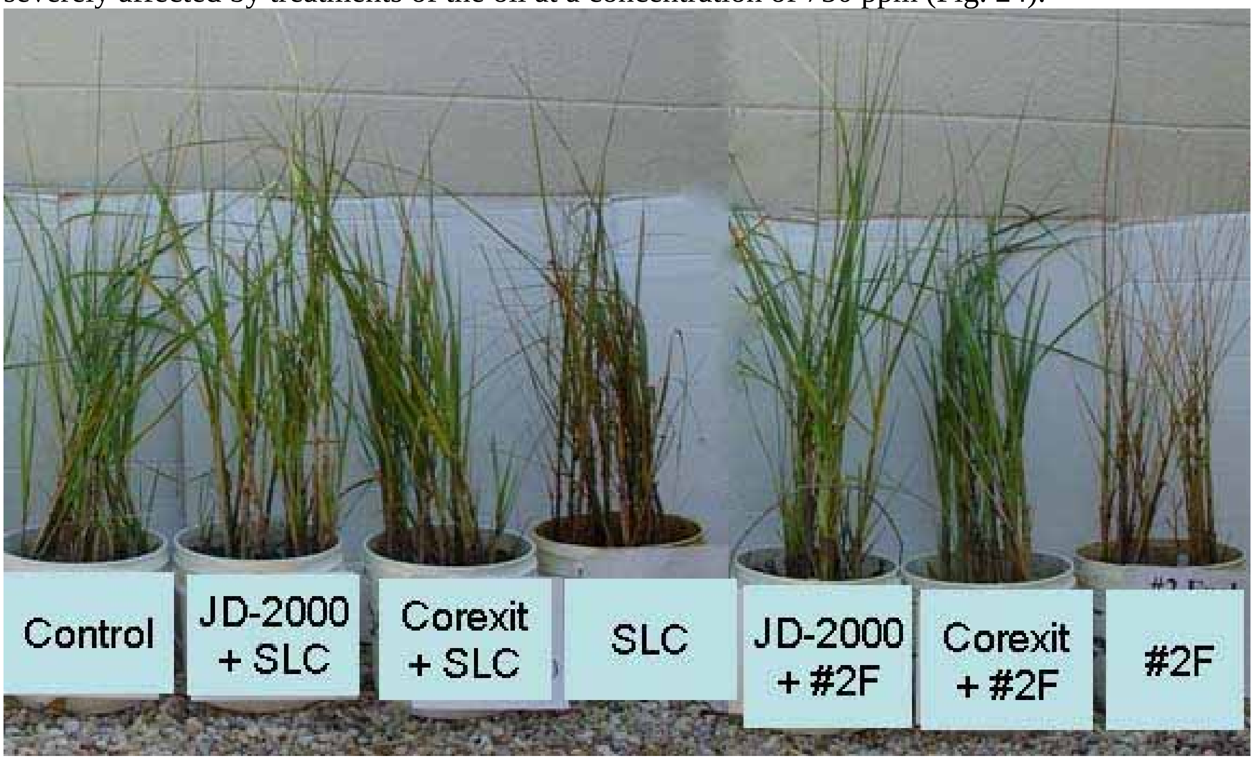


Fig. 24. The visual condition of *Spartina alterniflora* grown in an organic substrate two weeks after the initiation of the summer treatment with either dispersed or undispersed oil at a concentration of 750 ppm. SLC—South Louisiana crude oil, #2F—No. 2 fuel oil, Corexit—Corexit 9500.

4.2.1. Effect of dispersed and undispersed oil (750 ppm) on leaf mortality and the concentration of the oil coating shoots of *Spartina alterniflora*

Simulating dispersant application in nearshore environments greatly reduced the detrimental effect of the oil on leaf mortality of the plants. Plant shoots and leaves were exposed to SLC oil or No. 2 fuel oil at a concentration of 750 ppm with or without dispersant JD-2000 or Corexit 9500 during the simulated high tide. Leaf mortality rate of *S. alterniflora* was significantly ($p<0.0001$) higher in treatments receiving SLC or No. 2 fuel oil than the control two and four weeks after treatment (Fig. 25). The plant leaf mortality rate was as high as 90% in the 750-ppm No. 2 fuel oil treatment two weeks after treatment (Fig. 25). Application of dispersants significantly decreased plant leaf mortality rate by the oil. Plant leaf mortality rates in treatments with 750 ppm of the JD-2000 dispersed SLC or No. 2 fuel oil were not significantly different from the control two and four weeks after treatment. Leaf mortality rate of *S. alterniflora*

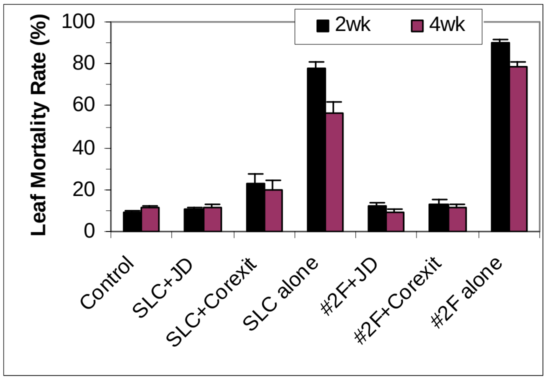


Fig. 25. Effect of dispersed and undispersed oil (750-ppm) on leaf mortality rate of *S. alterniflora* two and four weeks after treatment.

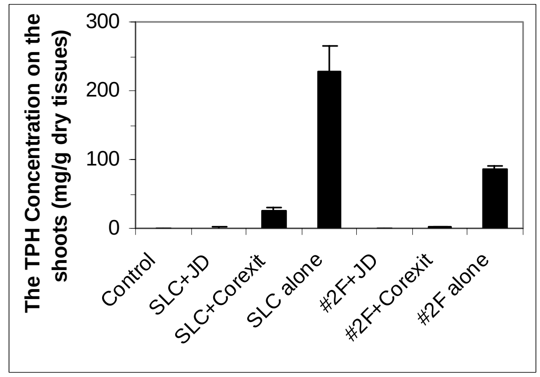


Fig. 26. Effect of dispersed and undispersed oil (750-ppm) on the TPH concentration of leaves and stems of *S. alterniflora* 24 hours after treatment.

in the treatment receiving 750 ppm of the Corexit 9500 dispersed SLC oil was significantly ($p<0.0001$) higher than the control, but was significantly ($p<0.0001$) lower than that of the treatment receiving 750 ppm of the oil alone (Fig. 25). Plant leaf mortality rate in the treatment receiving 750 ppm of the Corexit 9500-dispersed No. 2 fuel oil was not significantly different from the control, indicating that Corexit 9500 is likely more effective for the lighter No. 2 fuel oil than the more viscous crude oil.

Twenty-four hours after treatment, we sampled two plant culms from each experiment unit. The culms were rinsed with DCM, and the amount of oil coating leaves and stems of *S. alterniflora* analyzed. The concentration of TPH coating the leaves and stems was significantly higher in treatments receiving SLC oil or No. 2 fuel oil than the control (Fig. 26). Furthermore, the concentration of TPH coating the leaves and stems in treatments receiving the Corexit 9500 dispersed SLC oil was significantly ($p<0.0001$) higher than the control (Fig. 22), but was significantly ($p<0.0001$) lower than that in treatments receiving the oil alone. However, concentrations of TPH coating the leaves and stems in treatments receiving the other dispersed oil were not significantly different from the control.

4.2.2 Effect of dispersed and undispersed oil (750 ppm) on community photosynthetic rate of *Spartina alterniflora*

Community photosynthetic rate of *S. alterniflora* was significantly ($p<0.005$) lower in treatments receiving 750 ppm of SLC oil or No. 2 fuel oil than the control one week after treatment. The community photosynthetic rate was almost completely inhibited by 750 ppm of SLC or No.2 fuel oil alone one week after treatment (Fig. 27). However, application of dispersants to 750 ppm of oil relieved the effect of the oil on photosynthetic rates. The community photosynthetic rate of *S. alterniflora* in treatments receiving 750 ppm of the JD-2000-dispersed SLC or No. 2 fuel oil were not significantly different from the control one week after treatment. However, the community photosynthetic rate of the treatment receiving 750 ppm of the Corexit 9500-dispersed SLC oil was significantly ($p<0.0001$) lower than the control, but was significantly ($p<0.0001$) higher than that of treatments receiving 750 ppm of the oil (Fig. 27). The community photosynthetic rate in the treatment receiving 750 ppm of the Corexit 9500 dispersed No. 2 fuel oil was significantly ($p<0.001$) higher than the Corexit 9500 dispersed SLC. Six weeks after treatment, community photosynthetic rate of *S. alterniflora* was significantly ($p<0.005$) lower in treatments receiving 750 ppm of No. 2 fuel oil than the control. Community photosynthetic rates of all other treatments were not significantly different from the control six weeks after treatment.

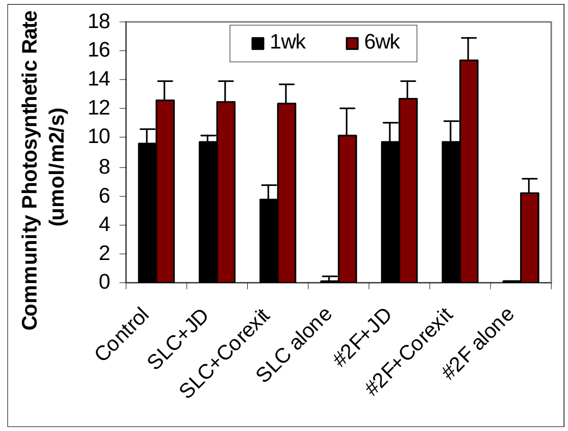


Fig. 27. Effect of dispersed and undispersed oil (750-ppm) on community photosynthetic rate of *S. alterniflora* one and six weeks after treatment.

4.2.3 Effect of dispersed and undispersed oil (750 ppm) on stem density of *Spartina alterniflora*

Live stem density of *S. alterniflora* in treatments receiving 750 ppm of SLC oil or No. 2 fuel oil was significantly ($p<0.0001$) lower than the control one month after treatment initiation (Fig. 28). However, application of dispersants to 750 ppm of oil reduced the impact of the oil on stem density. Live stem densities in treatments of 750 ppm of the JD-2000-dispersed SLC or No. 2 fuel oil were not significantly different from the control one month after treatment. Dead stem densities of *S. alterniflora* were significantly higher in treatments receiving 750 ppm of SLC oil ($p<0.005$) or No. 2 fuel oil ($p<0.0001$) than the control one month after treatment. Dead stem densities in treatments receiving the 750-ppm JD-2000 or Corexit 9500-dispersed oil were not significantly different from the control one month after treatment. Two months after treatment, live stem densities were significantly lower in treatments receiving 750 ppm of SLC oil ($p<0.005$) or No. 2 fuel oil ($p<0.0001$) than the control; dead stem densities were significantly higher in treatments receiving 750 ppm of SLC oil or No. 2 fuel oil ($p<0.005$) than the control. However, two months after treatment, live and dead stem densities in the 750-ppm JD-2000 or Corexit 9500-dispersed oil treatments were not significantly different from the control.

4.2.4 Effect of dispersed and undispersed oil (750-ppm) on aboveground biomass of *Spartina alterniflora*

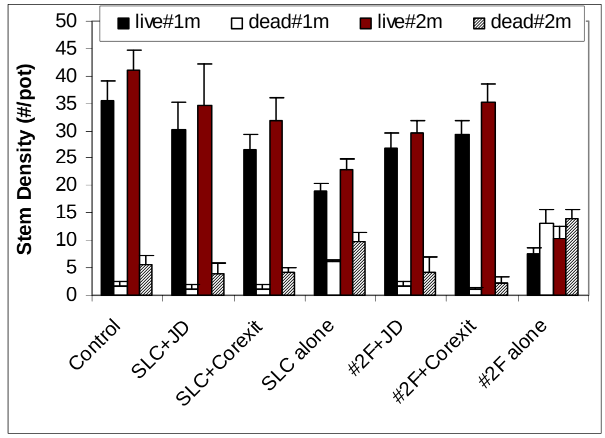


Fig. 28. Effect of dispersed and undispersed oil (750-ppm) on stem density of *S. alterniflora* one and two months after treatment.

Two months after treatment, plant aboveground biomass was harvested to determine the short-term effect of oil and dispersed oil on plants. Live aboveground biomass of *S. alterniflora* was significantly lower in treatments receiving SLC oil ($p<0.05$) and No. 2 fuel oil ($p<0.0005$) than the control (Fig. 29). Live aboveground biomass was not significantly different for all other treatments compared to the control (Fig. 29). Dead aboveground biomass of *S. alterniflora* was significantly ($p<0.005$) higher in treatments receiving SLC or No. 2 fuel oil than the control. However, dispersant application, both JD-2000 and Corexit 9500, relieved the impact of the oil on *S. alterniflora*; live and dead aboveground biomass in treatments receiving 750 ppm of SLC or No. 2 fuel oil dispersed by either JD-2000 or Corexit 9500 were not significantly different from the control.

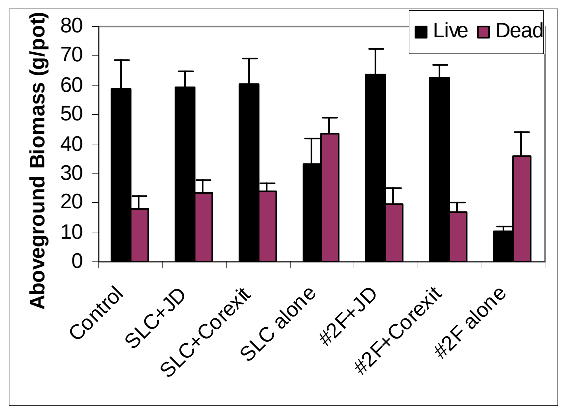


Fig. 29. Effect of dispersed and undispersed oil (750-ppm) on aboveground biomass of *S. alterniflora* two months after treatment.

4.2.5 Effect of dispersants on concentrations of TPH, alkanes, and PAHs in the marsh soil

One week after treatment, concentrations of TPH in the soil were significantly higher in treatments receiving oil or dispersant oil than the control (Fig. 30). However, dispersant application did not significantly affect the TPH concentration in the soil. In addition, concentrations of total targeted alkanes (Fig. 31A) and PAHs (Fig. 31B) were higher in all oil treatments compared to the control. The higher concentrations of alkanes and PAHs in all treatments receiving No. 2 fuel oil than those receiving SLC oil were most likely correlated to the higher percentage in the composition of the original No. 2 fuel oil than SLC oil.

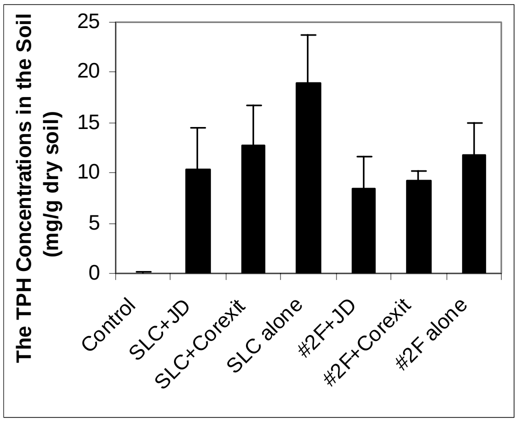


Fig. 30. Effect of dispersed and undispersed oil (750-ppm) on the TPH concentration in the marsh soil one week after treatment.

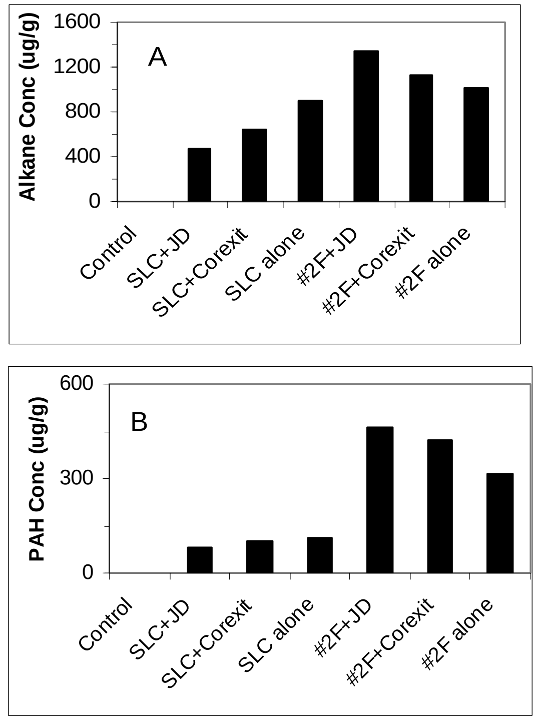


Fig. 31. Effect of dispersed and undispersed oil (750-ppm) on concentrations of alkanes (A) and PAHs (B) in the marsh soil one week after treatment. Values are composited from five samples of the same treatment.

4.2.6 Effect of dispersed and undispersed oil (750-ppm) on microbial populations in the marsh soil

Populations of heterotrophic microbes were significantly higher in treatments that received SLC oil or No. 2 fuel oil than in the other treatments (Fig. 32A). Populations of petroleum hydrocarbon degraders were significantly higher in all treatments receiving oil or dispersed oil than in the control (Fig 32B). However, populations of petroleum hydrocarbon degraders in treatments with dispersed oil were not significantly different from those with undispersed oil.

4.2.7 Effect of dispersed and undispersed oil (750 ppm) on the regrowth of *Spartina alterniflora* after the first harvest of aboveground biomass

The first harvest of aboveground biomass of *Spartina alterniflora* took place two months after treatment initiation for evaluating the short-term effect of oil and dispersed oil. We allowed the plants to regrow from their belowground rhizomes for four months until the end of the plant-growing season to evaluate the mid-term effect of oil or dispersed oil on the plants. Community photosynthetic rate of *S. alterniflora* (33A) was not significantly different among all treatments, suggesting a recovery of plants from the initial impact of the oil. However, stem density (Fig. 33B) and aboveground biomass (Fig. 33C) of *S. alterniflora* were still significantly lower in the treatment receiving 750 ppm of No. 2 fuel oil than in the control and other treatments. The stem density and aboveground biomass of the other treatments, including the treatment of SLC oil alone, were not significantly different from the control. It should be noted that all shoots regrew from their rhizomes, and were alive.

4.2.8 Effect of dispersed and undispersed oil (750 ppm) on the regrowth of *Spartina alterniflora* after the second harvest of aboveground biomass

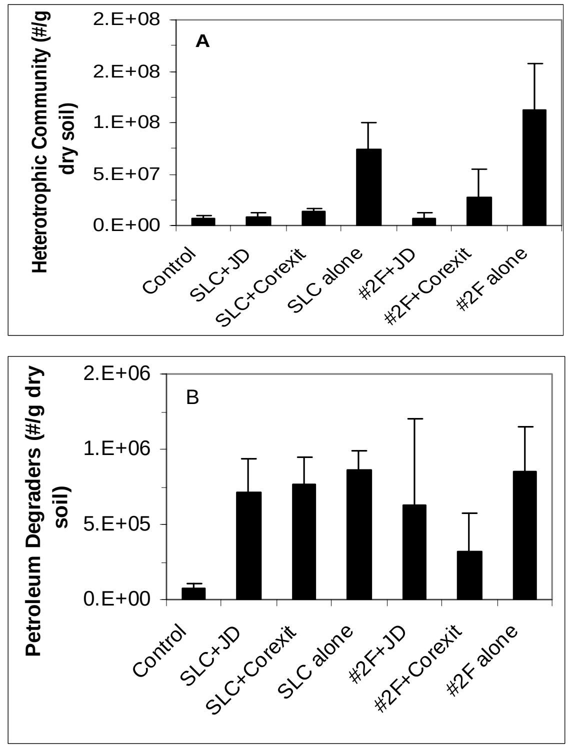


Fig. 32. Effect of dispersed and undispersed oil (750-ppm) on heterotrophic (A) and petroleum degrader (B) populations in the soil two weeks after treatment.

After the second harvest of aboveground biomass of *Spartina alterniflora* at the end of the growing season for evaluating the mid-term effect of oil and dispersed oil, we allowed the plants to regrow from their belowground rhizomes for another six months in the spring and early summer to evaluate the long-term effect of oil or dispersed oil on the plants. One year after treatment, community photosynthetic rate (Fig. 34A), stem density (Fig. 34B), and aboveground biomass (Fig. 34C) of the re-grown *Spartina alterniflora* were not significantly different among all treatments, suggesting the full recovery of the plants from the initial impact of the oil. In addition, heterotrophic microbe and oil degrader populations were not significantly different among all treatments (data not shown). The TPH concentration in the soil of treatments receiving 750 ppm of SLC oil and No. 2 fuel oil alone was significantly higher than in the other treatments (Fig. 34D). The concentration of total targeted alkanes in the soil of the 750-ppm No. 2 fuel oil treatment was noticeably higher than the other treatments even one year after treatment (Fig. 35A). The concentration of PAHs was higher in treatments receiving 750 ppm of SLC or No. 2 fuel oil compared to the 750-ppm dispersed SLC or No. 2 fuel oil one year after treatment (Fig. 35B). Furthermore, concentrations of PAHs in all oil treatments except for No. 2 fuel were generally higher than those of alkanes one year after the treatment (Figs. 35A and 35B) although the PAH concentrations in the soil were lower than those of alkanes one week after treatment initiation (Figs. 31A and 31B), suggesting that PAHs were more difficult to degrade than alkanes.

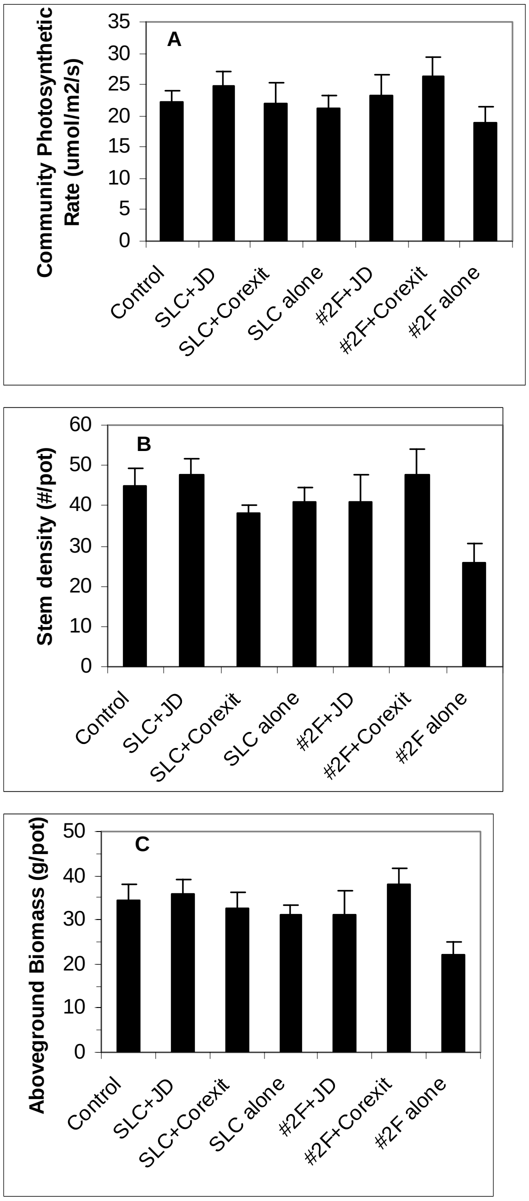


Fig. 33. Effect of dispersed and undispersed oil (750-ppm) on community photosynthetic rate (A), stem density (B) and aboveground biomass (C) of the regrown *S. alterniflora* six months after treatment.

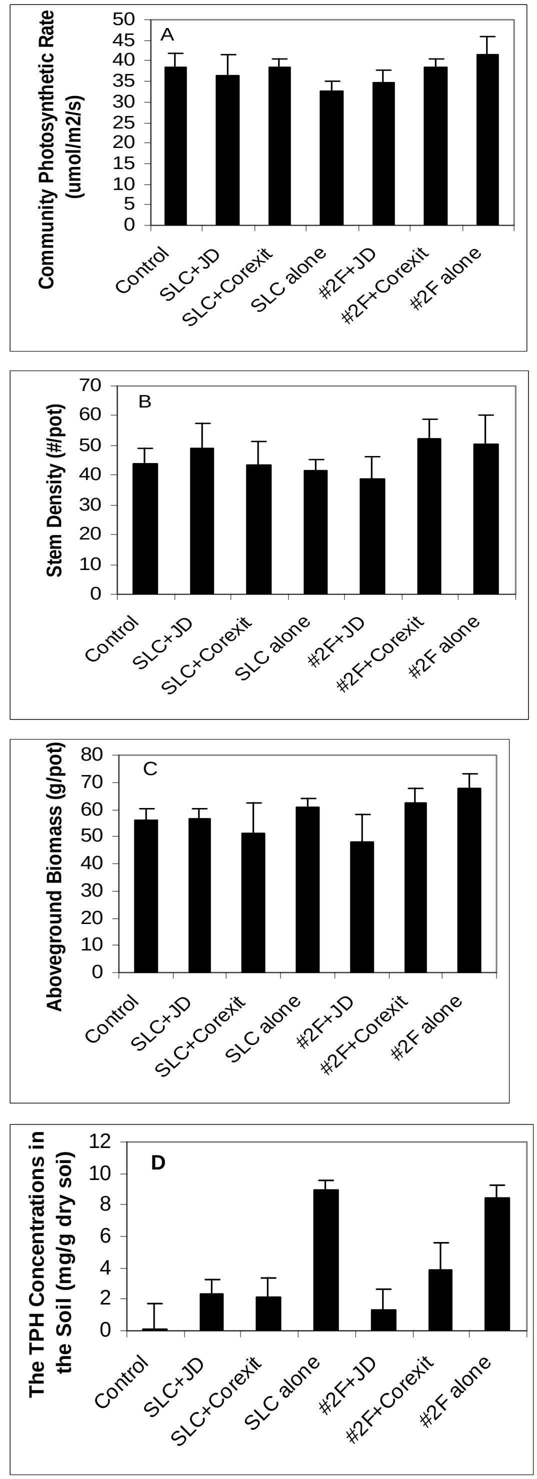


Fig. 34. Effect of dispersed and undispersed oil (750-ppm) on community photosynthetic rate (A), stem density (B), aboveground biomass (C) of the regrown *S. alterniflora*, and the TPH concentration in the soil (D) 12 months after treatment.

4.3 Effect and efficacy of dispersants applied to low concentrations (150 ppm) of SLC and No. 2 fuel oil during the fall, when plants are inactively growing in the salt marsh

This experiment simulated application of dispersants in nearshore environments in the inactive fall plant-growing season for salt marsh habitat protection. The type of marsh used in this experiment was an organic substrate salt marsh collected from the same location as the summer experiment. The purpose of this experiment was to determine if applying dispersants in the fall season, when plants are in the inactive growing stage, was as effective as in the summer when plants are in the active growing stage.

4.3.1 Effect of dispersed and undispersed oil (150-ppm) on leaf mortality of *Spartina alterniflora*

Simulating dispersant application in nearshore environments in the fall season also greatly reduced the detrimental effect of oil on salt marsh plants (Fig. 36).

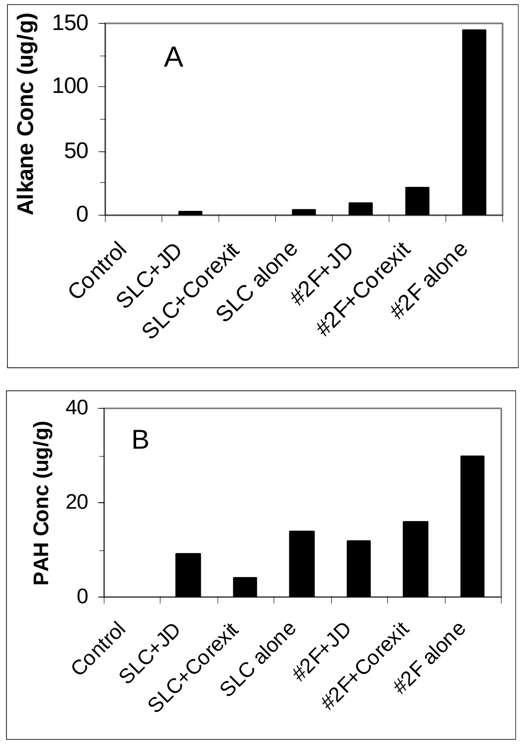


Fig. 35. Effect of dispersed and undispersed oil (750-ppm) on concentrations of alkanes (A) and PAHs (B) in the marsh soil 12 months after treatment. The values are composited from five samples of the same treatment.

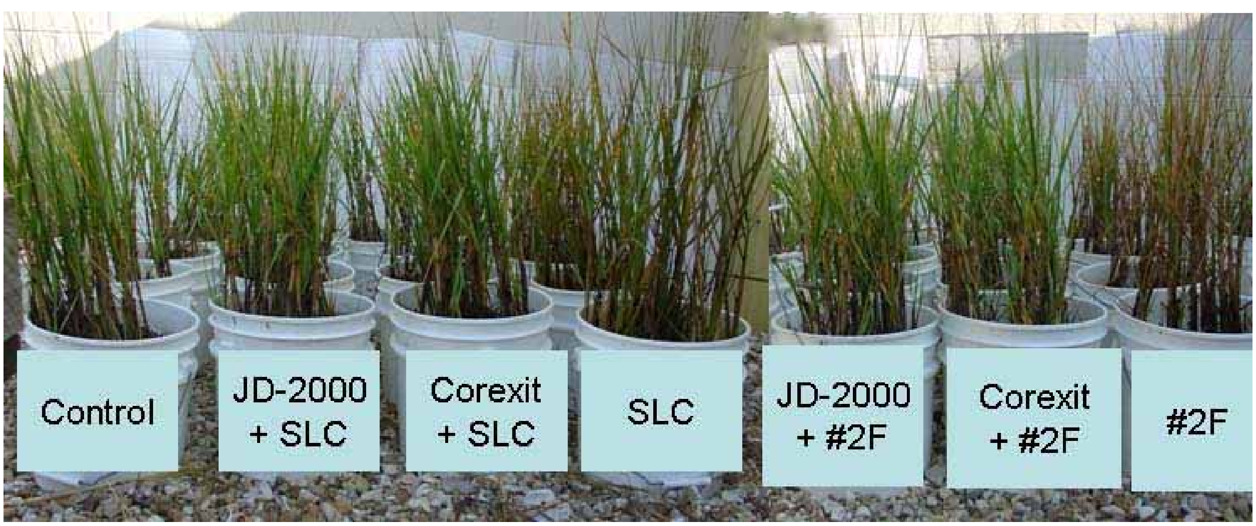


Fig. 36. The visual condition of *Spartina alterniflora* grown in an organic substrate two weeks after the initiation of the fall treatment with either dispersed or undispersed oil at a concentration of 150 ppm. SLC—South Louisiana crude oil, #2F—No. 2 fuel oil, Corexit—Corexit 9500.

Shoots and leaves of *S. alterniflora* were exposed to SLC oil or No.2 fuel oil at a concentration of 150 ppm with or without dispersant JD-2000 or Corexit 9500 during the simulated high tide in the fall season. One month after treatment, leaf mortality rate of *S. alterniflora* was significantly ($p<0.0001$) higher in treatments receiving SLC oil or No. 2 fuel oil than in the control (Fig. 37). In addition, the leaf mortality rate was significantly ($p<0.0001$) higher for No. 2 fuel oil than for SLC oil. Two months after treatment, plant leaf mortality rate of *S. alterniflora* was still significantly ($p<0.0001$) higher in treatments receiving SLC oil or No. 2 fuel oil than in the control (Fig. 37). However, with addition of dispersant JD-2000 or Corexit 9500, the impact of the oil on plant leaves was greatly relieved; plant leaf mortality rates in treatments receiving either the JD-2000 or Corexit 9500 dispersed oil were not significantly different from the control at both one and two months after treatment (Fig. 37).

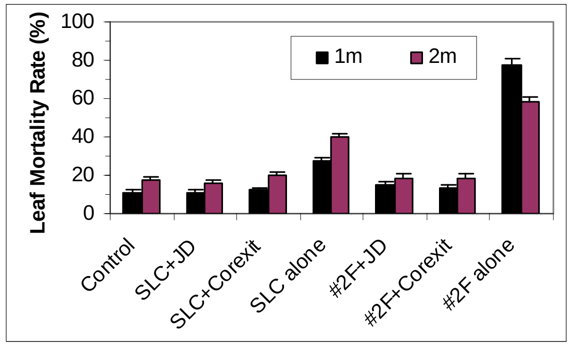


Fig. 37. Effect of dispersed and undispersed oil (150-ppm) on leaf mortality rate of *S. alterniflora* one and two months after the fall oil treatment.

4.3.2 Effect of dispersed and undispersed oil (150-ppm) on community photosynthetic rate of *Spartina alterniflora*

Community photosynthetic rates of *S. alterniflora* in treatments receiving 150 ppm of SLC oil ($p<0.0005$) or No. 2 fuel oil ($p<0.0001$) were significantly lower than the control three weeks after treatment (Fig. 38).

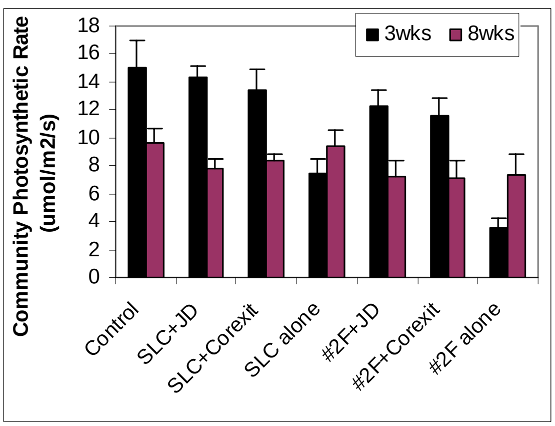


Fig. 38. Effect of dispersed and undispersed oil (150-ppm) on community photosynthetic rate of *S. alterniflora* three and eight weeks after the fall oil treatment.

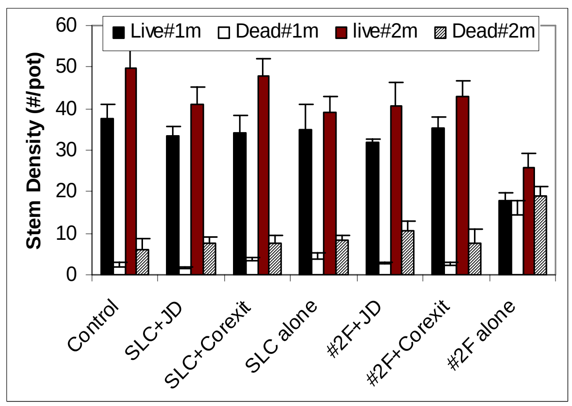


Fig. 39. Effect of dispersed and undispersed oil (150-ppm) on stem density of *S. alterniflora* one and two months after the fall oil treatment.

However, community photosynthetic rates in treatments receiving 150 ppm of SLC oil or No. 2 fuel oil were not significantly lower than the control eight weeks after treatment initiation. Application of JD-2000 and Corexit 9500 to both SLC and No. 2 fuel oil significantly relieved the impact of the oil on community photosynthetic rates; community photosynthetic rates of treatments receiving dispersed oil were not significantly different from the control three and eight weeks after the fall oil treatment.

4.3.3 Effect of dispersed and undispersed oil (150 ppm) on stem density of *Spartina alterniflora*

Live stem density of *S. alterniflora* in the treatment receiving 150 ppm of No. 2 fuel oil was significantly ($p<0.001$) lower than the control one and two months after treatment (Fig. 39). Dead stem density of *S. alterniflora* in the treatment receiving No. 2 fuel oil was significantly higher than the control one ($p<0.0001$) and two months ($p<0.005$) after treatment. However, live and dead stem densities of *S. alterniflora* in the other treatments were not significantly different from the control one and two months after treatment.

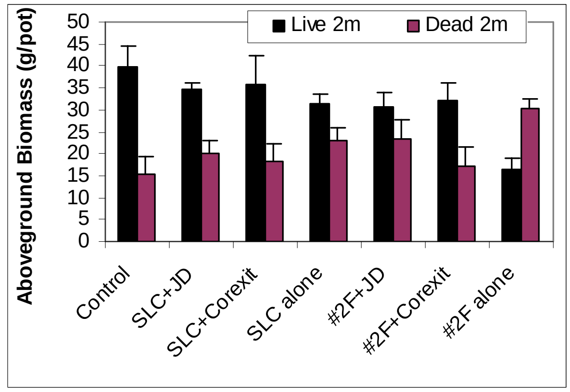


Fig. 40. Effect of dispersed and undispersed oil (150-ppm) on the aboveground biomass of *S. alterniflora* two months after the fall oil treatment.

4.3.4 Effect of dispersed and undispersed oil (150 ppm) on aboveground biomass of *Spartina alterniflora*

Two months after treatment, aboveground biomass was harvested to determine the short-term effect of oil and dispersed oil on plants. Live aboveground biomass of *S. alterniflora* was significantly ($p<0.0005$) lower in the treatment receiving No. 2 fuel oil than the control (Fig. 40). Dead aboveground biomass of *S. alterniflora* was significantly ($p<0.01$) higher in the treatment receiving No. 2 fuel oil than the control. Live and dead aboveground biomass of *S. alterniflora* in all other treatments was not significantly different from the control.

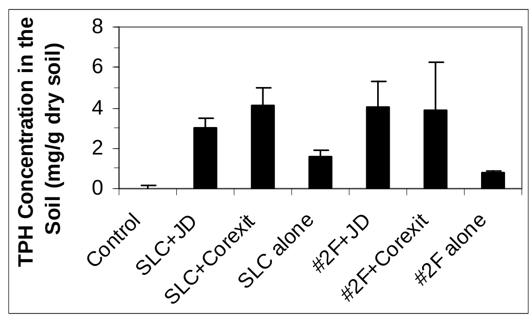


Fig. 41. Effect of dispersed and undispersed oil (150-ppm) on the TPH concentration in the marsh soil one week after the fall oil treatment.

4.3.5 Effect of dispersants on the TPH concentration in the marsh soil

One week after treatment, the TPH concentration in the soil was significantly higher in treatments receiving oil or dispersant oil than the control (Fig. 41). However, dispersant application did not significantly affect the TPH concentration in the soil.

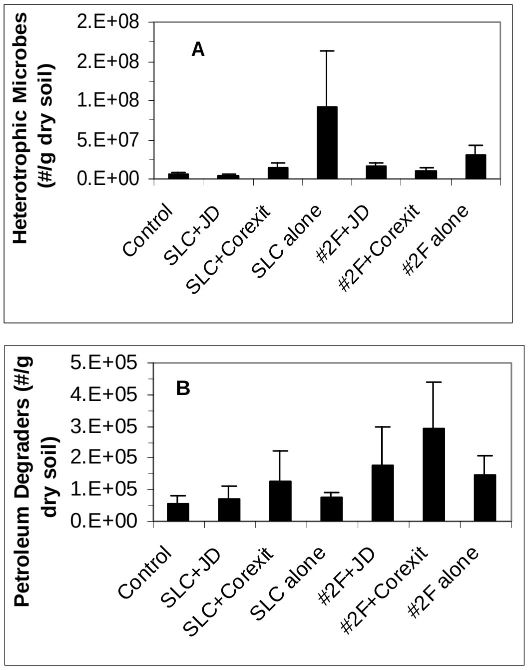


Fig. 42. Effect of dispersed and undispersed oil (150-ppm) on the heterotrophic (A) and petroleum degrader (B) populations in the soil two weeks after the fall oil treatment.

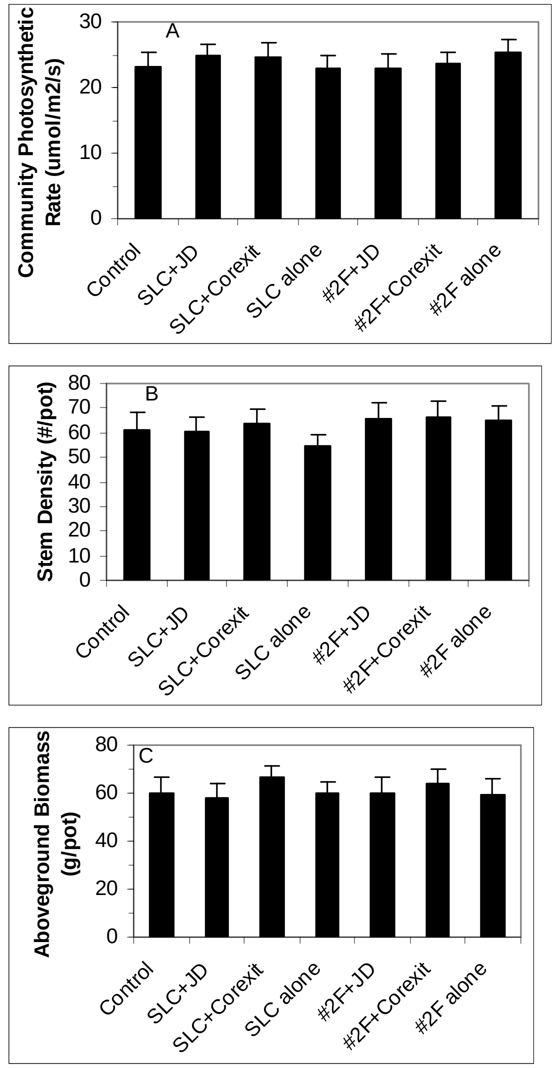


Fig. 43. Effect of dispersed and undispersed oil (150-ppm) on the community photosynthetic rate (A), stem density (B) and aboveground biomass (C) of the regrown *S. alterniflora* 12 months after the fall oil treatment.

4.3.6 Effect of dispersed and undispersed oil (150 ppm) on microbial populations in the marsh soil

Populations of heterotrophic microbes (Fig. 42A) and oil degraders (Fig. 42B) in the soil were not significantly different among all treatments. Large variation existed within treatments.

4.3.7 Effect of dispersed and undispersed oil (150 ppm) on the regrowth of *Spartina alterniflora* one year after treatment

After the harvest of aboveground biomass of *Spartina alterniflora* at the end of the growing season for evaluating the short-term effect of oil and dispersed oil, we allowed the plants to regrow from their belowground rhizomes for 10 months to evaluate the long-term effect of oil or dispersed oil on the plants. We did not harvest aboveground biomass six months after treatment because the plants had not grown well in the early spring after the harvest. One year after treatment, community photosynthetic rate (Fig. 43A), stem density (Fig. 43B), and aboveground biomass (Fig. 43C) of the regrown *Spartina alterniflora* were not significantly different among all treatments, suggesting the full recovery of the plants from the initial impact of the oil. Alkanes and PAHs in the soil for all treatments were not detected one year after treatment.

5.0 Discussion and Importance to Oil Spill Response/Restoration

The present study, which simulated dispersants applied in nearshore and estuarine environments, demonstrated that dispersants can greatly relieve the impact of oil that coats aboveground plant components. Without dispersant application, both South Louisiana crude oil and No. 2 fuel oil at a concentration as low as 150 ppm greatly impacted the salt marsh grass, *Spartina alterniflora*. Compared with the control, significantly higher leaf mortality rate and dead stem density, and significantly lower community photosynthetic rate, live stem density, and live aboveground biomass occurred with undispersed oil in a two-month period after treatment. Dispersant application, both JD-2000 and Corexit 9500, significantly reduced the impact of the oil on the plants; plant leaf mortality rate, photosynthetic rate, plant stem density, and aboveground biomass in treatments receiving the dispersed SLC or No. 2 fuel oil were not significantly different from the control in the short-, mid- and long-term for both the organic marsh substrate and sandy marsh substrate. Furthermore, in the present study, dispersant application both in the active plant growing season in the summer and in the inactive plant growing season in the fall greatly relieved the impact of SLC oil and No. 2 fuel oil on *Spartina alterniflora*. Therefore, regardless of marsh substrate type and application season, simulating application of dispersants in nearshore environments greatly reduced the adverse effect of a low concentration of oil at 150 ppm of SLC and No. 2 fuel oil on the plant.

Without dispersants, a high concentration of oil (750 ppm of SLC oil or No. 2 fuel oil) resulted in much greater impact on *S. alterniflora* than did the low oil concentration. The high concentration (750 ppm) of undispersed oil resulted in as much as 90% leaf mortality, and completely inhibited plant photosynthesis in the short term. The undispersed oil also caused significantly higher dead stem density and dead aboveground biomass, and significantly lower live-stem density and live aboveground biomass compared to the control. However, the application of dispersants to the high concentration (750 ppm) of oil also significantly reduced the impact of oil on the plant. Leaf mortality rate, plant photosynthetic rate, plant stem density, and aboveground biomass in treatments receiving the dispersed SLC or No. 2 fuel oil were significantly improved compared to treatment with oil alone for the short-term (two months after treatment) and the mid-term (six months after treatment). In addition, Corexit 9500 was not as effective as JD-2000 for reducing the impact of the high concentration of SLC oil on *S. alterniflora,* although it still significantly reduced the impact of the oil on the plants compared to treatment by SLC oil alone.

In this study, it appeared that the impacts of oil and dispersed oil on plants were caused primarily by oil coating the plant shoots. Without dispersants, a considerable amount of oil coated the plant leaves and stems in experiments with both low concentration (150 ppm) and high concentration (750 ppm) of oil, resulting in a higher mortality rate and a lower photosynthetic rate, live-stem number, and aboveground biomass compared to the control. However, with the application of dispersants, little oil coated the plants shoots and leaves, and the impact on the plants was limited. On the other hand, concentrations of oil retained in the soil were generally not significantly different between treatments of dispersed and undispersed oil, and amounts of TPH in the soil were less than 20 mg/g. Studies (Lin and Mendelssohn 1998 and 2004; Lin *et al*. 2002) have indicated that *Spartina alterniflora* is able to grow in soil with a TPH concentration as high as 320 mg/g dry soil, which is more than 15 times higher than the concentration of the present study. These data suggest that the impact of the oil on the salt marsh grass, *S. alterniflora*, in the present study primarily resulted from the oil coating the plant shoots and leaves, rather than from the oil retained in the soil.

The strategy of the present study was to investigate the efficacy of applying dispersants in nearshore environments before oil moved into adjacent coastal marshes. The present study differs from a handful of previous studies (Baker *et al*. 1984; Lane *et al*. 1987; Little and Scales 1987), in which oil came in contact with marsh plants before dispersant application. Baker *et al*. (1984) reported that the dispersant BP1100WD was ineffective in cleaning an oiled salt marsh, reduced *Spartina anglica* density in one to two years, and resulted in short-term loss of *Salicornia* spp. Similarly, salt marshes exposed to weathered crude oil and the dispersant Corexit 9527 showed that dispersed oil had a greater impact on *S. alterniflora* than the oil alone (Lane *et al*. 1987). Application of the dispersant BP Enersperse 1037 to coastal salt marshes contaminated with weathered Nigerian crude, fuel oil, and mousse showed that dispersed oil was more destructive to *Spartina* and *Aster* than untreated oil (Little and Scales 1987). In contrast, other studies (Smith *et al*. 1984; DeLaune *et al*. 1984) demonstrated that dispersants applied to *Spartina alterniflora* contaminated by Louisiana crude oil provided short-term benefits to plant photosynthesis although they did not have long-term effects on plant biomass. In the field trial, Duke *et al*. (2000) demonstrated that death of mangrove trees was significantly lower in the plots treated with dispersed weathered crude oil compared to oil alone.

The principal biological benefit of dispersant use is that it prevents oil from stranding in sensitive habitats (NRC 1989), such as coastal salt marshes. The present study clearly demonstrated that applying dispersants reduced the oil adhering to marsh plants, with significantly lower oil amount on plant shoots and leaves than occurred without dispersants for both South Louisiana crude oil and No. 2 fuel oil. Generally, oil tends to stick to the surface of various objects. In a simulated oil spill in a nearshore surf-zone, Page *et al*. (2000) reported that little oil adsorbed to sediments in the chemically dispersed oil treatment. In comparison, approximately 49% of the applied oil adsorbed to sediment or other surfaces when the treatment was crude oil alone. In addition, in a 24-inch pipeline rupture in Nigeria, which released 40,000 bbl of light crude oil into the marine environment, dispersants played an important role in limiting and preventing shoreline and estuarine mangrove habitats from oiling (Olagbende *et al*. 1999). The dispersed oil may move into marsh with tides or currents, but most of dispersed oil appears to move out of marshes with the ebbing tide because dispersed oil is not as adhesive to the surface of plant aboveground components as oil alone. Therefore, the strategy of applying dispersants in nearshore environments before oil comes in contact with adjacent coastal marshes appears to have a great potential for coastal marsh habitat protection.

Experiments in the present study were conducted in an open-ended greenhouse in which environmental conditions were held relatively constant compared to conditions in the field. Thus, the effect and efficacy of dispersants were examined with natural variability minimized. In addition, the open-ended greenhouse lets weather variables, such as temperature, humidity and day-length, change with the season; thus, the results are more realistic. Furthermore, in the present study, intact sods of salt marsh dominated by *Spartina alterniflora* were extracted from coastal marshes with minimal disturbance of marsh sediment structure and plants. Using intact, undisturbed marsh sods as experimental units ensured that the results would be more similar to those in the field. However, it should be noted that the current study was not conducted in large-scale field conditions; thus field investigations may still be needed to further verify the efficacy of dispersants applied in nearshore environments for habitat protection.

Federal oil-spill response agencies, such as US-EPA, NOAA, and USCG, generally pre-approve the use of dispersants in open water deeper than 10 meters (about 30 feet). Dispersant use was considered to be a major factor in reducing shoreline oiling. In any particular situation, the decision to use dispersants involves balancing the potential advantages of dispersant use--removing oil from the water surface and avoiding some shoreline impacts--with the potential disadvantages, such as impacts to plankton or other water column organisms (NOAA 2007).

The current research investigated the potential effect and efficacy of dispersant application in nearshore environments for coastal habitat protection. The study is compatible with the statement of the National Research Council (2005), that there were significant gaps in the knowledge needed to make sound decisions regarding the use of dispersants in areas that were nearshore, shallow, or had restricted flushing rates. In these areas, the simplified assumptions that were used in the risk analysis for open-water setting were insufficient.

Recommendations and applications of the present study

1. Dispersants may be applied to an oil slick before the slick comes in contact with sensitive coastal habitats, such as coastal salt marshes. Oil slicks detrimentally impact salt marsh plants, such as the dominant salt marsh plant, *Spartina alterniflora*. However, oil that has been dispersed before it comes in contact with adjacent coastal marshes had little effect on marsh plants, not only in the short-term but also in the long-term. Under no circumstances, may we apply dispersants directly onto marsh vegetation or on an oil slick inside marshes. Oil that has coated the surface of plant leaves and stems is hard to disperse because oil tends to affiliate with leaf cuticle (leaf surface wax). In addition, the application of dispersants onto oil slicks inside marshes most likely cannot be effective because there is not enough mixing energy for dispersant action inside the marshes. For both scenarios (applying onto vegetation or on an oil slick inside marshes), applied dispersants most likely add extra toxicity to habitats.

2. Dispersants are more effective in dispersing light oil such as No. 2 fuel oil than viscous oil such as crude oil. In the present study, dispersants applied to a high concentration (750 ppm) of the heavier crude oil were less effective for relieving oil impacts on marsh plants than when they were applied to the same concentration of a lighter No. 2 fuel oil. Thus, it is better to apply dispersants as soon as possible before oil weathering or before oil emulsification. Both weathered and emulsified oil may increase the viscosity of oil, thus reducing dispersion effectiveness.

3. Various dispersants may have different dispersion effectiveness and toxicity, which may affect the extent to which they are used. Corexit brand dispersants, such as Corexit 9527 and Corexit 9500, are stockpiled in the United States, probably because of their longer development history and the brand recognition developed by the Exxon Company. However, the present results indicated that the dispersant JD-2000, also listed in NCP, was more effective than Corexit 9500 for relieving the impacts of heavier oil at a high concentration. In addition, JD-2000 is effective in dispersing oil

in both salt and fresh water; however, Corexit 9500 disperses oil in salt water only (EPA, 2001). JD-2000 might have the advantage of keeping oil dispersed even when oil dispersed in salt water moves with tides or currents into low-salinity or fresh water. Thus, it would be helpful to further evaluate the effectiveness of JD-2000 in wave tank and field conditions. Stockpiling JD-2000 may provide oil-spill response groups with more options.

4. Dispersants relieved the oil impact on marsh plants by application in both summer and fall seasons, although cold weather may affect oil viscosity and thus reduce dispersion effectiveness. In addition, the study showed that dispersants reduced oil impact on marsh plants regardless of marsh soil type (organic or sandy substrate). Thus, dispersants may be applied in widely varied environmental conditions for the protection of sensitive coastal habitats.

5. Dispersants do not adversely affect soil microbes. The results of the study suggested that dispersants enhanced oil degradation. For low concentrations of oil such as the 150-ppm oil treatments, alkanes and PAHs in the sediment were completely degraded after one year whether or not dispersants were applied. However, for high concentrations of oil such as the 750-ppm oil treatment, concentrations of TPH, alkanes, and PAHs were lower for the dispersed than for the undispersed oil treatment one year after treatment initiation, especially when n-alkanes and PAHs were enriched by No. 2 fuel. This suggested that application of dispersants accelerated oil degradation in the soil.

6.0 Technology Transfer

The primary deliverables of this project are the final report, publication in scientific journals, and a number of presentations at various conferences, scientific meetings, technical seminars and workshops. The results were presented at oil-spill conferences, and workshops such as the International Oil Spill Conference (IOSC), the Arctic and Marine Oilspill (AMOP) Technical Seminar, NOAA’s Oil Spill Response Workshop, and NATO/CCMS Oil Spill Response Workshop. All of these conferences and workshops have large audiences that include oil spill response groups from local, state, federal agencies, foreign governments, petroleum industries, environmental industries, and academia. Coastal salt marshes are vulnerable to nearshore oil spills because of their location within the coastal zone. Furthermore, the salt marsh plant, *Spartina alterniflora*, is the dominant marsh species along the Northern Gulf of Mexico and Atlantic coasts. Thus, the results of this study have the potential for widespread usage.

7.0 Achievement and Dissemination

The published manuscript:
Lin, Q. and I.A. Mendelssohn 2005. Dispersants as Countermeasures in Nearshore Oil Spills for Coastal Habitat Protection. The Proceedings of the *2005 International Oil Spill Conference*. American Petroleum Institute, Washington, D.C. 447-451 pp.

Numerous oral presentations at various conferences, scientific meetings, and workshops:
Lin, Q. and I.A. Mendelssohn. Dispersants as oil spill countermeasures for the remediation and restoration of sensitive coastal habitats, NOAA’s Emerging Research in Oil Spill Response and Restoration Workshop, NOAA, Silver Spring, Maryland, March 21-22, 2005.

Lin, Q. and I.A. Mendelssohn. Dispersants as countermeasures in nearshore oil spills for coastal habitat protection. The *2005 International Oil Spill Conference*. Miami, Florida, May 15-19, 2005.
Lin, Q. and I.A. Mendelssohn. Effectiveness of dispersants on relief of oil spill impacts for coastal salt marshes. The 28th Arctic and Marine Oilspill Technical Seminar. Calgary, Canada, June 6-9, 2005.
Lin, Q. and I.A. Mendelssohn. Dispersants as oil spill countermeasures for the remediation and restoration of sensitive coastal habitats. NOAA's Coastal Response Research Workshop, Durham, NH., March 13-14, 2006.
Lin, Q. and I.A. Mendelssohn. Effectiveness of dispersants as crude oil spill countermeasures for sensitive coastal habitat protection, The 29th Arctic and Marine Oilspill Technical Seminar. Vancouver, Canada, June 6-8, 2006.
Lin, Q. and I.A. Mendelssohn. Effectiveness of dispersants for coastal habitat protection as a function of types of oil and dispersant, The 2006 Third Annual NATO/CCMS Oil Spill Response Workshop, Dartmouth, Nova Scotia, Canada, October 11-13, 2006

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