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SURVIVAL AND GROWTH OF THE DOMINANT SALT MARSH GRASS *SPARTINA ALTERNIFLORA* IN AN OIL INDUSTRY SALINE WASTEWATER

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Saline oil produced water (PW) is the largest wastewater stream in the oil exploration and production processes. Although eventual disposal of PW into shallow coastal waters occurs nearby coastal wetlands, no studies regarding its toxicity to higher plants were found in our literature review. To fill this knowledge gap and evaluate the potential use of this halophyte for PW phytoremediation the salt marsh grass Spartina alterniflora was grown in five PW concentrations and no PW treatment control for seven weeks. The oil & grease, NaCl, and ammonium ($N\text{-}NH4^{+}$) concentrations in the PW were 120 mg L^{-1}, 30 g L^{-1}, and 381 mg L^{-1}, respectively. Plants grown in 30% PW and 10% PW achieved survival rates (75%) significantly higher than plants grown in 100% PW (35% survival). LT50 of S. alterniflora to raw PW with 120 mg L^{-1} of oil & grease (100% PW) was estimated at 30 days. Root and sprout biomass were significantly stimulated by PW; plants grown in 10% to 50% PW concentrations were 70–300% more productive than those in control, 80% PW and 100% PW, respectively. No significant inhibitory effects on survival or growth were detected for concentrations of PW less than 80% when compared to control. Our results pointed out that S. alterniflora grows in saline oil PW and its potential use to phytoremediate this effluent should be evaluated.

KEY WORDS produced water, halophyte, toxicity, phytoremediation

INTRODUCTION

A complex mixture of water co-produced with oil is called produced water (PW), formation water or oilfield brine. It contains variable concentrations of inorganic salts, trace elements, such as heavy metals and naturally occurring radionuclides, dispersed and dissolved hydrocarbons and chemicals added to the production system of a well (Stephenson, 1992; Oliveira and Oliveira, 2000; CAPP, 2001; Neff, 2002; OGP, 2005; Dórea *et al.*, 2007). PW is the largest wastewater stream in the oil exploration and production

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processes (Stephenson, 1992; Utvik, 1999; Sirivedhin and Dallbauman, 2004; Veil *et al.*, 2004; Al-Masri, 2006; Lu *et al.*, 2006) and large volumes are generated worldwide (Lin, 1999; Veil *et al.*, 2004; CAPP, 2001; Neff, 2002; OGP, 2005). The volume of PW brought to the surface depends on the extraction technology utilized, reservoir characteristics, and rate of oil extraction. At some sites, the amount of PW may be tenfold the quantity of oil produced (Stephenson, 1992; Campos *et al.*, 2002; OGP, 2005). Produced water must be removed before the crude oil can be refined (Neff, 2002) and after separation it is usually either injected into a well or discharged after treatment to surface waters (Lin, 1999; Utvik, 1999; CAPP, 2001; Neff *et al.*, 2006).

Many isolated compounds of PW are toxic to a number of organisms (Peterson *et al.*, 1996; Henderson *et al.*, 1999; Neff, 2002; OGP, 2002; Thomas *et al.*, 2004; Durell *et al.*, 2006; Tollefsen *et al.*, 2006; Lu *et al.*, 2006). Chronic and acute responses of marine organisms (fish, invertebrate larvae and mysids) exposed to PW have also been shown (Washburn, Stone, and MacIntyre, 1999; Stephens *et al.*, 2000; Raimondi and Boxshall, 2002; Elias-Samlalsingh and Agard, 2004). However, potential negative effects of PW disposal into near- and offshore waters are still controversial (Gray, 2002; Ekins, Vanner, and Firebrace, 2007).

Produced water PW may not represent a risk to offshore marine life and deleterious effects might be limited to the immediate vicinity of the point of discharge (Girling, 1989; Reed and Lewis, 1994; Strømgren *et al.*, 1995; Odeigah, Nurudeen, and Amund, 1997; Negri and Heyward, 2000; Gray, 2002; Holdway, 2002; Jerrez Vegueira, Godoy, and Miekely, 2002). Nevertheless, bioaccumulation and biomagnification may occur when PW is disposed into shallow coastal waters with low hydrodynamics (Neff, 2002); particularly, nearby coastal wetlands and other habitats vegetated by higher plants that can incorporate toxic compounds from the water column as well as the sediment. Thus, the toxicity to coastal wetland organisms must be known and efficient ways to treat PW must be developed in order to avoid any threat to coastal sites.

The treatment of PW usually comprises physical, chemical and biological steps and is one of the most costly procedures in the oil industry (Curtice and Dalrymple, 2004). An alternative method could be the utilization of artificial wetlands ("phytoremediation"), which are cost-saving compared to traditional methods (Knight, Kadlec, and Ohlendorf, 1999; Gratão *et al.*, 2005). The typically high salt content of PW poses a treatment problem, particularly for biological steps (Campos *et al.*, 2002). In order to develop a phytoremediation system which efficiently treats PW, salt-tolerant plants must be selected. Although the use of wetlands to reduce toxic loads of PW have been reported (Anonymous, 1997; Canning, 1998; Knight, Kadlec, and Ohlendorf, 1999; Myers *et al.*, 2001; Ji, Sun, and Ni, 2007), the plant species involved and their quantitative growth responses to PW exposure were not specified. Additionally, no studies regarding lethal toxicity of PW to salt marsh higher plants were found in our review of the literature.

Spartina alterniflora Loisel. (smooth cordgrass), a widely dispersed salt-tolerant plant of the American continent (Costa and Davy, 1992), has already been used for the restoration of salt marshes impacted by oil spills (Bergen *et al.*, 2000; Vitaliano *et al.*, 2002). Despite the fact that *S. alterniflora* responses to oil contamination have been investigated (DeLaune *et al.*, 1984; Lin and Mendelsohn, 1996; Lindau *et al.*, 1999; Smith and Proffitt, 1999; Pezeshki *et al.*, 2000; Pezeshki, DeLaune, and Jugsujinda, 2001; Lin *et al.*, 2002bb, 2002aa; Lindau, DeLaune, and Jugsujinda, 2003), knowledge about its viability to grow and potentially treat PW are still lacking. This study, therefore, aims to quantify the survival and growth capacity of *S. alterniflora* under different concentrations of saline oil PW in a hydroponics experiment. This analysis is essential to evaluate the potential use of this halophyte for PW phytoremediation.

MATERIALS AND METHODS

Experimental Design

Spartina alterniflora plants from a natural salt marsh in Rio Grande (RS, Southern Brazil, 32°02′36″ S; 52°04′09″ W) were collected within an 8 m^2 patch in order to reduce genetic variability. Tillers were individualized, planted in a mix of fine sand and commercial soil (1:1) in 0.3 m^2 plastic trays and acclimated in an unheated greenhouse for one month prior to experiments. After that, one hundred twenty healthy plants between 20–55 cm (37.5 ± 0.9 cm; mean ± standard error) were gently removed from the trays and individual culms (shoots with intact roots and rhizomes free of soil) were kept in a hydroponics system mounted in 3 L glass vessels. All dead leaves and sprouts were removed from the culms. The plants were fit in polystyrene buoys as used in fishing nets in a way that only the roots were submerged.

Saline oil PW was supplied by Almirante Soares Dutra Oil and Derivates Terminal ‘TEDUT’, Osório (RS, Brazil; Petrobrás—Petróleo Brasileiro S.A.). The PW was drained from a tank containing a mixture of four different crude oil types, with a density of 24.7° API. The oil & grease, NaCl, and ammonium (N-$NH4^+$) concentrations in the PW used were 120 mg L^{-1}, 30 g L^{-1} and 381 mg L^{-1}, respectively.

The glass vessels with individual culms were filled with 2.5 L of six distinct concentrations of PW (100%, 80%, 50%, 30%, 10%, and 0%) by adding distilled water. Estimated initial concentrations of total oil & grease, NaCl, and ammonium for each treatment are presented in Table 1. Each PW concentration treatment level was replicated 20 times. The no PW treatment level (control) was maintained with 10 g NaCl L^{-1} in order to guarantee optimal growth of *S. alterniflora* (between 5 and 30 g NaCl L^{-1}; Bradley and Morris, 1992; Cunha, Asmus, and Costa, 2005; USDA, 2000) and to allow comparisons between this control and saline PW treatment levels. The pH values for all treatments were always around 7.0–7.5. Once a week 20 ml of Hoagland standard nutrient solution (described by Bannister, 1976) were added to each replicate and the evaporated water was replaced by a similar volume of distilled water to maintain initial salinity. Half to full Hoagland solution is recommended to hydroponic toxicity tests with aquatic macrophytes (EPA, 1996; Doucette *et al.*, 2005; Armstrong *et al.*, 2008), however PW used had a high nitrogen concentration, element known to have significant positive effect on growth of *S. alterniflora* either in the form of ammonium or nitrate (Cavalieri, 1983; Bradley and Morris, 1992; Dai and Wiegert, 1997). Low concentration of Hoagland solution (about 1% per week) was designed to meet micronutrients’ requirements of *S. alterniflora* and control treatment (no PW) to be representative of dissolved macronutrients available in the interstitial water of non-eutrophied salt marsh soils (*e.g.*, dissolved nitrogen ranged between 0.14 and 7 mg L^{-1}, which is equivalent to 0.01–0.5 mM nitrogen; Jefferies, 1977; Bradley and Morris, 1992). Thus, the five remaining PW treatment levels were used to examine both toxicity and stimulation by the PW complex mixture.

After seven weeks, live and dead aerial shoots, roots, and new sprouts were separated, dried at 60°C for 72 hours and weighted (nearest 0.01 g). The daily averages of minimum and maximum temperatures inside the unheated greenhouse were 11.5 ± 0.5°C and 30.7 ± 1.1°C (mean ± standard error), respectively.

Table 1 Initial concentrations of Oil & Grease, NaCl, and ammonium estimated for each saline oil PW treatment

	Treatment					
	Control	10% PW	30% PW	50% PW	80% PW	100% PW
Oil & Grease ($mg\ L^{-1}$)	0	12	36	60	96	120
NaCl ($g\ L^{-1}$)	10	3	9	15	24	30
Ammonium ($mg\ N\text{-}NH4^{+}\ L^{-1}$)	0	38	114	191	305	381

Data Analysis

Survival. The survival curves of *S. alterniflora* plants were created using the survival rates for each treatment level at ten days intervals and fitting a 2nd order polynomial trend line to the data; all r^2 obtained were higher than 0.95. Survival was compared among treatment levels by the Chi-Square test, considering a 0.05 probability level (Kleinbaum, 1996). Since statistical significant differences among survivals were found, pair wise comparisons were made using the two-sample log-rank test at the 0.01 probability level.

Growth. Statistical differences in biomass of live and dead shoots, roots and new sprouts of *S. alterniflora* plants were tested among PW treatment levels through Analysis of Variance (ANOVA) and the *post hoc* Least Significant Difference (LSD) test was used when significant differences at the 0.05 probability level were found among PW treatments (Sokal and Rohlf, 1981). The assumption of normality and homogeneity of variances were tested by Kolmogorov-Smirnov and Levene's statistical tests (Zar, 1984), respectively. To achieve normality, all biomass data were log transformed.

RESULTS

Survival

The survival of *S. alterniflora* plants (Figure 1) was significantly affected by PW concentration (Chi-Square = 12.2; $p < 0.05$). After 30 days the survival curve of 100% PW plants was markedly different, reaching 50% survivors, whereas mortality was less than 20% in the other treatment levels. After seven weeks, pair wise comparisons by the log-rank test showed that the low survival of plants grown in 100% PW (35% survival) was significantly different ($p < 0.01$) from survival observed by those grown in 30% PW and 10% PW; both with 75% survival. The survival in the control (55%) was not significantly different from PW treatments. Despite the lack of significance, the 30% and 10% PW treatments presented survival rates 36% higher than the control, whilst the 100% PW treatment was 63% lower than the later one.

Growth

The average values of all biomass components of *S. alterniflora* plants grown in 100% PW were similar to those of plants grown in 80% PW. However, the reduced sample size

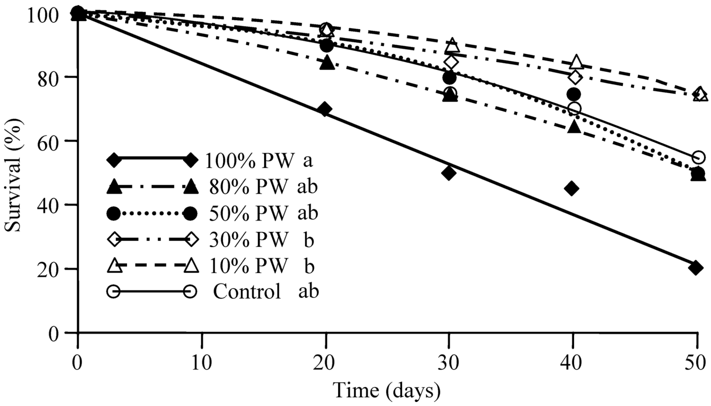

Figure 1 Survival curves comparing performance of *S. alterniflora* in six concentrations of oil produced water. Survival curves of the PW concentrations with the same letters are not significantly different.

in 100% PW (due to low survival), leading to a large mean variation, seems to explain the absence of statistical differences between 100% PW and treatment levels with medium/low concentrations of PW (50%, 30%, and 10%).

After seven weeks of cultivation, average live and dead shoot biomass did not show significant differences ($p > 0.05$) among PW treatment levels (Figure 2A, 2B). On the other hand, plants grown in low/medium concentrations of PW (10% to 50% PW) produced more root and sprout biomass than those grown in control and 80% to 100% PW (Figure 2C, 2D). Root biomass of 50% PW and 30% PW treatment levels were 160% and 180% significantly higher than 80% PW ($p < 0.05$), as well as the two former concentrations were 70% and 290% significantly higher than the control, respectively. Except for the control treatment level, the sprout biomass increased with decreasing PW concentrations. The average sprout biomass of 30% PW and 10% PW were, in this order, 300% and 280% significantly higher than the 80% PW treatment level, as well as 270% and 260% significantly higher than the control.

DISCUSSION

The smooth cordgrass *Spartina alterniflora* demonstrated to be highly tolerant to saline oil produced water exposure since no significant inhibitory effects on its survival or growth were detected in concentrations of PW less than 80%, when compared to plants grown in a control solution without PW with nutrient availability similar to non-eutrophied salt marsh soils. However, when exposed to raw PW (100% PW) with 120 mg L^{-1} of oil & grease half of the plants died within 30 days. This survivorship is indicative of the LC50 of *S. alterniflora* to PW; thus, the specific toxicity of the PW used. High mortality and the inhibition on the formation of sprouts and new roots observed in 80–100% PW are not likely to be the result of nutrient deficiencies because such deficiencies would be much more evident in 10–30% PW treated plants. No studies regarding PW lethal toxicity to higher plants were found in our review of the literature. Comparing this PW LC50 to reported median lethal and effect concentrations, *S. alterniflora* is more resistant to PW than most marine organisms (Neff, 2002). Part of this resistance is explained by the fact that stems and leaves of *S. alterniflora* had no direct contact with PW. Lin and Mendelssohn

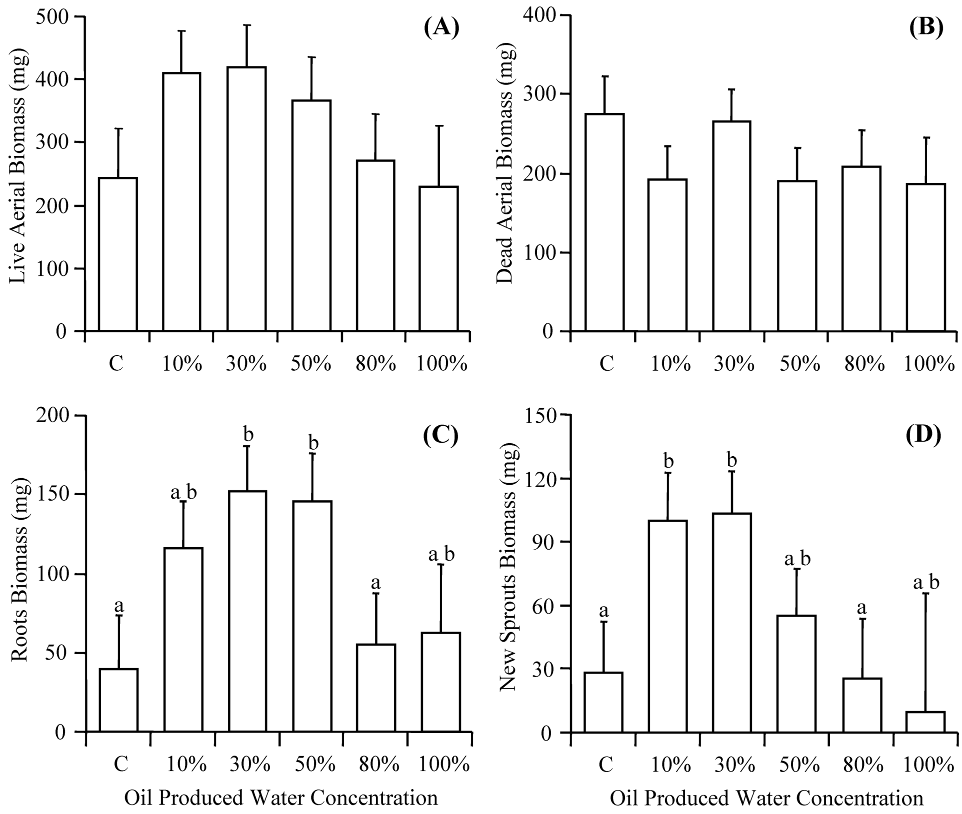

Figure 2 Effect of oil produced water concentration on the live (A) and dead (B) aerial biomass of original shoots, roots (C), and new sprouts (D) biomass of *S. alterniflora* after seven weeks of hydroponics cultivation. Values are means with standard errors. * No significant differences were found for live (A) and dead (B) aerial biomass. Means with the same letters are not significantly different.

(1998) demonstrated that both *S. alterniflora* and *S. patens* were very sensitive to oil coating their aerial parts, thus preventing gas exchange. Freire, Cammarota, and Sant' Anna Jr. (2001), Myers *et al.* (2001), and Neff (2002) emphasized that PW varies widely in chemical composition; hence one should be cautious when comparing results of toxicity between freshwater and marine organisms. For instance, the oil & grease content of the PW used in this study (120 mg L^{-1}) was intermediate among other studies, which ranged from 11 to 283 mg L^{-1} (Doran *et al.*, 1998; Utvik, 1999; Freire, Cammarota, and Sant' Anna Jr., 2001; Myers *et al.*, 2001; Campos *et al.*, 2002; Lu *et al.*, 2006). Although being different pollutants, PW and crude oil are products of the oil extraction process and thus contain many similar constituents. Lin and Mendelssohn (1996) reported lower mortality and growth sensitivity of *S. alterniflora* to South Louisiana crude oil than *Spartina patens*. In a greenhouse study, DeLaune *et al.* (2003) did not find mortality of *S. alterniflora* planted in soil with 2 L m^{-2} of South Louisiana crude oil and Arabian Medium crude oil but found a low survival rate for *S. patens* exposed one month to similar oil types and concentrations. Roughly converting, this 2 L m^{-2} oil concentration would represent about 6.7 mg of oil L^{-1} in the soil used by DeLaune *et al.* (2003), thus less oil than in the 10% PW treatment (Table 1) where *S. alterniflora* showed high survival.

Besides oil & grease, other toxic constituents are present in this effluent. Lead, chromium, and nickel are often among the most abundant heavy metals but their concentrations vary between individual wells (Henderson *et al.*, 1999; Holdway, 2002). However, previously, *S. alterniflora* showed to be highly tolerant to heavy metals, partly explained by its ability to immobilize these elements due to air transport through aerenchymatic tissue and rhyzosphere oxidation (Lacerda, Freixo, and Coelho, 1997) and also by absorbing them, as revised by Weis and Weis (2004). *S. alterniflora* tolerance to salinity, oil pollution and heavy metals can be particularly useful in dealing with salty oil spill sites and for the treatment of oil polluted effluents like PW. Such treatment systems, in addition to be cost-saving, could also provide economic return from the generated *Spartina* biomass (Qin, Xie, and Jiang, 1998).

Diluted PW stimulates *S. alterniflora* growth. One to three-fold growth increases of new sprouts and root biomass of *S. alterniflora* were observed in low PW concentrations (10% and 30% PW) compared to the control (without PW). Lower survival (although not significant) and less growth of *S. alterniflora* in control level than diluted PW (10–30%) may be related to nutrient deficiency (*e.g.*, dissolved nitrogen was kept around 0,48 mg N L^{-1} by weekly addition). Increased plant growth following the fertilization have been found in *Spartina* (Cavalieri, 1983; Dai & Wiegert, 1997) and other salt marsh plants (Jefferies, 1977; Drake & Ungar, 1989) above a minimum nitrogen level of 7–14 mg L^{-1}. Nitrogen is highly demanded by *S. alterniflora* (Smart & Barko, 1980) and high ammonium concentration of the PW used in the present experiment seems to be responsible for *S. alterniflora* growth stimulation. In other studies, nitrogen content in leaves and maximum photosynthetic rates of *S. alterniflora* were greatly enhanced after receiving ammonium-nitrate fertilizer leading to a markedly increase of plant growth (Mendelssohn, 1979; Howes, Dacey, and Goehringer, 1986; Dai and Wiegert, 1997). Lin *et al.* (2002b) also reported an enhancement of *S. alterniflora* growth at low oil concentration (7 mg g^{-1} dry soil; $\approx$1500 mg oil L^{-1} of wet soil) after three months of a greenhouse experiment where this species was transplanted into soil contaminated with distinct No.2 fuel oil dosages (0–456 mg oil g^{-1} dry soil). In this study, below ground biomass was 42% higher in the 1500 mg oil L^{-1} of wet soil than in the control treatment level. Recently, Ji, Sun, and Ni (2007) evaluating the efficiency of a surface flow constructed wetland with reed (unspecified species) for heavy oil PW treatment concluded that total plant biomass in wetlands receiving PW was 5% to 20% higher than for PW free wetlands. These authors concluded that the oil PW had mainly positive impacts on reed's health parameters. Growth enhancement of *S. alterniflora* by PW may also be related to microbe mediated processes. Salt tolerant bacteria (Bertrand *et al.*, 1990) and ectomycorrhizal fungi (Meharg and Cairney, 2000) are capable of degrading hydrocarbons, reclaiming soil structure and enhancing re-establishment of plants in oil spill sites.

Our results pointed out vegetative propagules of *S. alterniflora* are able to grow in saline oil PW. Additional research is required to evaluate if nutrient status may mediate PW toxicity in *S. alterniflora* and the potential use of this species for PW phytoremediation.

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