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Evidence for reduced post-spill recovery by the halophyte *Sporobolus iocladus* (Nees ex Trin.) Nees in oil-contaminated sediments

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Abstract

The germination behavior of *Sporobolus iocladus* seeds including germination percentage, accumulated germination percentage, the average incubation period to germination and germination velocity was studied under laboratory conditions. Treatments included six salinity regimes (0, 70, 140, 210, 280 and 350 mM NaCl) and three sources of oil hydrocarbons; Light Arabian Crude, polynuclear aromatic hydrocarbons (PAHs) including diaromatic or triaromatic hydrocarbons (in crude oil equivalent concentrations, COEC).

The average incubation period needed for seeds to germinate was significantly longer for seeds germinated in 350 mM NaCl (6 ± 1.16 days) compared with the control (4 ± 00 days). The accumulated germination percentage gradually decreased with increasing salinity (control: 90 ± 10, while 350 mM NaCl: 63 ± 8.8). Oil hydrocarbons significantly affected all germination parameters of *S. iocladus* seeds regardless of salinity levels. COEC of di- and triaromatic hydrocarbons suppressed seed germination more than crude oil. Seeds exposed to diaromatic hydrocarbons failed to germinate. Hydrocarbon's salinity interaction significantly reduced the number of germinated *S. iocladus* seeds.

It is concluded that hydrocarbon pollutants adversely affect *S. iocladus* through reducing germination. It is also suggested that the toxic effect of hydrocarbons on seeds is not solely mediated through their interaction with salinity. The ecological implications of these findings are discussed in relation to other studies on the post-spill recovery of halophytes. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: PAHs; NaCl; Halophytes; Naphthalene; Anthracene; Seed germination

1. Introduction

High salinity and oil pollution are two major factors threatening the already scarce natural vegetation coverage in most of the oil producing countries in the arid Arabian Gulf. Until recently, spraying crude oil around pipelines and oil exploration plants to stabilize sandy soils was a common practice. In addition, salinity represents a serious problem to the natural plant coverage in the region due to the low rainfall and high rates of evaporation.

Germination of halophyte seeds in arid regions, and the soil conditions to which seedlings and mature plants will be exposed are critical (Mohammed and Sen, 1990; Khan and Rizvi, 1994; Pujol et al., 2000). Despite the ability of halophyte seeds to maintain viability for long periods during exposure to saline conditions (Woodell, 1985; Mayer and Poljakoff-Mayber, 1989; Keiffer and Ungar, 1995), germination is generally negatively affected by increasing salinity (Woodell, 1985; Gehlot and Sen, 1996; Gul and Weber, 1999; Pujol et al., 2000).

Oil spills have both short- and long-term impacts on natural plant coverage (Mendelssohn et al., 1990). In oil-contaminated sediments, specific long-term recovery of many perennial halophytes depends on changes made to their reproductive ability, viability of the soil-stored seed bank, and rate of seedling recruitment after the spill. However, the effect of oil hydrocarbons on seed germination is poorly understood and rarely considered. Polychlorinated biphenyls (PCBs) were suggested to have an anesthetic effect on cell membranes, which enhance the imbibition process and consequently increasing the percentage of germination (Gobas et al., 1991; Ferasol et al., 1995). In contrast, the adverse effect of salinity appears to be aggravated by the presence of oil hydrocarbons. Page et al. (1985) suggested that toxic

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hydrocarbons might cause membrane damage leading to excessive salt accumulation in the plant tissue.

Sporobolus iocladus is a perennial halophyte widely distributed in many countries in Arabian Peninsula including Saudi Arabia (Migahid, 1974). In the United Arab Emirates, *S. iocladus* occurs in inland marshy areas of western and central regions of Abu Dhabi (Western, 1989; Böer and Gliddon, 1998). These areas endure intense activities associated with oil and gas production, and considerable quantities of oil are stored and transported through.

Until the present study, it was not known whether oil hydrocarbon contaminants affected salt tolerance in halophytes, including *S. iocladus*, at the stage of seed germination. The present study explores the effect of different forms of hydrocarbons including diaromatics, triaromatics and crude oil hydrocarbons on the germination *S. iocladus* seeds under saline conditions.

2. Materials and methods

Spikes with seeds from healthy population of *S. iocladus* were collected from the central area of Abu Dhabi in April 1999. Under laboratory conditions, seeds were removed from the spikes, thoroughly mixed, and stored in the dark in paper bags at room temperature (about 25°C) until use for germination tests. Seeds showed 100% germination in distilled water within the first 24 h after being surface sterilized using 50% ethyl alcohol and soaked for 1 h prior to the viability test. Floating seeds were considered non-viable and were discarded. Preliminary experiments showed no significant effect of light on germination and 25°C is the optimum temperature for germination. At this temperature, salinity of about 600 mM NaCl showed no germination response.

Three sources of hydrocarbons were used in the present study; Light Arabian Crude, naphthalene (diaromatic hydrocarbon), and anthracene (triaromatic hydrocarbon). The latter two treatments represent the most common classes of polynuclear aromatic hydrocarbons (PAHs) in the aqueous extract of crude oil. Individual PAHs were prepared in crude oil equivalent concentrations (COEC: the sum of concentrations of all diaromatics or triaromatics in the crude oil expressed as $\mu g\ ml^{-1}$ naphthalene or anthracene, respectively). Concentrations of 7 and 14 $\mu g\ ml^{-1}$ of naphthalene and anthracene, respectively, were dissolved in six different NaCl concentrations (0, 70, 140, 210, 280, and 350 mM NaCl). Similar concentrations were separately added to crude oil treatments. All solutions were kept refrigerated at 4°C until use.

A total of 720 seeds were used in this experiment; three replicates (10 seeds each) for six different salinities and three hydrocarbon treatments. Germination was carried out in $50 \times 9\ mm^2$ Pyrex petri dishes lined with filter papers with tight-fitting lids. Five ml of test solution were used from the stock solution for every plate. To prevent the physical coating of crude oil on seeds in the oil treatments, two filter papers were used for every plate, the first was saturated with 5 ml of crude oil and the second moistened with distilled water and placed over the first one. Five ml of each stock solution were then added to each petri dish. Seed germination was monitored every other day for two weeks and germinated seeds were removed from dishes after each counting. The germination rate was estimated using a modified Timson index of germination (Timson, 1965) as described in Pujol et al. (2000) using the following formula:

$$GV = \sum G/t,$$

where GV is the germination velocity, G is the percentage germination at 2-day intervals, and t is the total germination period.

Germination data were arcsine square transformed before statistical analysis. This transformation improved the normality of the distribution of the data and made group variance more homogenous. Data were then analyzed with one-way ANOVA using general linear module, GLM, of Systat 7.0 (Wilkinson, 1997). A two-way ANOVA was also used to demonstrate the effect of interaction between various salinities and oil hydrocarbons on germination percentage and velocity.

3. Results

Oil hydrocarbons negatively affected all germination parameters of *S. iocladus* seeds. Oil hydrocarbons significantly reduced the accumulated germination percentage ($F = 138.84$, $P < 0.001$), the average incubation period ($F = 697.152$, $P < 0.000$), and the rate of germination ($F = 180.822$, $P < 0.000$). Seeds of *S. iocladus* appear to be less sensitive to this mild salinity range than to hydrocarbons. Salinity ranging from 0 to 350 mM NaCl negatively affected the accumulated germination percentage ($F = 4.67$, $P < 0.01$) while the average incubation period and velocity of germination were not affected. Similarly, the interaction between sources of hydrocarbons and salinity showed no significant differences in these two germination parameters (average incubation period and velocity of germination). The accumulated germination percentage of successfully germinated seeds, however, was significantly affected by the interaction of hydrocarbons and salinity ($F = 2.58$, $P < 0.01$) (Table 1).

The accumulated percentage of germination was only significantly lower than the control (0 mM NaCl) when salinity increased in the absence of oil hydrocarbons to

Table 1
Results of ANOVA for the effect of hydrocarbons and NaCl concentration on the accumulated germination percentage, average incubation period and germination velocity in *S. iocladus* seeds

Source of variation	*df*	Ms	*F*-ratio	*P*
Accumulated germination percentage				
Salinity (*S*)	5	0.114	4.67	$< 0.01^{**}$
Oil hydrocarbon (*O*)	3	3.389	138.84	$< 0.001^{***}$
$S \times O$	15	0.063	2.58	$< 0.01^{**}$
Error	48	0.024		
Average incubation period				
Salinity (*S*)	5	0.069	1.274	ns
Oil hydrocarbon (*O*)	3	38.005	697.152	$< 0.000^{***}$
$S \times O$	15	0.058	1.057	ns
Error	46	0.055		
Germination velocity				
Salinity (*S*)	5	0.144	2.235	ns
Oil hydrocarbon (*O*)	3	11.632	180.822	$< 0.000^{***}$
$S \times O$	15	0.087	1.351	ns
Error	48	0.064		

ns: Not significantly different at $P > 0.05$.

350 mM NaCl (Table 2). There were no significant differences recorded between the control and other salinity levels. Fig. 1 shows that at any given salinity treatment the presence of hydrocarbons significantly reduced the germination percentage from that of the control. Both crude oil and triaromatic hydrocarbons (e.g. anthracene) were similar but less suppressive to germination than the diaromatic compounds (e.g. naphthalene) particularly at lower salinity range (0–140 mM NaCl) (Table 2). Seeds treated with naphthalene showed no germination at any salinity. At the higher salinity range (210–280 mM NaCl) accumulated germination percentage was significantly higher ($P < 0.0001$) in crude oil than in the triaromatics treatment (Fig. 1 and Table 2).

As shown in Tables 3 and 4, the average incubation period to break dormancy and germination velocity were only affected by oil hydrocarbons. Lower molecular weight PAHs (e.g. naphthalene) appear to be more suppressive to seed germination than higher molecular weights (e.g. anthracene).

4. Discussion

The success of a halophyte population in desert environment is greatly dependent on the germination responses of their seeds. Seed germination in halophytes usually occurs during a period when soil salinity is minimum. A great deal of variation however, exists in their responses to salinity (Khan and Ungar, 1996, 1997a,b,c; Gul and Weber, 1999). Maximum halophyte germination was reported to occur in distilled water or under reduced salinity (Khan and Ungar, 1984; Khan and Weber, 1986). Halophytes also vary in their upper limits of salt tolerance (Ungar, 1996). Increasing salinity may reduce both germination velocity (Khan and Ungar, 1984; Ungar, 1996) and accumulated seed germination or delay the initiation of the germination process (Philipupillai and Ungar, 1984; Keiffer and Ungar, 1995). Baskin and Baskin (1995) suggested that the mean NaCl concentration that reduces germination by 10% in halophytes is about 350 mM. Several halophytes, however, were reported to tolerate up to 1000 mM NaCl

Table 2
Effect of hydrocarbons and NaCl concentration on the accumulated germination percentage of *S. iocladus* seeds

NaCl (mM)	Hydrocarbons				Overall
	Control	Crude	Diaromatic	Triaromatic	
0	90 ± 10.0 aA	30 ± 5.8 aB	(NG) aC	50 ± 15.3 aB	43 ± 10.7
70	80 ± 10.0 abA	40 ± 10.0 aB	(NG) aC	53 ± 12.1 aB	43 ± 9.6
140	70 ± 5.8 abA	30 ± 5.8 aB	(NG) aC	27 ± 8.8 aB	32 ± 8.0
210	60 ± 0.0 abA	43 ± 6.7 aB	(NG) aC	17 ± 3.3 aD	30 ± 7.2
280	70 ± 10.0 abA	40 ± 5.8 aB	(NG) aC	7 ± 3.3 aD	29 ± 8.8
350	63 ± 8.8 bA	33 ± 6.7 aB	(NG) aC	10 ± 5.8 aD	27 ± 7.8
Overall	72 ± 3.7	36 ± 2.7	(NG)	27 ± 5.5	

NG: No germination. Values with similar small letters in columns or capital letters in rows are not significantly different at $P > 0.05$ (Tukey's test).

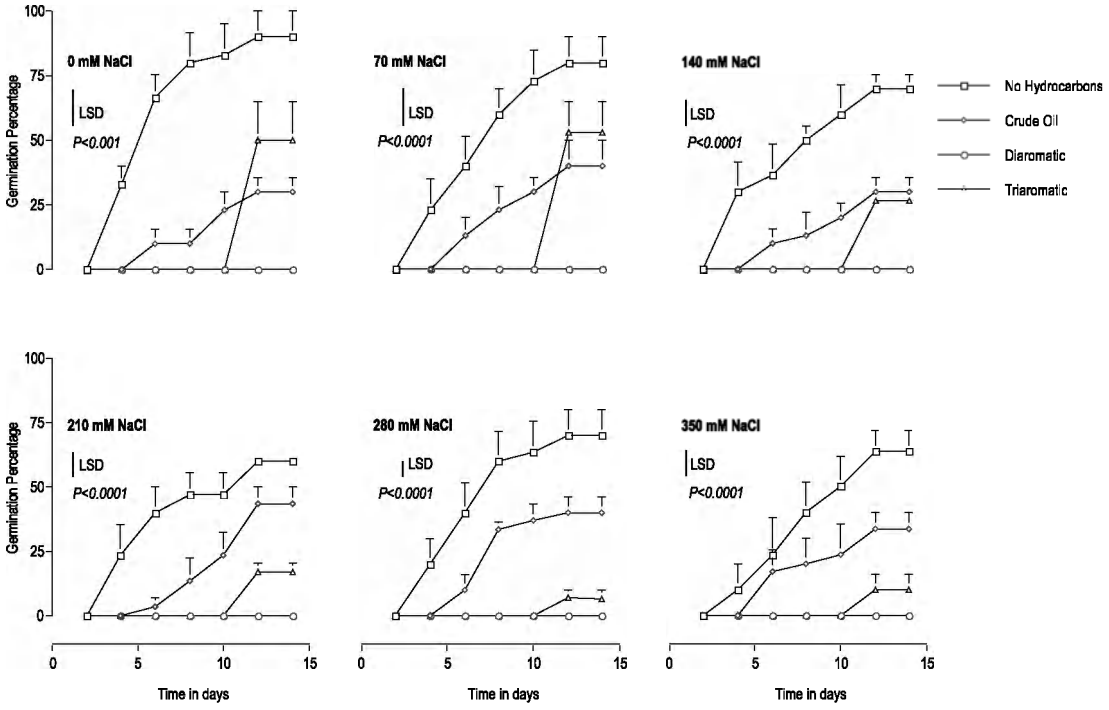

Fig. 1. Percentage germination of *S. iocladus* seeds in 0, 70, 140, 210, 280 and 350 mM NaCl and diaromatic, triaromatic and crude oil hydrocarbons. Error bars represent SE. Vertical lines represent fisher's least significant difference (LSD).

Table 3
Effect of hydrocarbons and NaCl concentration on the germination velocity of *S. iocladus* seeds

NaCl (mM)	Hydrocarbons				Overall
	Control	Crude	Diaromatic	Triaromatic	
0	5.2 ± 0.72 aA	1.0 ± 0.32 aB	NG aC	0.7 ± 0.24 abB	1.7 ± 0.63
70	4.0 ± 0.56 aA	1.4 ± 0.41 aB	NG aC	0.8 ± 0.19 aB	1.6 ± 0.47
140	3.7 ± 0.51 aA	1.0 ± 0.31 aB	NG aC	0.4 ± 0.14 abB	1.3 ± 0.45
210	3.2 ± 0.66 aA	1.1 ± 0.31 aB	NG aC	0.3 ± 0.05 abD	1.2 ± 0.41
280	3.6 ± 0.84 aA	1.6 ± 0.27 aB	NG aC	0.1 ± 0.05 bD	1.3 ± 0.48
350	2.6 ± 0.87 aA	1.3 ± 0.49 aA	NG aB	0.2 ± 0.09 abC	1.0 ± 0.38
Overall	3.7 ± 0.31	1.2 ± 0.13	NG	0.4 ± 0.09	

Values with similar small letters in columns or capital letters in rows are not significantly different at $P > 0.05$ (Tukey's test).

Table 4
Effect of hydrocarbons and NaCl concentration on the incubation period of *S. iocladus* seeds to first day of germination

NaCl (mM)	Hydrocarbons				Overall
	Control	Crude	Diaromatic	Triaromatic	
0	4.0 ± 0.00 aA	7.3 ± 1.33 aB	NG aC	12.7 ± 0.67 aD	6.0 ± 1.44
70	4.7 ± 0.67 abA	6.7 ± 0.67 aB	NG aC	12.0 ± 0.00 aD	5.8 ± 1.31
140	4.0 ± 0.00 aA	6.7 ± 0.67 ab	NG aC	12.7 ± 0.67 aD	5.8 ± 1.40
210	4.7 ± 0.67 abA	8.0 ± 1.16 aB	NG aC	12.7 ± 0.67 aD	6.3 ± 1.43
280	4.7 ± 0.67 abA	6.7 ± 0.67 aB	NG aC	12.0 ± 0.00 aD	4.7 ± 1.27
350	6.0 ± 1.16 bA	8.0 ± 2.00 aB	NG aC	12.0 ± 0.00 aD	6.0 ± 1.43
Overall	4.7 ± 0.28	6.9 ± 0.49	NG	12.4 ± 0.20	

Values with similar small letters in columns or capital letters in rows are not significantly different at $P > 0.05$ (Tukey's test).

during germination (Khan, 1991; Khan and Gul, 1998). In the present study, the accumulated germination percentage in *S. iocladus* was the most affected germination parameter with increasing salinity, showing an average reduction of 27% when salinity increased from 0 to 350 mM NaCl (Table 2). No clear changes were observed for

any of the other characteristics by increasing salinity within this range. Decreasing the accumulated percent of germination of halophytes is attributed by many authors to the development of osomatically enforced seed dormancy at high salinities (Ungar, 1996; Gul and Weber, 1998).

Oil hydrocarbons in all of the tested forms (di- or tri- or crude mixture) significantly delayed the onset of germination, slowed the germination velocity and reduced the accumulated number of successfully germinated *S. iocladus* seeds. Diaromatic hydrocarbons (e.g. naphthalene) appear to be toxic to the seed embryo as it completely suppressed the germination after successful imbibition in all salinities including the control. Oil hydrocarbon interaction with NaCl concentration range used in the present study significantly reduced the accumulated number of germinated *S. iocladus* seeds. For example, at 280 mM NaCl, seeds showed 70% germination in the absence of any hydrocarbons, which was reduced to 40%, 7%, and 0% in the presence of crude oil, triaromatic and diaromatic hydrocarbons, respectively. This would suggest that oil hydrocarbons might affect a number of processes of direct control of seeds germinability including the integrity of the plasma membranes. Several previous studies described the cytotoxic effect of aromatic hydrocarbons. Oil toxic components, benzene and formaldehyde, were reported to reduce both transmembrane sodium gradient and calcium contents in cells of the blue mussels *Mytilus edulis* (Borseth et al., 1995). Benzene is reported to increase membrane fluidity in *Rhodococcus* sp. leading to an increase in the ratio of saturated to unsaturated fatty acids (Gutierrez et al., 1999). Similarly, PCBs were suggested to react with the plasma membrane of many microorganisms leading to structural modification affecting membrane function as a selective barrier and as a matrix for enzymes (Sikkema et al., 1995; Wilke, 1997). In the present study, naphthalene, perhaps due to its lower molecular weight, is the most potent inhibitor to germination of all treatments. However, reductions in germination percentage in all oil treatments in the absence of NaCl prove that these hydrocarbons have a toxic effect on *S. iocladus* seeds, which is not solely mediated through their interaction with salinity.

Many authors have studied the effect of oil pollution on the performance of many marsh plants, particularly the coastal species *Juncus roemerianus* and *Sparina alterniflora* (Smith et al., 1984; Mendelssohn et al., 1990; Pezeshki and DeLaune, 1993). It was earlier suggested that effect of oil on these vegetations and the subsequent recovery depends on many factors including oil type and concentration and the time of the spill (Webb et al., 1981). Most of these studies, however, were mainly focusing on the short-term vegetative recovery of the plant. In inland salt desert environments, where hypersaline conditions occur frequently, seeds of many halophytes may remain dormant until the hypersaline condition is alleviated (Ungar, 1995). Under these circumstances seed banks may play an important role in determining the long-term recovery of the affected vegetation. It is thereby suggested that in studying post-spill recovery in perennial halophyte vegetations the repercussions on both reproductive ability of the species and viability of the soil-stored seed bank should be considered.

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