EVALUATION OF TOLERANCE LIMITS FOR RESTORATION AND PHYTOREMEDIATION WITH *SPARTINA ALTERNIFLORA* IN CRUDE OIL-CONTAMINATED COASTAL SALT MARSHES

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ABSTRACT

Information and knowledge about the oil tolerance of native coastal plants are limited, but are essential for restoring and remediating oil impacted habitats. Spartina alterniflora is the dominant native salt marsh plant species along the Northern Gulf of Mexico and the Atlantic coast; the presence of this species is important to sustain healthy coastal salt marshes. The crude oil tolerance limits of S. alterniflora and its capacity to phytoremediate South Louisiana crude (SLC) oil in marsh sediments were investigated in a greenhouse. Spartina alterniflora was transplanted into marsh sediments contaminated with SLC at concentrations of 0, 40, 80, 160, 320, 640 and 800 mg/g. Plant photosynthetic rate, stem density, shoot height, aboveground biomass and belowground biomass were analyzed periodically during a one-year plant growth cycle to determine the oil tolerance limits of S. alterniflora. Most plant parameters were significantly lower at 320 and 640 mg/g of SLC oil in the soil compared to the control. No plants survived at the 800 mg/g SLC oil dosage. The tolerance limits of Spartina alterniflora were estimated between 320 mg/g and 640 mg/g of SLC oil in the soil. Furthermore, phytoremediation with S. alterniflora enhanced oil degradation; residual concentrations of total petroleum hydrocarbons in the phytoremediation treatment with S. alterniflora were significantly lower than those in oiled, unvegetated sediments for both the surface and subsurface soil at the 40 and 160 mg/g oil dosages one year after treatment. The current study shows the promise of using coastal marsh vegetation for restoring crude oil-impacted coastal wetlands and enhancing oil spill cleanup.

INTRODUCTION

Risks of oil spills in Coastal zones are relatively high because of intensive petroleum production, transportation and refining in this area. Oil spills both in the coastal marshes and on the sea, which may move onshore and come in contact with the shoreline, can cause substantial damage to sensitive habitats, such as coastal salt marshes. A number of studies (Alexander and Webb, 1985; Mendelssohn *et al*., 1990; Lin and Mendelssohn, 1996, 1998; Lin *et al*., 1999; Pezeshki *et al*., 2000; Lin *et al*., 2002) have investigated the effect of petroleum hydrocarbons on coastal wetland plants. Others have investigated the response options, attempting to draw a clear line between those actually effective and those not (Hoff, 1995). Futhermore, planting seedlings to accelerate Spartina restoration in heavily impacted marshes areas is becoming more and more a recognised practice (Bergen et al., 2000)

However, to our knowledge, data documenting the oil tolerance limits of plants rarely exist. Lin *et al.* (2002) determined the dose-response relationship of *Spartina alterniflora* to *No.* 2 fuel oil in a 3-month short-term experiment and found that this species was relatively tolerant to *No.* 2 fuel oil. Oil tolerance limits are especially important if we are to use plants in phytoremediation attempts to restore and clean up oil contaminated habitats. However, the successful use of phytoremediation depends, in part, on identifying the petroleum hydrocarbon concentration that will allow successful transplant establishment into the contaminated soil.

Wetlands generally are in remote locations that are relatively inaccessible to heavy machines, and these habitats are highly sensitive to physical disturbance (Lin *et al*. 1999). Phytoremediation, the use of vegetation for the *in-situ* treatment of contaminated soil and sediment, may be especially useful for wetland environments because it provides a less intrusive approach than conventional mechanical clean-up methods. A number of studies demonstrated the potential of phytoremediation for the cleanup of petroleum hydrocarbons (Reilley *et al*., 1996; Lin and Mendelssohn, 1998; Lin *et al*., 1999b; Liste and Alexander, 2000; Harvey et al., 2002; Widdowson *et al*., 2005; White *et al*., 2006). We have previously utilized *S. alterniflora* for phytoremediating weathered crude oil in sediments (Lin and Mendelssohn, 1998). However, the information regarding the effectiveness of phytoremediation with wetland species is generally lacking, but is essential for successful remediation of oil-impacted habitats.

The objectives of this study were to determine (1) the tolerance limits of *Spartina alterniflora* to crude oil in salt marsh sediments for potentially restoring oil-contaminated habitats, and (2) evaluate the effectiveness of phytoremediation by *S. alterniflora* transplants on oil degradation in wetland environments. Coastal salt marshes are generally more vulnerable to oil spills due to their location within the coastal zone. *Spartina alterniflora* is a dominant species along the Northern Gulf of Mexico and Atlantic coasts, and often forms a mono-species salt marsh community. Determination of its oil tolerance limits and phytoremediation effectiveness has potentially widespread practical application.

MATERIALS AND METHODS

Experimental procedures

The sediment was collected from a salt marsh in Cocodrie, Louisiana, USA. After storage in the dark at ambient temperature for a month, the sediment was homogenized with a cement mixer. The original plant rhizomes and roots were removed during mixing. South Louisiana crude oil, artificially weathered by 25% (v/v), was applied to the homogenized salt marsh sediments and mixed thoroughly with a cement mixer at concentrations of 0, 40, 80, 160, 320, 640 and 800 mg g^{-1} dry soil. Each treatment of mixed sediment-oil was then transferred to a bucket (23 cm tall and 23 cm in diameter), with each treatment replicated three times. Eight (8) culms (shoots with intact roots, free of soil) of *Spartina alterniflora* were transplanted into each bucket. In addition, treatments receiving oil concentrations of 0, (no oil) 40 and 160 mg g^{-1} dry soil, but without transplants, were also included in the experiment to determine the role of phytoremediation by *S. alterniflora* for oil cleanup effectiveness. The experiment was conducted in a greenhouse for a one-year plant growth cycle. The water table in the experimental units was maintained under a simulated diurnal tidal regime, with 12 hours of high tide having water level 4 cm above the soil surface and 12 hours of low tide with water level 15 cm below the soil surface. A slow-release fertilizer (Osmocote 14-14-14, Scotts-Sierra Horticultural Products) was applied 5 times during the experiment, resulting in a total of 500 kg ha^{-1} of N, 218 kg ha^{-1} of P, and 415 ha^{-1} of K (Lin *et al*., 1999b).

Analyses of plant responses

Stem density of *S. alterniflora* was measured by directly counting the number of stems in each experimental unit. Canopy height of *S. alterniflora* was measured with a ruler in each experimental unit to the nearest 1 cm. Plant community photosynthetic rates of *S. alterniflora* were measured by using a portable infrared photosynthesis system (CID CI-301PS) and a large transparent chamber. The soil surface was covered with a 4-cm water layer during measurement. 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 s were calculated based on the area of the soil surface, expressed as μmol $CO_2/m^2/s$. Aboveground biomass of *S. alterniflora* was harvested at the end of the experiment 12-months after the transplantation, separated into live and dead components, and dried at 65ºC until a constant weight was achieved. Belowground biomass of *S. alterniflora* at 0-8 cm and 8-16 cm soil depths was separated and determined at the end of the experiment by washing off soil from the roots and rhizomes and drying at 65ºC to a constant weight.

Residual total petroleum hydrocarbons (TPH) in the soil

At the termination of the experiment, the 0-2 cm surface and 10-12 cm subsurface soils were sampled, extracted with dichloromethane (DCM), and analyzed gravimetrically. The DCM extracts were transferred to pre-weighed dishes, the DCM evaporated, and the un-evaporated oil remaining in the dishes were weighed to the nearest 0.0001 g. The TPH concentration was calculated and expressed as mg g^{-1} dry soil (Lin and Mendelssohn, 1996).

Statistical analysis

Statistical analysis was conducted with the Statistical Analysis System (SAS 9.1.3). Treatment effects were analyzed with general linear models (Proc GLM). Duncan's multiple range tests were used to evaluate statistical differences among individual treatment levels. Significant differences are reported at the 0.05 probability level, unless otherwise stated. In addition, the relationship between plant growth variables and oil dosages was calculated with SAS regression models (Proc REG).

RESULTS

Initial short-term effect of crude oil in the soil on *Spartina alterniflora*

The growth responses of *Spartina alterniflora* transplants depended upon concentrations of crude oil in the sediment. Four month after transplantation, numerous new shoots grew from the belowground rhizomes, especially in the control and low oil dosages. However, dead shoots from the initial transplant shock and original transplant senescence in the winter 4 months after transplantation made it hard to document the effects of oil on new growing plants. New growing plants indicate the marsh regeneration capability, which is important for sustaining healthy wetlands. Thus we clipped off all shoots of the original transplants for better documenting new growing plants. We analyzed various non-destructive plant variables, including stem density, shoot height, and photosynthetic rate. The crude oil in the sediment had significant ($p<0.0005$) effects on stem density of new growing plants 4 months after transplantation (Fig. 1A); stem density of *S. alterniflora* was significantly lower at crude oil dosages ≥ 320 mg/g, indicating that high concentrations of crude oil in the soil inhibited new shoot production from their belowground rhizomes. At the 800 mg/g oil dosage, no original transplants survived, and no new shoots grew, thus we do not include data of the 800 mg/g dosage in the figure. Four months after transplantation, stem densities (SD) of new growing plants were negatively correlated to crude oil dosage (X) ($SD_{4m} = -0.02632X + 24.5$, $R^2=0.66$, $p < 0.0001$). The $EC_{50\text{-}4m}$ value for stem density was estimated at 475 mg g^{-1} dry soil.

Crude oil in the sediment restrained shoot height of new growing plants. Canopy height of *S. alterniflora* 4 month after transplantation was significantly ($p<0.0001$) lower in crude oil dosages ≥ 80 mg/g compared to the control, and decreased with increasing crude oil dosages (Fig. 1B). Canopy height (CH) was negatively correlated with the crude oil dosages (X) ($CH_{4m} = -0.0556X + 49.8$, $r^2=0.79$, $p< 0.0001$). The $EC_{50\text{-}4m}$ value for canopy height was estimated at 410 mg g^{-1} dry soil.

Shorter shoots and fewer stems might result in less aboveground biomass. However, we did not want to harvest the new growing shoots for biomass analysis 4 months after transplantation because we needed to analyze the long-term effect of oil on the plants. The community photosynthetic rate, measuring the plant net photosynthetic rate in a given area of soil surface, provides no-destructive estimation of live aboveground biomass. The crude oil in the sediment significantly ($p<0.0001$) affected on community photosynthetic rate of new growing plants 4 months after transplantation (Fig. 1C); the community photosynthetic rate at oil dosages ≥ 80 mg/g was significantly lower than the control. Community photosynthetic rate (CPS) was negatively correlated with the crude oil dosages (X) ($CPS_{4m} = -0.0174X + 13.1$, $r^2=0.62$, $p< 0.0001$). The $EC_{50\text{-}4m}$ value for the community photosynthetic rate was estimated at 235 mg g^{-1}.

Effect of crude oil in the soil on *Spartina alterniflora* 8 months after transplantation

Eight months after transplantation, non-destructive variables were analyzed to determine the mid-term effect of oil on the marsh plants. The crude oil in the sediment had no significant ($p=0.102$) effect on stem density of *S. alterniflora* 8 months after transplantation (Fig. 2A). No new stems emerged at the 800 mg/g dosage, indicating the 800 mg/g dosage completely killed the plants. However, regression still indicated that the stem density (SD_{8m})

decreased with increasing oil dosages (SD_{8m} = -0.029X + 54.4; r^2=0.38. p<0.01). In addition, oil significantly (p<0.0005) affected plant shoot height; canopy height of *S. alterniflora* at oil dosages ≥ 160 mg/g were significantly lower than the control (Fig. 2B). Regression between canopy height (CH_{8m}) and oil dosages (X) indicated that the shoot height decreased with increasing oil dosages (CH_{8m} = -0.05X + 72.7; r^2=0.74. p<0.0001). Community photosynthetic rate further indicated that oil still stressed the plants 8 months after transplantation. Community photosynthetic rates at oil dosages ≥ 160 mg/g were significantly (p<0.0001) lower than the control (Fig. 2C). Regression also indicated that community photosynthetic rate (CPS_{8m}) decreased with increasing oil dosages (X) (CPS_{8m} = -0.05X + 72.7; r^2=0.74. p<0.0001).

Effect of crude oil in the soil on *Spartina alterniflora* one year after transplantation

One year after transplantation, the difference in the measured variables between oil treatments and the control diminished. Stem densities were not significantly (p=0.3743) different among all oil dosages ranging from 0 to 640 mg/g (data not shown). Twelve months after transplantation, we harvested the plants for analyzing above- and below-ground biomass. Aboveground biomass of *S. alterniflora* was significantly lower at oil dosages ≥ 320 mg/g than the control (Fig. 3A). Aboveground biomass (AB) was negatively correlated with crude oil dosages (X) (AB = -0.055X + 187, r^2=0.55, p < 0.001).

The effect of crude oil in the soil on belowground biomass of *S. alterniflora* was more severe (Fig. 4) compared to the aboveground component. We separated belowground biomass into 2 depth ranges, 0-8 cm (surface) and 8-16 cm (subsurface). Few roots and rhizomes were able to penetrate into subsurface sediment at high crude oil dosages (Fig. 3B); the belowground biomass at the 8-16 cm depth at oil dosages ≥ 80 mg/g was significantly (p<0.0001) lower than the control, and consistently decreased with increasing crude oil dosages. The subsurface belowground biomass (BB_{sub}) was negatively correlated with crude oil concentrations in the sediment (X) (BB_{sub} = -0.069X + 40.2, R^2=0.66, p < 0.0001). The $EC_{50\text{-}12m}$ value for the subsurface belowground biomass was estimated at 234 mg g^{-1}. In addition, the belowground biomass of *S. alterniflora* at the 0-8 cm depth (surface soil) at oil dosages ≥ 320 mg/g was significantly (p<0.005) lower than the control. Belowground biomass at the surface soil (BB_{surf}) was negatively correlated with crude oil dosages (X) (BB_{surf} = -0.099X + 85, R^2=0.60, p < 0.0005). The total belowground biomass (combined biomass of the 0-8 cm and 8-16 cm) in crude oil dosages > 160 mg/g was significantly lower than the control (Fig. 4). Total belowground biomass (BB_{tot}) of *S. alterniflora* decreased with increasing crude oil dosages (X) (BB_{tot}= -0.169X + 125, R^2=0.68, p < 0.0001). The $EC_{50\text{-}12m}$ value for the total belowground biomass was estimated at 358 mg g^{-1}.

Effectiveness of phytoremediation with *Spartina alterniflora* on concentrations of residual oil in the soil

In this experiment, we also included treatments receiving 40 and 160 mg/g SLC oil dosages but without *S. alterniflora* transplants. By comparing the residual oil concentrations in the sediments with and without transplants, we could determine if phytoremediation by *S. alterniflora* enhanced SLC oil degradation. Phytoremediation by *S. alterniflora* reduced the residual oil concentration in the soil. One year after treatment-initiation, the concentration of residual total petroleum hydrocarbons (TPH) in the treatment with phytoremediation was significantly (p<0.0001) lower than that without transplants at both 0-2 cm soil-depth and 10-12 cm depth for the 40 mg/g oil dosage (Fig. 4A). In addition, the concentration of residual total petroleum hydrocarbons (TPH) in the treatment with phytoremediation was significantly (p<0.0005) lower than that without transplants at both 0-2 cm soil-depth and 10-12 cm depth for the 160 mg/g oil dosage (Fig. 4B).

DISCUSSION

High concentrations of oil in the soil detrimentally affected plants, especially in the initial transplantation period. Four months after transplantation, plants stem density, shoot height and community photosynthetic rate greatly decreased with increasing oil dosages. All plant parameters measured at high oil dosages were less than half of those of the control. No plants survived at the 800 mg/g crude oil dosage. The initially severe impact of the oil on the plants most likely resulted from the high oil concentrations in the soil, especially from those light hydrocarbon components. In the initial 4 months, the time was not long enough for considerable oil degradation. In addition, the oil was relatively fresh, with a high percentage of light hydrocarbon components. Generally, light hydrocarbon components are more toxic than heavy ones (Alexander and Webb, 1985; Lin and Mendelssohn, 1996; Lin et al., 2002). Lin et al (2002) demonstrated that *No.* 2 fuel oil, which is enriched with light hydrocarbon components, was highly toxic to *Spartina alterniflora*. However, toxicity of crude oil containing less light hydrocarbon components than fuel oil was relatively low to the same species (Lin and Mendelssohn, 1996). Therefore, high concentrations and relatively enriched light components most likely resulted in severe impact of the oil on the plants at the initial transplantation period.

Eight and 12 months after transplantation, plant stem density at high oil dosages kept up with that of the control. In addition, the difference in shoot height and community photosynthetic rate between high dosages and the control decreased at 8 months compared to the 4 months after transplantation. All these suggested that toxicity of oil would have decreased with time. Naturally microbial degradation takes place in the soil (Salminen *et al*., 2004). Moreover, gradually growing plant biomass with time may decrease additional oil concentration through phytoremediation process (Lin and Mendelssohn, 1998; Lin *et al*., 1999b; Liste and Alexander, 2000; Harvey *et al*., 2002; White *et al*., 2006). In general, light hydrocarbons not only possess a higher toxicity, but they also degrade more easily than heavy ones. Previous studies (Salminen *et al*., 2004; Rahman *et al*., 2003) demonstrated that the light hydrocarbon components were more readily degraded than the heavy ones, even under natural degradation conditions (Salminen *et al*., 2004). Considerable amounts of hydrocarbons (especially light hydrocarbon components) might have degraded in 8 to 12 months, thus leaving oil with a lower toxicity to the plants as compared to the initial period. Previous studies (Lin and Mendelssohn, 1996, 1998) suggested that toxicity of oil decreased with time. A relatively un-weathered crude oil initially inhibited growth of *S. alterniflora* at 8 L m^{-2} of SLC oil (Lin and Mendelssohn, 1996), but the same oil, weathered and naturally degraded in the sediment for more than two years, even stimulated growth of *S. alterniflora* transplanted into marsh soils with oil dosages occurred as high as 24 L m^{-2} or 400 mg g^{-1} dry soil compared the control (Lin and Mendelssohn, 1998).

The present study showed that plant belowground components were more severely stressed by oil compared to aboveground shoots. In the present study, aboveground biomass was significantly lower at oil dosages ≥ 320 mg/g than the control; belowground biomass at the 8-16 cm soil depth, however, was significantly lower at oil dosages ≥ 80 mg/g than the control. Few belowground roots and rhizomes at high oil dosages were able to penetrate into the subsurface soil. In a previous study (Lin and Mendelssohn, 2002) demonstrated a similar pattern of little belowground penetration in a *No.* 2 fuel oil-contaminated soil. Therefore, measurements of oil effects with biological end-points based solely on aboveground responses may underestimate the potential

impacts of petroleum hydrocarbon spills, especially when the oil has migrated below the soil surface.

A number of studies have indicated the potential of phytoremediation in reducing the concentrations of petroleum hydrocarbons (Reilley *et al*., 1996; Lin and Mendelssohn, 1998; Lin *et al*., 1999b; Liste and Alexander, 2000; Harvey et al., 2002; White *et al*., 2006). In the present study, phytoremediation with *S. alterniflora* significantly reduced the residual TPH in the sediments at the 40 and 160 mg/g crude oil dosages for both surface soil and subsurface soil as compared to the same oil dosages in the control treatments. The enhanced degradation might involve several mechanisms. For example, many wetland plants, such as *S. alterniflora*, are able to transport atmospheric oxygen to their rhizosphere. Oxygen is important for complete oil degradation, and aerobic oil degradation is generally more effective in degrading oil than anaerobic degradation (Hambrick *et al*., 1980; Salminen *et al*., 2004). In addition, *S. alterniflora* might produce root exudates that may promote microbial numbers and increase their activity in the rhizosphere, which in turn could enhance oil degradation (Hegde and Fletcher, 1996, Yoshitomi and Shann, 2001). Enzymes such as cytochrome P450, peroxidases and dehalogenases excreted by plant roots into their rhizophere might also enhance oil degradation (Harvey *et al*., 2002). In addition, the transplants might uptake and metabolize the hydrocarbons within their tissues, thus, reducing hydrocarbon concentrations in the soil. Our future research may identify these different mechanisms.

CONCLUSIONS

Oil concentrations in the soil are important factors controlling the successful transplantation of the coastal salt marsh plant, *Spartina alterniflora*, in oil-contaminated wetlands. High concentrations of SLC oil in the sediment significantly suppressed growth variables such as plant stem density, shoot height, community photosynthetic rate, aboveground biomass and belowground biomass. The tolerance limit of *S. alterniflora* to South Louisiana crude oil was estimated between 320 to 640 mg g^{-1}. Phytoremediation with *S. alterniflora* enhanced degradation of TPH in the sediments. One year after treatment initiation, concentrations of TPH in the treatment with phytoremediation by *S. alterniflora* were 61% and 46% of those of the un-vegetated sediment for the surface and subsurface soil, respectively, for the 40 mg/g crude oil dosage, and 66% and 70%, respectively, for the 160 mg/g oil dosage. The results of this study indicated the potential of simultaneous restoration and remediation of oil-contaminated wetlands by transplanting dominant native marsh plants, like *S. alterniflora*.

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BIOGRAPHY

Qianxin Lin is an assistant professor at Louisiana State University. His research primarily focuses on the effect and fate of oil spill in coastal wetland habitats, bioremediation, phytoremediation, *in-situ* burning, and dispersant application as a means of oil spill cleanup, remediation and habitat restoration.

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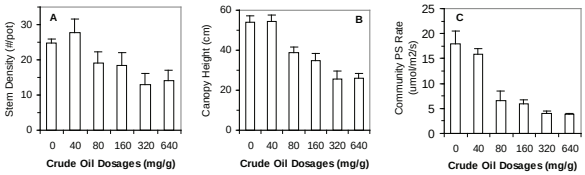

FIG. 1. EFFECT OF SOUTH LOUISIANA CRUDE OIL IN THE SEDIMENT ON LIVE STEM DENSITY (A), CANOPY HEIGHT (B) AND COMMUNITY PHOTOSYNTHETIC RATE (C) OF THE NEW GROWING *SPARTINA ALTERNIFLORA* 4 MONTHS AFTER TRANSPLANTATION. VALUES ARE MEANS WITH SE.

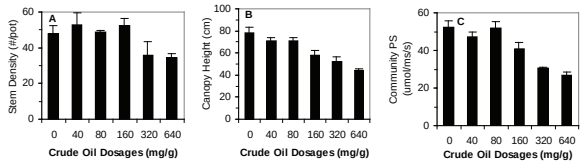

FIG. 2. EFFECT OF SOUTH LOUISIANA CRUDE OIL IN THE SOIL ON STEM DENSITY (A), CANOPY HEIGHT (B) AND COMMUNITY PHOTOSYNTHETIC RATE (C) OF *SPARTINA ALTERNIFLORA* 8 MONTHS AFTER TRANSPLANTATION. VALUES ARE MEANS WITH SE.

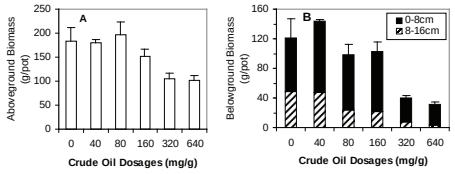

FIG. 3. EFFECT OF CRUDE OIL IN THE SOIL ON THE ABOVEGROUND (A) AND BELOWGROUND (B) BIOMASS OF *SPARTINA ALTERNIFLORA* 12 MONTHS AFTER TRANSPLANTATION. VALUES ARE MEANS WITH SE.

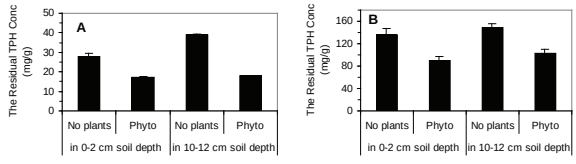

FIG. 4. EFFECT OF PHYTOREMEDIATION WITH *SPARTINA ALTERNIFLORA* ON RESIDUAL TOTAL PETROLEUM HYDROCARBONS (TPH) IN THE SOIL OF THE 40 MG/G (A) AND 160 MG/G (B) DOSAGES 12-MONTHS AFTER TREATMENT. VALUES ARE MEANS WITH SE.