

Dispersant Effects on Fresh Marsh Vegetation: Toxicity Evaluation and Oil Remediation

Qianxin Lin
and
Irving A. Mendelsohn

Wetland Biogeochemistry Institute
School of the Coast and Environment
Louisiana State University
Baton Rouge, LA 70803

**Technical Report Series
169-30-4150**

2003

DISCLAIMER

This report was prepared under contract between Louisiana State University and the Louisiana Oil Spill Coordinator's Office/Office of the Governor, Louisiana Applied and Educational Oil Spill Research and Development Program. The contents of this document do not necessarily reflect the views and policies of the Louisiana Oil Spill Coordinator's Office-Office of the Governor or those of the Louisiana Applied and Educational Research and Development Program, nor does mention of trade names or commercial products constitute endorsement or recommendation for use by the state of Louisiana.

REPORT AVAILABILITY

Additional copies of this report may be obtained by writing to:

Louisiana Applied and Educational Oil Spill Research and Development Program (OSRADP)
2003 Deliverables
258 A/B Military Science Building
Baton rouge, Louisiana 70803
Telephone Number: (225) 578-3477 FAX Number (225) 578-0403

or from

The Louisiana Oil Spill Coordinator's Office-Office of the Governor
1885 Wooddale Blvd., 12th Floor
Baton rouge, Louisiana 70806
Telephone Number: (225) 922-3230 FAX Number (225) 922-3239

CITATION

Lin, Q., and I. A. Mendelssohn. 2003. Dispersant effects on fresh marsh vegetation: Toxicity evaluation and oil remediation. Louisiana Applied and Educational Oil Spill Research and Development Program, OSRADP Technical Report Series 169-30-4150, 22 pp.

TABLE OF CONTENTS

	Page
1.0	Introduction.....1
1.1	Goal and objectives.....3
2.0	Materials and methods3
2.1	Phase 1. Toxicity to marsh plants of dispersant and dispersed oil applied to the soil3
2.1.1	Task 1. Dose-response relationship between dispersant applied to the soil and the growth of the marsh plant, <i>Sagittaria lancifolia</i>3
2.1.2	Task 2. Dose-response relationship between chemically dispersed oils applied to the soil and the growth of the marsh plant, <i>Sagittaria lancifolia</i>4
2.2	Phase 2. Potential and effectiveness of nearshore dispersant application on habitat protection and oil remediation4
2.3	Methods5
2.4	Statistical analysis.....6
3.0	Results.....6
3.1	Task 1 of Phase 1. Dose-response relationship between dispersant applied to the soil and the growth of the marsh plant, <i>Sagittaria lancifolia</i>6
3.2	Task 2 of Phase 1. Dose-response relationship between dispersed oils applied to the soil and growth of the marsh plant, <i>Sagittaria lancifolia</i>10
3.3	Phase 2. Effect of oil type, concentration and dispersant application on plant responses and oil remediation.....13
4.0	Discussion17
5.0	Conclusions.....18
6.0	References.....20

LIST OF FIGURES

Figure	Description	Page
1	<i>Sagittaria lancifolia</i> growth status after different concentrations of the dispersant JD-2000 applied to the soil.....	7
2	Dose-response relationship between photosynthetic rates of <i>Sagittaria lancifolia</i> and different dosages of the dispersant JD-2000 in the soil at different times after treatment.	8
3	Dose-response relationship between leaf mortality rate of <i>Sagittaria lancifolia</i> and different dosages of the dispersant JD-2000 in the soil at different times after treatment.	8
4	Dose-response relationship between the aboveground biomass of <i>Sagittaria lancifolia</i> and different dosages of the dispersant JD-2000 in the soil 10 months after treatment.....	9
5	<i>Sagittaria lancifolia</i> growth status after different concentrations of JD-2000 dispersed diesel (0-145,800 ppm from left to right) were applied to the soil.....	10
6	Photosynthetic rates of <i>Sagittaria lancifolia</i> three weeks (6a) and six months (6b) after different concentrations of the JD-2000 dispersed oil were applied the soil.....	11
7	Dose-response relationship between leaf mortality rates of <i>Sagittaria lancifolia</i> and different dosages of the JD-2000 dispersed oil in the soil three weeks (7a), six weeks (7b) and six months (7c) after the treatment.	12
8	Dose-response relationship between aboveground biomass of <i>Sagittaria lancifolia</i> and different dosages of JD-2000 dispersed oil in the soil seven months after treatment	13
9	Effect of oil type and dispersant application on intact marsh sods dominated by <i>Sagittaria lancifolia</i> two weeks after the 2000 ppm (9a) and 50 ppm (9b) dispersed oil treatment.	13
10	Effect of dispersant, oil type, and dosage on photosynthetic rate of <i>Sagittaria lancifolia</i> three weeks after treatment.....	14
11	Effect of dispersant, oil type and dosage on stomatal resistance of <i>Sagittaria lancifolia</i> three weeks after treatment.....	14
12	Effect of dispersant, oil type and dosage on mortality rate of <i>Sagittaria lancifolia</i> three weeks after treatment	15
13	Effect of dispersant, oil type, and dosage on total aboveground biomass for all plant species seven months after treatment.	15
14	Effect of dispersant, oil type, and dosage on total petroleum hydrocarbon (TPH) concentration in the soil at 0-1.5 cm soil depth one week after treatment	16
15	Effect of dispersant, oil type, and dosage on the C10-C32 petroleum components in the soil at 0-1.5 cm soil depth one week after treatment.....	16
16	Effect of dispersant, oil type, and dosage on total petroleum hydrocarbon (TPH) concentration in the soil at 0-1.5 cm soil depth seven months after treatment.....	16

ACKNOWLEDGMENTS

We would like to thank the sponsor of this research, the Louisiana Applied and Educational Oil Spill Research and Development Program (OSRADP). Funding from the OSRADP and assistance from Dr. Donald Davis and his staff were essential to the successful completion of this research. In addition, we greatly appreciate Drs. Anita George-Ares, David E. Fritz, Charlie B. Henry, Chacko J. John, James E. Myers, and Albert D. Venosa for their valuable contributions to the experimental design. Also, we thank Joannie Docter of GlobalMark Resources for providing the dispersant JD-2000 sample.

Abstract

Oil spills in nearshore or estuarine environments may eventually be moved into coastal marshes by currents, tides and winds. The spills then strand inside these habitats and impact marsh organisms. One strategy for habitat protection and oil remediation may be to apply dispersants to the spilled oil before the oil drifts into coastal marshes. The overall goal of this project was to determine the potential use of dispersants as an oil spill countermeasure in nearshore environments where spilled oil may move into coastal marshes and impact sensitive habitats. Specifically, the objectives of the proposed project were to: (1) evaluate the toxicity of dispersants on coastal fresh marsh plants by determining the dose-response of plants to dispersants, (2) evaluate the effect of different dispersed oils (crude oil and diesel fuel) on coastal fresh marsh plants by determining the dose-response of plants to dispersed oils, (3) compare the effect of dispersed oils (diesel and crude) in simulated nearshore dispersant application on marsh habitat protection, and (4) determine the effect of dispersants on oil remediation. The dose-response of the fresh marsh plant *Sagittaria lancifolia* to the dispersant JD-2000 indicated that plant tolerance to this dispersant was relatively high. The marsh plant *S. lancifolia* was not impacted by the dispersant JD-2000 at dosages $\leq 4,000$ ppm based on plant photosynthetic rate, plant mortality rate, and plant aboveground biomass. Our results show that *Sagittaria lancifolia* can recover at dispersant dosages as high as 16,000 ppm, because the toxicity of the dispersant decreased during the 10 month experiment. In an experiment that determined the dose-response relationship and toxicity of dispersed oils to the fresh marsh plant *Sagittaria lancifolia*, plant photosynthetic rate, plant mortality rate, and plant aboveground biomass were negatively affected by the JD-2000 dispersed diesel at 16,200 ppm applied to the soil substrate. The LC_{50} (6 weeks) of dispersed diesel to *Sagittaria lancifolia* was estimated at 20,000 ppm. However, dispersed South Louisiana crude oil did not detrimentally affect *S. lancifolia* even at an oil dosage of 145,800 ppm. This indicated that the toxicity of the dispersed oil primarily resulted from the oil itself, not from the dispersants. In an experiment that simulated oil dispersed before coming in contact with marshes, the dispersant JD-2000 greatly relieved the adverse effects of both diesel and crude oil. Without the dispersant, both diesel and crude oil significantly decreased photosynthetic rate and increased mortality even at a 50-ppm dosage. Two thousand ppm of diesel without the dispersant resulted in $> 60\%$ mortality of aboveground components. In contrast, neither the dispersed crude nor the dispersed diesel significantly affected *S. lancifolia* compared to the no-oil control. Furthermore, application of the dispersant significantly reduced oil adsorption to the marsh sediment. Therefore, dispersant application greatly reduced oil impact on fresh marsh vegetation and sediment, indicating the potential of using dispersants as alternative countermeasures for oil spills in nearshore or estuarine environments.

Dispersant Effects on Fresh Marsh Vegetation: Toxicity Evaluation and Oil Remediation

1.0. Introduction

Deleted: Summary¶

¶ Oil spills in nearshore environments, or in wetland creeks, can move into marshes by tides and winds, strand inside marshes, and impact marsh organisms. One strategy might be to apply dispersants onto the spilled oil before the oil drifts into marshes. Dispersants have received considerable attention for open water oil spills. However, little information is available on the toxicity and remediation of chemically dispersed oil, especially in wetland environments. The objectives of the research are to evaluate the potential of using dispersants in nearshore and marsh environments by comparing and determining the toxicity and oil remediation of 'new generation' dispersants and dispersed oils on different marshes with different oil types, concentrations, dispersant type and spill scenarios. The first year experiment was conducted with fresh marsh sods. For the dose-response study during the first year, results of plant photosynthetic rate, plant growth and plant mortality after 3 and 6 weeks indicated chemical dispersed diesel applied to the soil substrate detrimentally affected *Sagittaria lancifolia* at 16,200 ppm. The plant was completely killed at 48,600 ppm and higher. LC_{50} of dispersed diesel was estimated at 24,000 ppm for 3 weeks and 20,000 ppm for 6 weeks. Dispersed crude oil did not detrimentally affect *S. lancifolia* even at an oil dosage of 145,800 ppm. For the experiment simulating oil dispersed before entering a marsh, the dispersant (JD-2000) greatly relieved the adverse effect of both diesel and crude oil coating the aboveground vegetation. Without the dispersant, both diesel and crude oil significantly decreased photosynthetic rate, and increased mortality even at a 50 ppm dosage. Two thousand ppm of diesel without dispersant resulted in $> 60\%$ mortality. In contrast, the dispersed oils did not significantly affect *S. lancifolia* compared to the no-oil control based on photosynthetic rate and plant mortality. In addition, dispersant application significantly reduced oil adsorption to the marsh sediment, with only about 10% of the oil concentration for the treatment with the dispersant compared to without dispersant at 2000 ppm concentration. Therefore, dispersant application greatly reduced oil impact on fresh marsh vegetation and sediment. During the second year, toxicity and effects of dispersed diesel and crude oil on plant response, microbial activity, and oil remediation in salt marsh sods will be evaluated. In the second year, Corexit 9500, marketed as a salt water dispersant, will be included in addition to JD-2000. Generally, salt marshes are more vulnerable to nearshore spills because of their location within the coastal zone. Furthermore, the sal

The risk of oil spills in the coastal zone is high because of intensive oil-related activities in this area. Not only can spilled oil harm coastal habitats and associated organisms, but cleaning up oil stranded in coastal marshes with dense vegetation may do more damage to these highly sensitive marshes than the oil itself. Preventing oil from stranding and coating sensitive coastal marshes is an important way to protect these habitats. Dispersants, which have received considerable attention for open, deep water oil spills, may be helpful, but require evaluation for oil spill countermeasure in the nearshore and in wetlands.

The use of dispersants for oil spill cleanup has attracted great attention since the Exxon Valdez oil spill in 1989. However, dispersants are controversial because of disagreement among scientists about their effectiveness and toxicity. These issues have been discussed since dispersants were introduced during the Torrey Canyon oil spill in 1967 (Cunningham et al. 1991; Venosa et al. 1999). Dispersants are chemicals that contain surfactants, or compounds that break liquid substances like oil into tiny droplets in the water column. This helps remove oil from the water surface, making it less likely that an oil slick will reach or strand in sensitive habitats, such as coastal marshes. Dispersant use has been recommended as a way to disperse oil slicks in the sea before coastal habitats can be reached, although minimal guidelines have been outlined (Page et al. 2000). Therefore, the effects of dispersants have mainly been 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). Most of these studies on marine organisms were acute toxicity tests. However, decisions to use oil spill response chemicals should not be based solely on aquatic toxicity (George and Clark 2000).

A number of studies have been conducted on the effects of dispersants on plants from salt to fresh water marshes, although dispersant application is not pre-approved in Louisiana or Texas for water less than 10 m deep (RRT-6-OCS 1996). Some studies indicated that dispersants, such as BP1100WD (Baker et al. 1984), Corexit 9527 (Lane et al. 1987), and BP Enersperse 1037 (Little and Scales 1987) were ineffective in cleaning an oiled salt marsh and had greater detrimental impact on *Spartina anglica*, *Salicornia* spp., *S. alterniflora* and *Aster* spp. than oils without applied dispersants. In contrast, other studies (Smith et al. 1984; DeLaune et al. 1984) demonstrated that dispersants applied to Louisiana crude oil contaminated *Spartina alterniflora* provided short-term benefits to plant photosynthesis. However, these studies found that the dispersants did not have long-term effects on plant biomass.

The dispersants used today are more effective and much less toxic than earlier products (NRC 1989). For example, a more recently marketed dispersant, Corexit 9500, contains the same surfactants as Corexit 9527 and an improved oliophilic solvent delivery system. The dispersant JD-2000, manufactured and marketed by Van Waters and Rogers in 2001, is especially effective for South Louisiana crude oil (EPA 2001) in both salt and fresh water. Both are high performance, biodegradable oil spill dispersants listed in the National Contingency Plan (EPA 2001). The efficacy of dispersants may vary with different spill scenarios. Dispersants generally are more effective in salt water than in fresh water. They also work better on less viscous oil, and in warmer temperatures (NRC 1989; Guyomarch et al. 1999; EPA 2001). Thus, dispersant use may be more effective in the near subtropical climate of Louisiana's coastal wetlands. However, little information is available on the toxicity and effects of dispersants on marsh habitats and the strategy of dispersant use in the nearshore to protect sensitive coastal habitats.

1.1. Goal and Objectives

The overall goal of the project was to determine the potential use of dispersants as oil spill countermeasures in nearshore environments where spilled oil may eventually move into coastal marshes with tide and wind, and impact sensitive wetland habitats. Specifically, the objectives of the proposed project were to: (1) evaluate the toxicity of dispersants on coastal fresh marsh plants by determining the dose-response of plants to dispersants, (2) evaluate different dispersed oils [South Louisiana crude oil (SLC) and diesel fuel] on coastal fresh plants by determining the dose-response of plants to dispersed oils, (3) use simulated nearshore applications to compare how well dispersed oils (diesel and SLC) protected the marsh habitats, and (4) determine the effect of dispersants on oil remediation of SLC and diesel fuel and how these dispersants influence dispersed oil adsorption to the marsh sediment.

2.0. Materials and Methods

The experiments were conducted in two phases. Phase 1 involved a determination of the dispersants' toxicity and the effect of chemically dispersed oils in the soil to fresh marsh plants. Phase 2 involved determining how well nearshore dispersant application protected habitat and promoted oil remediation.

2.1. Phase 1. Toxicity to Marsh Plants of Dispersant and Dispersed Oil Applied to the Soil

2.1.1. Task 1. Dose-Response Relationship Between Dispersant Applied to the Soil and the Growth of the Marsh Plant, *Sagittaria lancifolia*

The fresh marsh plant, *Sagittaria lancifolia*, was collected from coastal Louisiana and transplanted to the potting substrate, Jiffy Mix plus. Plants were maintained in the greenhouse and grown until they reached a satisfactory size and health. The dispersant JD-2000 (a product of Van Waters and Rogers, Cincinnati, OH) was used because JD-2000 disperses oil in both fresh water and salt water (Corexit-9500, another dispersant-candidate dispersing oil effectively only in salt water, will be included in the salt marsh experiment). JD-2000 is a dispersant listed in the National Contingency Plan (EPA 2001). JD-2000, ranging from 0, 250, 500, 1000, 2000, 4000, 8000, and 16000 ppm in concentration, was applied to the substrate containing healthy *Sagittaria lancifolia* to determine the dose-response relationship between the chemical dispersant and growth of the marsh plant. This toxicity test was designed to provide reference data of the dispersants' toxicity to plants in comparison with the standard bioassay test aquatic organisms. Each dosage was replicated three times, resulting in a total of 24 experimental units. Short- and long-term toxicity and effect of the dispersant to the plants were analyzed by measuring photosynthetic rate and plant leaf mortality rate. The LC₅₀ of the dispersant on the plants was determined at different times after the treatment. Long-term toxicity of the dispersant was analyzed by measuring the photosynthetic rate, mortality rate, and plant aboveground biomass in the second growing season (10 months after dispersant application).

2.1.2. Task 2. Dose-Response Relationship Between Chemically Dispersed Oils Applied to the Soil and the Growth of the Marsh Plant, *Sagittaria lancifolia*

As in Task 1, the fresh marsh plant, *Sagittaria lancifolia*, was collected, transplanted, maintained in the greenhouse, and grown until it reached a satisfactory size and health. The dispersant JD-2000 was pre-mixed with oils at a ratio of 1:20 (within the manufacturer's recommended ratio of 1:10 to 1:50 of dispersant to oil). The dispersed oil, ranging from 0, 600, 1800, 5400 16200, 48600 and 145800 ppm of weathered South Louisiana crude oil or diesel fuel (25% weathered by v/v), was applied to the substrate containing healthy *Sagittaria lancifolia*. This was done to determine the dose-response relationship between chemically dispersed crude oil or diesel and the growth of the fresh marsh plant. This toxicity test provided reference data for environmental assessments. Each dosage was replicated three times, resulting in a total of 42 experimental units. Toxicity of the dispersed SLC and diesel to the plants was assessed by measuring photosynthetic rate and plant leaf mortality rate. The LC₅₀ of the dispersed crude oil and dispersed diesel on the plants was determined. Long-term toxicity of the dispersed crude oil and diesel was analyzed by measuring the same parameters and plant aboveground biomass in the following growing season.

2.2. Phase 2. Potential and Effectiveness of Nearshore Dispersant Application on Habitat Protection and Oil Remediation

Oil spills in open water, nearshore environments, and estuaries may be moved into marshes by winds and tides. The oil then strands and impacts the marshes. It is extremely difficult to clean up stranded oil in marshes because coastal marsh habitats are highly sensitive to mechanical disturbance. Thus, one strategy may be to apply dispersants to the spilled oil before the oil comes in contact with marshes. We designed the following experiment to evaluate the effect of concentrations of dispersed crude and diesel on marsh plant growth and oil remediation.

Intact soil sods from a low salinity marsh dominated by *Sagittaria lancifolia*, but containing several subdominant species, were extracted from coastal Louisiana. These sods were used in a mesocosm experiment conducted in the greenhouse.

The following treatments simulated the application of dispersants on oil spilled in nearshore environments, estuaries, and wetland canals before the oil drifted into marshes. Dispersed oil may move into the marsh with tides or currents, but most dispersed oil appears to move out with the ebbing tide because dispersed oil does not adhere to the surface of sediment particles or plant aboveground components.

The treatments were as follows:

- 50 ppm weathered SLC or weathered diesel fuel dispersed in the water column by the dispersant JD-2000 [the concentration of dispersed oil in actual spills can approach 50 ppm at 1 m depth even in open water (National Research Council, 1989)]
- 2000 ppm weathered SLC or weathered diesel fuel dispersed in the water column by the dispersant JD-2000 (the concentration of oil dispersed in wetland creeks, canals, or the nearshore can be much higher than in open water because of the shallow water environment)
- 50 ppm weathered SLC or diesel fuel without JD-2000
- 2000 ppm weathered SLC or diesel fuel without JD-2000

A treatment with neither oil nor dispersant applied to the sods served as the control.

We simulated nearshore dispersed oil moving in and out of wetlands by generating a tide with the appropriate concentrations of the oil or dispersed oil in the water so that 80% of the plant height was covered for a 30-minute period. We then caused the water to recede to 10 cm above the soil surface for the rest of 12-hr high tide period. For the 12-hr low tide, the water table was at 10 cm below the soil surface. The standing water moved out of the sods from a hole on the wall of the buckets at the soil surface level, and water below the soil surface drained out of the sods through a hole at 10 cm below the soil surface. The water level was brought back to 10 cm above the sediment during the next high tide and 10 cm below the sediment during the next low tide for the following days until all oil was adsorbed to the sediment, and no oil floated back to the tidal water during the high tide. Thereafter, we maintained the water table fluctuation between 0 and 10 cm below the soil surface throughout the experiment. The experimental design was completely randomized with a 2 x 2 x 2 factorial treatment arrangement [two dispersant levels (with and without dispersant), two oil concentration levels (50 and 2000 ppm) and two oil types (SLC and diesel)], plus an overall control. Each treatment-level combination was replicated four times, resulting in a total of 36 experimental units.

The experiment lasted a full growth cycle. The effects and toxicity of the oils and the dispersed oils were analyzed by measuring photosynthetic rate, stomatal resistance, plant stem density, plant mortality rate, and plant aboveground biomass. In addition, the C10 to C32 of petroleum hydrocarbons and the DCM extractable total petroleum hydrocarbons adsorbed in the sediment were analyzed one week after treatment initiation and at the end of the experiment to evaluate the effects of the dispersants on oil removal and remediation.

2.3. Methods

Photosynthetic and stomatal resistance rates. The photosynthetic rate and stomatal resistance were measured with a portable photosynthesis system (CI-301PS, CID, Inc.). Sample air, taken 5 m aboveground to obtain relatively stable CO₂ concentrations, was utilized during photosynthetic rate measurements. Measurements were conducted at a quantum flux density of 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$. An intact, attached, and fully expanded young leaf was enclosed in the leaf chamber. Photosynthetic rate (CO₂ exchange) and stomatal resistance were expressed in units of $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ and $\text{m}^2\text{s}/\text{mol}$, respectively.

Plant mortality rate. Mortality rate was calculated using the ratio of the dead leaf numbers to the total leaf numbers.

Aboveground biomass. Aboveground biomass was harvested at the end of the experiment. Plant tissues for each plant species were separated and dried at 65 °C to a constant weight.

The C10-C32 petroleum components analyzed by GC/FID. Soil samples collected from a depth interval of 0 to 2 cm were mixed with anhydrous sodium sulfate to facilitate extraction. The samples were then extracted with dichloromethane (DCM) and analyzed by capillary gas chromatography with flame ionization detection (GC/FID). Results were corrected for background extractable material by comparison with oil free soil blanks. Gas chromatographic separations used a 30 m, 0.25 mm inner diameter column with a 5% phenyl-95 percent dimethylpolysiloxane (DB-5) stationary phase. The initial GC temperature was 50 °C for two

minutes followed by temperature programming to 320 °C at 15 °C /minute. The temperature was held at 320 °C for an additional 12 minutes.

The DCM extractable TPH in the sediment. Total DCM extractable total petroleum hydrocarbons (TPH) in the sediment were analyzed gravimetrically using a modification of the procedure outlined in EPA method 9071. Soil samples collected from a depth interval of 0 to 2 cm were mixed with anhydrous sodium sulfate and extracted with DCM. The extract was then transferred to a pre-weighed dish, the remaining extraction solvent evaporated, and the un-evaporated oil remaining in the dish weighed to the nearest to 0.0001 g. After extraction, each sediment sample was dried at 65 °C and weighed. Total hydrocarbon concentration in the sediment was calculated and expressed as mg hydrocarbons g⁻¹ dry soil. The gravimetric method was used to measure total petroleum hydrocarbons in the experiment because the GC/FID method analyzes only certain oil components, ranging from about C10 to C32 hydrocarbons.

2.4. Statistical Analysis

Statistical analysis was conducted with the Statistical Analysis System (SAS, version 8.02). Plant parameters, soil variables, microbial responses, and petroleum hydrocarbon concentrations were analyzed with general linear models (GLM). Duncan's Test was used to evaluate statistical differences of the main factors when no interaction occurred. The least square means test was used to evaluate statistical differences between treatment-level combinations if interaction between main factors occurred. Significant differences were reported at the 0.05 probability level unless otherwise stated.

3.0. Results

3.1. Task 1 of Phase 1. Dose-Response Relationship Between Dispersant Applied to the Soil and the Growth of the Marsh Plant, *Sagittaria lancifolia*

Responses of plants to dispersants are generally slower than responses of marine organisms. *Sagittaria lancifolia* did not show any visual symptoms 96 hours (four days) after the dispersant treatment (Fig. 1 a). Plant leaves turned brown at high dispersant concentrations seven days after the treatment (Fig. 1b), and the impacts became more severe three weeks after the treatment (Fig. 1c). However, the plant recovered at dispersant dosages ≤ 8000 ppm three months after the treatment (Fig. 1d). Six and 10 months after the application of the dispersant (Figs. 1e and 1f), the seeds of *Sagittaria lancifolia* were able to germinate, and seedlings grew in the soil receiving 16,000 ppm of JD-2000.



Fig. 1a *Sagittaria lancifolia* growth four days after different concentrations of the dispersant JD-2000 (0-16,000 ppm from left to right) were applied to the soil.

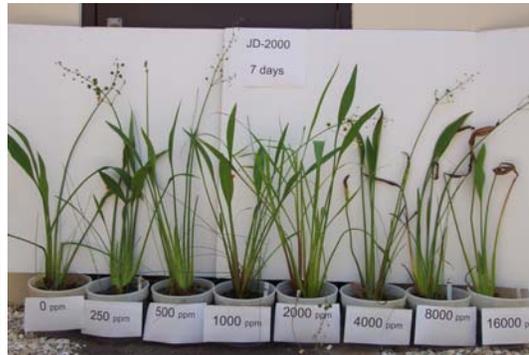


Fig. 1b *Sagittaria lancifolia* growth seven days after different concentrations of the dispersant JD-2000 (0-16,000 ppm from left to right) were applied to the soil.



Fig. 1c *Sagittaria lancifolia* growth three weeks after different concentrations of the dispersant JD-2000 (0-16,000 ppm from left to right) were applied to the soil.



Fig. 1d *Sagittaria lancifolia* growth three months after different concentrations of the dispersant JD-2000 (0-16,000 ppm from left to right) were applied to the soil.



Fig. 1e *Sagittaria lancifolia* growth six months after different concentrations of the dispersant JD-2000 (0-16,000 ppm from left to right) were applied to the soil.



Fig. 1f *Sagittaria lancifolia* growth 10 months after different concentrations of the dispersant JD-2000 (0-16,000 ppm from left to right) were applied to the soil.

Photosynthetic rate

Plant photosynthetic rate (Fig. 2) was impacted at high dispersant dosages four days after the treatment even though no visual symptoms were observed (Fig. 1a). Four days after the treatment, plant photosynthetic rate was significantly lower than the control at a dispersant dosage of 16,000 ppm. Plant photosynthetic rate was suppressed even more seven days after the treatment, with a significantly lower photosynthetic rate at dispersant dosages ≥ 4000 ppm. However, photosynthetic rates of *Sagittaria lancifolia* at dispersant dosages ≤ 8000 ppm were not significantly different from the control three months after the treatment. We measured photosynthetic rate only on healthy leaves because existing old leaves were senescent and new leaves emerged. Seven months after the treatment, photosynthetic rates in all treatments were not significantly different from each other. But we should note that for the 16,000-ppm dosage, the photosynthetic measurements were taken on the seedlings generated from seeds in the next spring because two out of three previously existing plants were killed at this dispersant dosage.

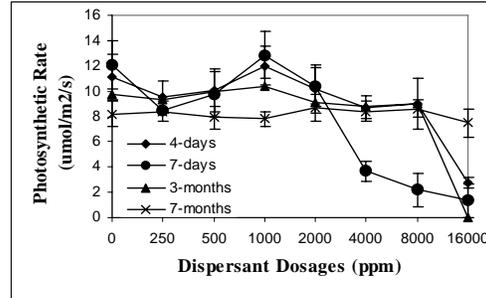


Fig. 2. Dose-response relationship between photosynthetic rates of *Sagittaria lancifolia* and different dosages of the dispersant JD-2000 in the soil at different times after the treatment.

Mortality

Dispersant application increased plant mortality rate at high dosages. No obvious visual symptoms of impacts occurred 96 hours (four days) after the treatment, with 0% mortality for all dispersant dosages (Fig. 3). The mortality rate increased with increasing dispersant concentrations 10 days after the treatment. However, dispersant concentration ≤ 2000 ppm did not affect the plants, with no significantly greater mortality rate than the control. The mortality rate significantly increased at 16,000-ppm dosage two months after the treatment. However, the mortality rate was not significantly different at dispersant concentrations ≤ 8000 ppm. Six months after the treatment, mortality rates were not significantly different among all dispersant treatments and the control since new shoots grew up in the growing season during the following spring. As mentioned above, plant seedlings resulting from seed germination

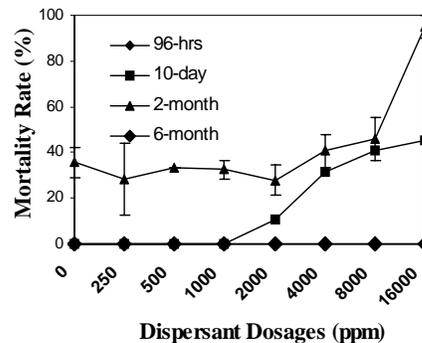


Fig. 3. Dose-response relationship between leaf mortality rate of *Sagittaria lancifolia* and different dosages of the dispersant JD-2000 in the soil at different times after the treatment.

grew at the 16,000-ppm dispersant level. This indicates that the 16,000-ppm dispersant-concentration was below the plant tolerance limits in the latter months of the experiment.

Biomass

Plant aboveground biomass was harvested in the next growing season 10 months after the treatment. Plant biomass at all dispersant concentrations was not significantly different among treatments (Fig. 4), indicating that the initial effect of the dispersant at high concentrations had disappeared. The dispersant did not have long-term effects on plant growth.

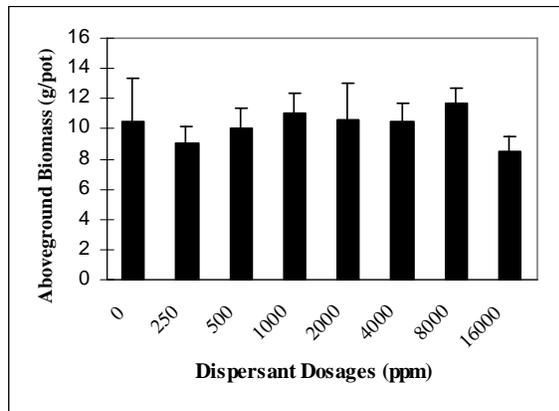


Fig. 4. Dose-response relationship between the aboveground biomass of *Sagittaria lancifolia* and different dosages of the dispersant JD-2000 in the soil 10 months after the treatment.

3.2. Task 2 of Phase 1. Dose-Response Relationship Between Dispersed Oils Applied to the Soil and Growth of the Marsh Plant, *Sagittaria lancifolia*

The effect of dispersed oil on marsh plants differed with oil types (Figs. 5a-f). The fresh marsh plant *Sagittaria lancifolia* did not show obvious symptoms throughout the nine month experimental period after the dispersed South Louisiana crude oil was applied to the soil at different dosages (Fig. 5a, 5c, and 5e). However, the dispersed diesel harmed the plant at high dosages in the soil (Figs. 5b, 5d, and 5f).



Fig. 5a. *Sagittaria lancifolia* growth two weeks after different concentrations of the JD-2000 dispersed SLC (0-145,800 ppm from left to right) were applied to the soil. The ratio of the dispersant to oil is 1:20.



Fig. 5b. *Sagittaria lancifolia* growth two weeks after different concentrations of the JD-2000 dispersed diesel (0-145,800 ppm from left to right) were applied to the soil. The ratio of the dispersant to oil is 1:20.

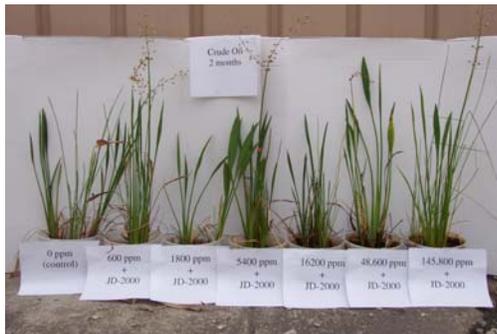


Fig. 5c. *Sagittaria lancifolia* growth two months after different concentrations of the JD-2000 dispersed SLC (0-145,800 ppm from left to right) were applied to the soil. The ratio of the dispersant to oil is 1:20.



Fig. 5d. *Sagittaria lancifolia* growth two months after different concentrations of the JD-2000 dispersed diesel (0-145,800 ppm from left to right) were applied to the soil. The ratio of the dispersant to oil is 1:20.



Fig. 5e. *Sagittaria lancifolia* growth nine months after different concentrations of the JD-2000 dispersed SLC (0-145,800 ppm from left to right) were applied to the soil. The ratio of the dispersant to oil is 1:20.



Fig. 5f. *Sagittaria lancifolia* growth nine months after different concentrations of the JD-2000 dispersed diesel (0-145,800 ppm from left to right) were applied to the soil. The ratio of the dispersant to oil is 1:20.

Photosynthetic Rates and Leaf Expansion Rate

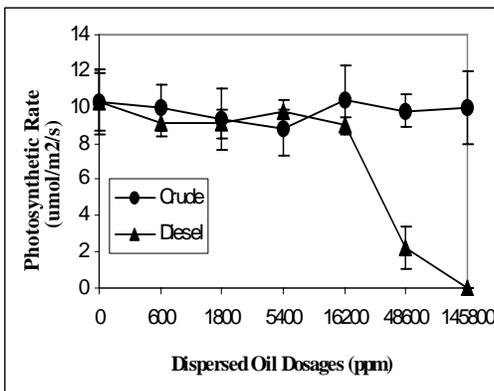


Fig. 6a. Photosynthetic rates of *Sagittaria lancifolia* three weeks after different concentrations of the JD-2000 dispersed oil were applied the soil.

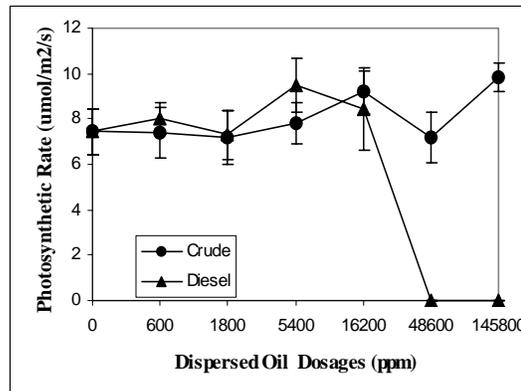


Fig. 6b. Photosynthetic rates of *Sagittaria lancifolia* six months after different concentrations of the JD-2000 dispersed oil were applied the soil.

The effects of the dispersed diesel and dispersed crude on the photosynthetic rates of the marsh plants were very different. Chemically dispersed diesel had a much greater toxicity than the dispersed South Louisiana crude oil. The photosynthetic rates of *S. lancifolia* were lower at high dosages of dispersed diesel. Three weeks (Fig. 6a) and six months (Fig. 6b) after the treatment, the photosynthetic rate of *S. lancifolia* was significantly lower than the control at dosages $\geq 48,000$ ppm of the dispersed diesel. However, the dispersed crude did not significantly affect the photosynthetic rate of *S. lancifolia* (Figs. 6a and 6b).

Mortality rate

Plant mortality rate, the ratio of dead leaf number to total leaf number, indicated that chemically dispersed diesel applied to the substrate at dosages $\geq 16,200$ ppm harmed *Sagittaria lancifolia* (Figs. 7a-c). The plant was completely dead at oil dosages $\geq 48,600$ ppm of dispersed diesel. The LC_{50} of dispersed diesel was estimated at 24,000 ppm of dispersed diesel for three weeks and 20,000 ppm for six weeks. Unlike dispersed diesel fuel, dispersed crude oil did not harm *S. lancifolia* even at the oil dosage of 145,800 ppm.

Aboveground biomass

High dosages of dispersed diesel applied to the soil substrate significantly impacted plant biomass even eight months after the treatment, with no live aboveground biomass in dosages of the dispersed diesel $\geq 48,600$ ppm (Fig. 8). However, dispersed diesel in dosages $\leq 16,200$ ppm did not significantly affect the aboveground biomass compared to the control. Dispersed South Louisiana crude oil did not significantly affect the plant biomass even at the highest dosage of 145,800 ppm in the present study (Fig. 8).

The dose-response results suggest that oil dispersed with “new generation” dispersants has a relatively low toxicity to marsh plants. Thus these dispersants may have considerable potential as oil spill countermeasures in nearshore and coastal marsh environments.

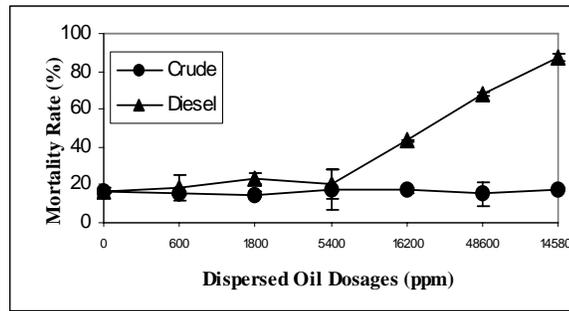


Fig. 7a. Dose-response relationship between leaf mortality rates of *Sagittaria lancifolia* and different dosages of the JD-2000 dispersed oil in the soil three weeks after the treatment.

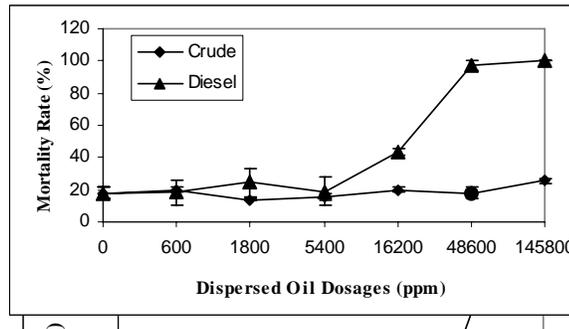


Fig. 7b. Dose-response relationship between leaf mortality rates of *Sagittaria lancifolia* and different dosages of the JD-2000 dispersed oil in the soil six weeks after the treatment.

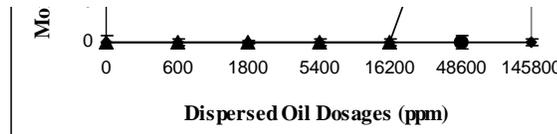


Fig. 7c. Dose-response relationship between leaf mortality rates of *Sagittaria lancifolia* and different dosages of the JD-2000 dispersed oil in the soil 6 months after the treatment.

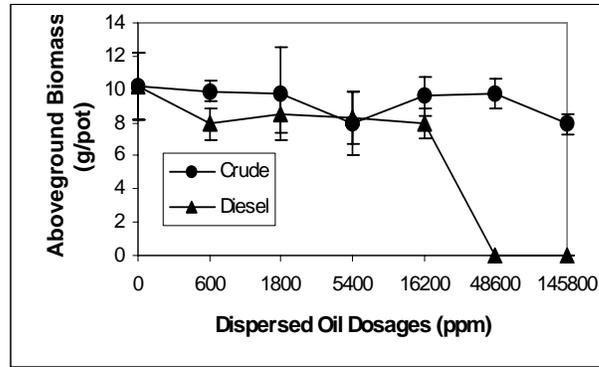


Fig. 8. Dose-response relationship between aboveground biomass of *Sagittaria lancifolia* and different dosages of the JD-2000 dispersed oil in the soil seven months after the treatment.

3.3. Phase 2. Effect of Oil Type, Concentration and Dispersant Application on Plant Responses and Oil Remediation

Photosynthetic Rate and Stomatal Resistance

Dispersant application simulation in the nearshore environment greatly reduced the detrimental effect of oil on marsh plants (Figs. 9a and 9b). Oil with and without the dispersant JD-2000 at both low and high dosages came in contact with the aboveground stems and leaves



Fig. 9a. Effect of oil type and dispersant application on intact marsh sods dominated by *Sagittaria lancifolia* two weeks after a 2000 ppm dispersed oil treatment. From left to right: control, 2000 ppm diesel, 2000 ppm diesel + dispersant JD-2000, 2000 ppm SLC, and 2000 ppm SLC + dispersant JD-2000. The ratio of dispersant to oil was 1:20.



Fig. 9b. Effect of oil type and dispersant application on intact marsh sods dominated by *Sagittaria lancifolia* two weeks after a 50 ppm oil treatment. From left to right: control, 50 ppm diesel, 50 ppm diesel + dispersant JD-2000, 50 ppm SLC, and 50 ppm SLC + dispersant JD-2000. The ratio of dispersant to oil was 1:20.

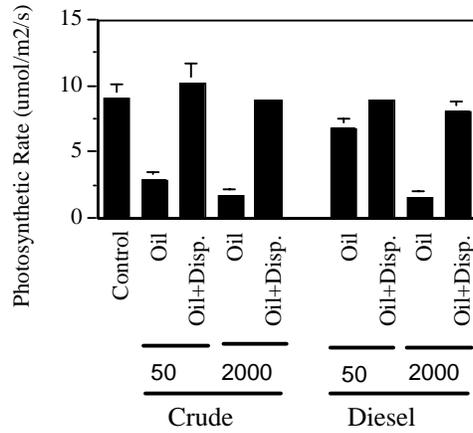


Fig. 10. Effect of dispersant, oil type and dosage on photosynthetic rate of *Sagittaria lancifolia* three weeks after the treatment. Control: neither oil nor dispersant; Oil: oil only; Oil+Disp.: Dispersant JD-2000 applied to oil; 50: 50 ppm of crude or diesel; 2000: 2000 ppm of oils.

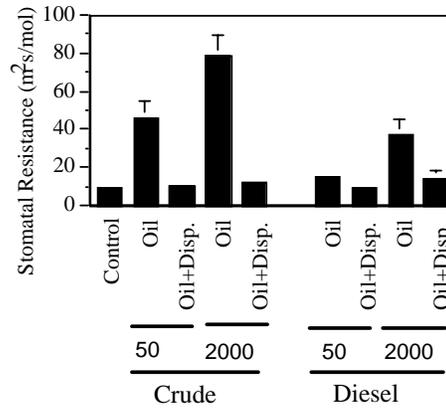


Fig. 11. Effect of dispersant, oil type and dosage on stomatal resistance of *Sagittaria lancifolia* 3 weeks after the treatment. Control: neither oil nor dispersant; Oil: oil only; Oil+Disp.: Dispersant JD-2000 applied to oil; 50: 50 ppm of crude or diesel; 2000: 2000 ppm of oils.

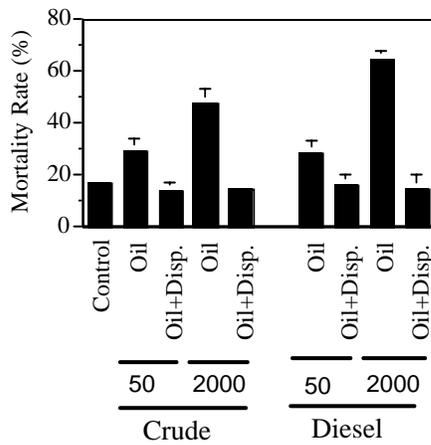


Fig. 12. Effect of dispersant, oil type and dosage on mortality rate of *Sagittaria lancifolia* three weeks after the treatment. Control: neither oil nor dispersant; Oil: oil only; Oil+Disp.: Dispersant JD-2000 applied to oil; 50: 50 ppm of crude or diesel; 2000: 2000 ppm of oils.

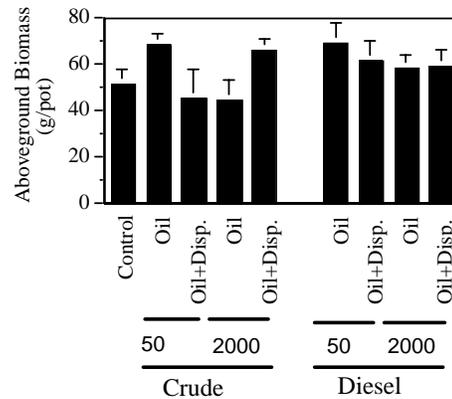


Fig. 13. Effect of dispersant, oil type and dosage on total aboveground biomass for all plant species seven months after the treatment. Control: neither oil nor dispersant; Oil: oil only; Oil+Disp.: Dispersant JD-2000 applied to oil; 50: 50 ppm of crude or diesel; 2000: 2000 ppm of oil.

during the high tide. Oil, especially diesel fuel, without the applied dispersant substantially impacted the plants (Figs. 9a and 9b). Photosynthetic rates of *S. lancifolia* (Fig. 10) were significantly lower in the treatment receiving oil without the dispersant than in the control. Without dispersant, the 2000 ppm oil (high dosage) had a greater adverse effect on photosynthetic rates than the 50 ppm oil (low dosage), although the low oil dosage significantly affected photosynthetic rates compared to the control (Fig. 10). However, dispersant application greatly relieved the adverse effect of oil on photosynthetic rates. Photosynthetic rates of *S. lancifolia* in the treatments receiving oil and dispersant (the dispersed oils), regardless of oil type and concentration, were not significantly different from the control.

It appears that the effect of crude oil on *Sagittaria lancifolia* is mostly caused by smothering the leaf surface and blocking gas exchange through stomata, whereas the effect of diesel was mostly caused by tissue damage of light oil components when no dispersant was applied. Visually, we can observe the differential effects of the two oil types: diesel-coated leaves turned brown and dried in less than one week, and crude oil coated leaves stayed green with a shiny oil film on the leaf surface for more than three weeks. In addition, results of stomatal resistance (Fig. 11) further confirmed the greater smothering effect of crude oil compared to diesel. Stomatal resistance, which provides a measurement of water transpiration resistance through the leaf surface, was significantly higher for *S. lancifolia* receiving the crude oil coating than the diesel coating, indicating physical stomatal block by oil. However, application of dispersant greatly alleviated the effect of the oil blocking stomata and significantly reduced the stomatal resistance. Six months after treatment, the plant photosynthetic rate was not significantly different between oil with dispersant and without dispersant, oil type, and oil concentration (date not shown). After six months, all live plant leaves were newly regrown and used for photosynthesis measurement; the oil coated leaves turned to senescence and were dead before the following spring and could not be used for photosynthesis measurement.

Plant Mortality Rate and Plant Biomass

Dispersant application greatly reduced oil impact on plants. The plant mortality rate was significantly higher in the treatment receiving oil alone than in the treatments receiving dispersed oils, especially for the high oil dosage (Fig. 12). Leaf mortality rates of *Sagittaria lancifolia* in the treatment receiving 2000 ppm of crude or diesel without dispersant were significantly higher than the treatments receiving 50 ppm of the oil. The mortality rates in the treatments receiving 50 ppm of crude or diesel without dispersant were significantly higher than the control. However, mortality rates in the treatment receiving crude or diesel with the dispersant were not significantly different from the control. This suggests that dispersants can relieve the immediate impact of oil on plant aboveground components.

Plant biomass harvested in the next growing season seven months after the treatment was not significantly different among the treatments (Fig. 13). The aboveground biomass was newly re-grown from rhizomes and was used for aboveground biomass analysis; the oil coated leaves senesced and died before the next spring, and were removed before the next growing season to better quantify the long-term effects.

Effect of Dispersant on Adsorption of Oil to the Marsh Sediment

Dispersant application greatly reduces oil adsorption to marsh sediment. TPH concentrations of the treatments receiving oils without dispersant application, ranging from 96 to 189 mg/g dry soil for the high oil dosage, were significantly higher than those with dispersant application and others (Fig. 14). However, TPH concentrations of treatments with dispersant applied to the high oil dosages were about 10% of the same oil dosage without the dispersant. Total C10 to C32 petroleum components

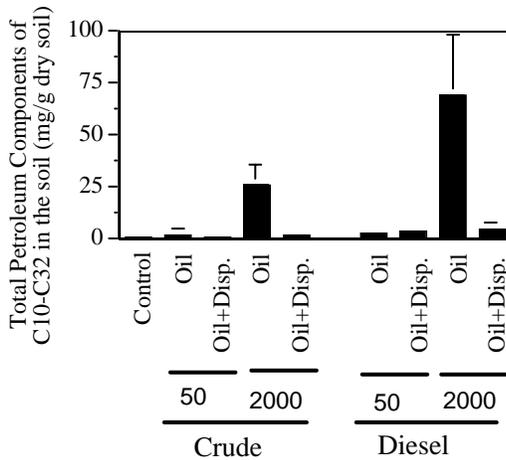


Fig. 15. Effect of dispersant, oil type and dosage on the C10-C32 petroleum components in the soil at 0-1.5 cm soil depth one week after the treatment. Control: neither oil nor dispersant; Oil: oil only; Oil+Disp.: Dispersant JD-2000 applied to oil; 50: 50 ppm of crude or diesel; 2000: 2000 ppm of oil.

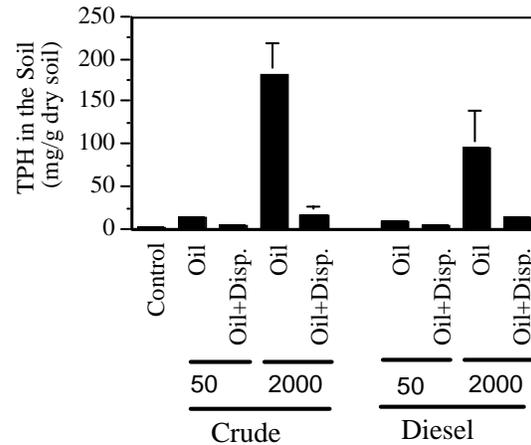


Fig. 14. Effect of dispersant, oil type and dosage on total petroleum hydrocarbon (TPH) concentration in the soil at 0-1.5 cm soil depth one week after the treatment. Control: neither oil nor dispersant; Oil: oil only; Oil+Disp.: Dispersant JD-2000 applied to oil; 50: 50 ppm of crude or diesel; 2000: 2000 ppm of oil.

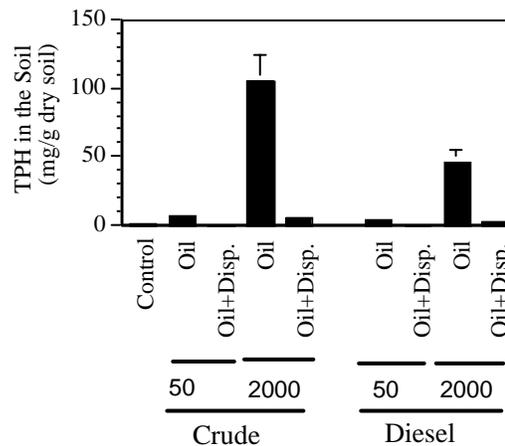


Fig. 16. Effect of dispersant, oil type and dosage on total petroleum hydrocarbon (TPH) concentration in the soil at 0-1.5 cm soil depth 7 months after the treatment. Control: neither oil nor dispersant; Oil: oil only; Oil+Disp.: Dispersant JD-2000 applied to oil; 50: 50 ppm of crude or diesel; 2000: 2000 ppm of oil.

(analyzed with GC/FID) in the treatments receiving oils without dispersant were significantly higher than those with dispersant application and others (Fig. 15). Oil concentrations of C10 – C32 were 26 and 69 mg/g dry soil at the high oil dosage for crude and diesel, respectively. The lighter hydrocarbon components (e.g. C10-C32, in diesel) are likely responsible for the greater oil penetration of leaf stomata and high toxicity to the plant. Seven months after the treatment, TPH concentrations of the treatments receiving oils without dispersant application, ranging from 42 to 102 mg/g for the high oil dosage, were still significantly higher than the concentrations in treatments with the same oil and dispersant application (Fig. 16).

4.0. Discussion

The current study indicated that plant tolerance to the dispersant JD-2000 was relatively high. The marsh plant *Sagittaria lancifolia* was not impacted by the dispersant JD-2000 at dosages $\leq 4,000$ ppm based on plant photosynthetic rate and plant mortality rate. *Sagittaria lancifolia* was able to recover at dispersant dosages as high as 16,000 ppm as the toxicity of the dispersant decreased with time. Better understanding the toxicity of dispersants to plants is important for more effective use of dispersants as oil spill countermeasures for habitat protection in the event of an oil spill. The current study demonstrated for the first time that the tolerance limits of plants to the dispersant JD-2000 was much greater than tolerances for the EPA standard bioassay organisms. The National Contingency Plan (EPA 2001) states that the LC_{50} (96-hr) for *Menidia beryllina* is 407 ppm and LC_{50} (48-hr) for *Mysidopsis bahia* is 90.5 ppm for the dispersant JD-2000. Gulf misid, the common name for the small shrimp species *Mysidopsis bahia*, is used as the test organism in a U.S. EPA bioassay test protocol. However, the present study indicated that LC_{50} for the fresh marsh plant, *Sagittaria lancifolia*, was greater than 4,000 ppm; this is more than 10 times higher than the test marine organism's LC_{50} .

Similar to the response of plants to dispersant alone, plant tolerance to the chemically dispersed oil was fairly high. The marsh plant *Sagittaria lancifolia* was not impacted by the JD-2000 dispersed diesel at dosages $\leq 16,200$ ppm based on plant photosynthetic rate and plant mortality rate. In addition, the JD-2000 dispersed South Louisiana crude oil at a dosage as high as 145,800 ppm, the highest test dosage in the present study, did not significantly impact *Sagittaria lancifolia*. The tolerance limits of this marsh plant to dispersed oil found in the current study were much greater than for marine organisms reported in other studies. The National Contingency Plan (EPA 2001) states that the LC_{50} (96-hr) for *Menidia beryllina* is 3.59 ppm and LC_{50} (48-hr) for *Mysidopsis bahia* is 2.19 ppm for the JD-2000 dispersed No. 2 fuel. The toxicity of No. 2 fuel oil is similar to that of diesel fuel. Thus, tolerance limits of the marsh plant appear to be up to one thousand times higher than the EPA test organisms. The LC_{50} (96-hr) of the Corexit-9527 and Corexit-9500 dispersed crude oils was 8.1 and 3.6 ppm in a shrimp (*Palaemon serenus*) bioassay and 28.5 and 14.1 ppm in the fish (*Macquaria novemaculeata*) larval bioassay (Gulec and Holdway 2000). Therefore, marine organisms seem to be more sensitive to dispersed oil than marsh plants.

In the present experiment that simulated dispersant applied in nearshore and estuarine environments, the dispersant greatly relieved oil coating impact on aboveground components. Without dispersant application, both diesel and crude oil severely impacted *Sagittaria lancifolia*. Dispersant application significantly reduced the oil's impact on the plant, with high photosynthetic rate and low mortality rate. Duke et al. (2000) reported that experimental application of dispersant Corexit-9527 to the weathered crude oil reduced mangrove tree mortality in a field trial. The

strategy of the present study is to apply dispersants in the nearshore before oil moves into coastal marshes. The present study differs from a handful of previous studies (Bake 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 dispersant and 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). Dispersant may increase oil availability to organisms (Wolfe et al. 1998, 2000) because dispersed oil is functionally soluble in water. In a confined area with limited water exchange, the availability of dispersed oil appears to be more profound. In contrast, other studies (Smith et al. 1984; DeLaune et al. 1984) demonstrated that dispersants applied to Louisiana crude oil contaminated *Spartina alterniflora* provided short-term benefits to plant photosynthesis although they did not have long-term effects on plant biomass. Therefore, the strategy of applying dispersants in nearshore environments before oil comes in contact with coastal marshes appears to have great potential for habitat protection.

The principal biological benefit of dispersant use is the prevention of oil from stranding in sensitive habitats (NRC 1989), such as wetlands. The present study clearly demonstrated that dispersant application to oil reduced the oil adsorption to the marsh sediment, with significantly lower oil concentration in the sediment than occurred without dispersant for both South Louisiana crude oil and diesel fuel. In a simulated oil spill in a nearshore surf-zone, Page et al. (2000) reported that no oil adsorbed to sediments in the chemically-dispersed oil treatment. This was compared to approximately 49% of the applied oil adsorbed to sediment or other surfaces for the treatment of crude without dispersant. 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 strategy of the present study is to apply dispersants in the nearshore before oil transports into the coastal marshes. Thus, dispersed oil may move into marshes with tides or currents, but, most dispersed oil appears to move out with the ebbing tide because dispersed oil does not adhere to the surface of sediment particles or plant aboveground components.

5.0. Conclusion

The current study elucidates the potential and effectiveness of dispersants as oil spill countermeasures for habitat protection and oil remediation in nearshore environments. The dose-response relationship of the fresh marsh plant *Sagittaria lancifolia* to the dispersant JD-2000 indicated that the plant tolerance to the dispersant was relatively high, more than 10 times higher than the EPA bioassay marine organism's response. In addition, the plant tolerance to the dispersed oil was relatively high; the JD-2000 dispersed South Louisiana crude oil applied to the soil did not detrimentally affect *S. lancifolia* even at an oil dosage of 145,800 ppm. By contrast, the JD-2000 dispersed diesel applied to the soil substrate did harm the marsh plant, *Sagittaria lancifolia*, at 16,200 ppm. The LC_{50} (6 weeks) of dispersed diesel was estimated at 20,000 ppm. It appeared that the toxicity of dispersed oil primarily resulted from the oil itself because the proportion of dispersant to oil was very low. Furthermore, dispersants greatly reduce oil impact

on vegetation and oil adsorption in habitats if oil is dispersed in nearshore environments before the oil comes in contact with a marsh. The dispersant greatly relieved the adverse effect of both diesel and crude oil coating the aboveground vegetation. Application of the dispersant significantly reduced oil adsorption to the marsh sediment. Therefore, dispersant application greatly reduced oil impact on fresh marsh vegetation and sediment, indicating the potential of using dispersants as alternative countermeasures for oil spills in nearshore environments.

6.0. References

- Baker, J.M., and Cruthers, J.H., Little, D.I., Oldham, J.H., Wilson, C.M. 1984. Comparison of the fate and ecological effect of dispersed and non-dispersed oil in a variety of marine habitats. In: Allen, T.E. (ed.), *Oil Chemical Dispersants: Research, Experience, and Recommendations*, STP 840. Philadelphia: ASTM. pp. 239-279.
- Cunningham, M. J., Sahatjian, A.K., Meyers, C. Yoshioka, A.G., Jordan, M.J. Use of dispersants in the United States: Perception or reality? Proceeding of the 1991 Oil Spill Conference, American Petroleum Institute, Washington, DC, pp 389-393.
- DeLaune, R.D., Jugsujinda, A., Pezeshki, S.R. 1998. Evaluation of habitat sensitivity to oiling: Effectiveness and impact of cleaner in removing oil from fresh water habitat, *Sagittaria lancifolia*. Louisiana Oil Spill Coordinator's Office/Office of the Governor, Louisiana Applied Oil Spill Research and Development Program, 97-004. 31 pp.
- Duke, N.C., Burns, K.A., Swennell, R.P., Dalhaus, O., Rupp, R.J. 2000. Dispersant use and a bioremediation strategy as alternate means of reducing impacts of large oil spills on mangroves: The Gladstone field trials. *Marine Pollution Bulletin*. 41:403-412.
- EPA. 2001. Guide to using the NCP product schedule notebook. 158 pp.
- Epstein, N., Bak, R.P.M. and Rinkevich, B., 2000. Toxicity of third generation dispersants and dispersed Egyptian crude oil on Red Sea coral larvae. *Marine Pollution Bulletin*. 40(6): 497-503.
- George A.A. and Clark, J.R., 2000. Aquatic toxicity of two Corexit(R) dispersants. *Chemosphere*. 40(8): 897-906.
- Gulec, I. and Holdway, A. D., 2000. Toxicity of crude oil and dispersed crude oil to ghost shrimp *Palaemon serenus* and larvae of Australian bass *Macquaria novemaculeata*. *Environmental Toxicology*. 15(2): 91-98.
- Guyomarch, J., Kerfourn, O, Merlino, F.X. 1999. Dispersants and demulsifiers: Studies in laboratory, harbor, and polludrom. Proceedings of the 1999 Oil Spill Conference, American Petroleum Institute: Washington, DC, pp.195-202.
- Lane, P.A. Vandermeulen, J.H., Crowell, M.J., Herche, L.R. 1987. Impact of experimentally dispersed crude oil on vegetation in a northwestern Atlantic salt marsh-preliminary observations. Proceedings of the 1987 Oil Spill Conference, American Petroleum Institute: Washington, DC, pp. 509-514.
- Little, I.D. Scales, D.L, 1987. Effectiveness of a type III dispersant on low energy shoreline, Proceedings of the 1987 Oil Spill Conference, American Petroleum Institute: Washington, DC, pp. 263-268.
- National Research Council (NCR) 1989. Using oil spill dispersants on the sea. National Academy Press, Washington, DC. 335 pp.
- Olagbende, O.T.; Ede, G.O.; Inyang, L.E.D.; Gundlach, E.R.; Gilfillan, E.S.; Page, D.S. 1999. Scientific and cleanup response to the IDOHO-QIT oil spill, Nigeria. *Environmental-Technology*. 20:1213-1222.
- Page, C.A. Bonner, J.S., Sumner, P.L., McDonald, T.J., Autenrieth, R.L., Fuller, C.B. 2000. Behavior of a chemically-dispersed oil and a whole oil on a near-shore environment. *Water Research*. 34(9): 2507-2516.
- Rhoton, L.S., Perkins, A.R., Richter, D.Z., Behr-Andres, C., Lindstrom, E.J. Toxicity of dispersants and dispersed oil to an Alaska marine. Proceedings of the 1999 Oil Spill Conference, American Petroleum Institute: Washington, DC.

- RRT-6-OCS. 1996. Pre-Approved Dispersant Use Manual, Version 2, EPA Region 6.
- Singer, M.M., George, S., Jacobson, S., Lee, I., Tjeerdema S.R., Sowby, L.M. 1994. Comparative effects of oil dispersants to the early life stages of topsmelt (*Atherinops affinis*) and kelp (*Macrocystis pyrifera*). *Environmental Toxicology and Chemistry*. 13(4): 649-655.
- Smith, C.J., DeLaune, R.D., Patrick, W.H. Jr., Fleeger, J.W. 1984. Impact of dispersed and undispersed oil entering a Gulf Coast salt marsh. *Environ. Toxic. and Chem.* 3:609-616.
- Venosa, A.D., Sorial, T.L., Uraizee, F., Richardson, T.L., Suidan, M.T. 1999. Research leading to revision in EPA's dispersant effectiveness protocol. Proceedings of the 1999 Oil Spill Conference, American Petroleum Institute: Washington, DC, 1019-1022.
- Wolfe, M.F., Schlosser, J.A., Schwartz, G.J.B., Singaram, S., Mielbrecht, E.E., Tjeerdema, R.S., Sowby, M.L. 1998. Influence of dispersants on the bioavailability of naphthalene from the water-accommodated fraction crude oil to the golden-brown algae, *Isochrysis galbana*. *Archives of Environmental Contamination and Toxicology*. 35(2): 274-280.
- Wolfe, M.F., Schwartz, G.J.B., Singaram, S., Mielbrecht, E.E., Tjeerdema, R.S., Sowby, M.L. 2000. Influence of dispersants on the bioavailability and trophic transfer of phenanthrene to algae and rotifers. *Aquatic Toxicology Amsterdam*. 48(1): 13-24.
- Wolfe, M.F., Schwartz, G.J.B., Singaram, S., Mielbrecht, E.E., Tjeerdema, R.S., Sowby, M.L. 2001. Influence of dispersants on the bioavailability and trophic transfer of petroleum hydrocarbons to larval topsmelt (*Atherinops affinis*). *Aquatic Toxicology Amsterdam*. 52(1): 49-60.

Summary

Oil spills in nearshore environments, or in wetland creeks, can move into marshes by tides and winds, strand inside marshes, and impact marsh organisms. One strategy might be to apply dispersants onto the spilled oil before the oil drifts into marshes. Dispersants have received considerable attention for open water oil spills. However, little information is available on the toxicity and remediation of chemically dispersed oil, especially in wetland environments. The objectives of the research are to evaluate the potential of using dispersants in nearshore and marsh environments by comparing and determining the toxicity and oil remediation of 'new generation' dispersants and dispersed oils on different marshes with different oil types, concentrations, dispersant type and spill scenarios. The first year experiment was conducted with fresh marsh sods. For the dose-response study during the first year, results of plant photosynthetic rate, plant growth and plant mortality after 3 and 6 weeks indicated chemical dispersed diesel applied to the soil substrate detrimentally affected *Sagittaria lancifolia* at 16,200 ppm. The plant was completely killed at 48,600 ppm and higher. LC₅₀ of dispersed diesel was estimated at 24,000 ppm for 3 weeks and 20,000 ppm for 6 weeks. Dispersed crude oil did not detrimentally affect *S. lancifolia* even at an oil dosage of 145,800 ppm. For the experiment simulating oil dispersed before entering a marsh, the dispersant (JD-2000) greatly relieved the adverse effect of both diesel and crude oil coating the aboveground vegetation. Without the dispersant, both diesel and crude oil significantly decreased photosynthetic rate, and increased mortality even at a 50 ppm dosage. Two thousand ppm of diesel without dispersant resulted in > 60% mortality. In contrast, the dispersed oils did not significantly affect *S. lancifolia* compared to the no-oil control based on photosynthetic rate and plant mortality. In addition, dispersant application significantly reduced oil adsorption to the marsh sediment, with only about 10% of the oil concentration for the treatment with the dispersant compared to without dispersant at 2000 ppm concentration. Therefore, dispersant application greatly reduced oil impact on fresh marsh vegetation and sediment. During the second year, toxicity and effects of dispersed diesel and crude oil on plant response, microbial activity, and oil remediation in salt marsh sods will be evaluated. In the second year, Corexit 9500, marketed as a salt water dispersant, will be included in addition to JD-2000. Generally, salt marshes are more vulnerable to nearshore spills because of their location within the coastal zone. Furthermore, the salt marsh plant, *Spartina alterniflora*, which is the dominate marsh species along the Gulf of Mexico and Atlantic coasts, is likely more sensitive than *Sagittaria* to oil coating because of the grass leaf surface structure of the former. Whether dispersants can relieve the impact of oils on *Spartina* marshes appears to be challenge. The experimental procedure, duration, and variables measured on plants, soil microbes, and oil chemistry will be similar to the first year experiment. The proposed project is closely related to the OSRADP's priority areas of fate and effects of dispersants on fresh, brackish and salt tolerant vegetation. The results from the proposed project will have widespread practical use in Louisiana, where the risk of oil spills in coastal marshes are high.

