

Emergence and Height of Plants Seeded in Crude Oil Contaminated Soil

Jamie D. Spiares

Kevin E. Kenworthy

*Department of Agribusiness, Agronomy, Horticulture and Range Management,
Tarleton State University, Stephenville, TX 76402*

Robert L. Rhykerd

Department of Agriculture, Illinois State University, Normal, IL 61790

ABSTRACT

Accidental contamination of soils with petroleum hydrocarbons results from oil production and shipping. Growing plants in these soils may enhance the rate and extent of remediation of these soils reducing their potential to contaminate surface and ground water. The objective of this study was to identify plants that are native, naturalized, or that have been successfully introduced to north central Texas which will emerge and grow in crude oil contaminated soil. A greenhouse study was conducted in seed flats with the dimensions of 55 x 28 x 3.2 cm (L x W x H) containing 20 individual rows. Rows were filled with a Windthorst sandy clay loam soil amended with 0 (control), 0.5, 5, or 10% unweathered crude oil (soil dry weight basis). Soil moisture was maintained near – 30kPa. Nineteen plant varieties were seeded in separate rows at a rate of ten seeds per row. Treatments were conducted in triplicate in a completely randomized design. Emergence and plant height were measured on days 7, 14, 21, and 28. After 28 days emergence had decreased by 79%, 90%, and 98% in soils with 0.5%, 5%, and 10% respectively when compared to controls. Decreases in plant height were 72%, 86% and 96% in soils with 0.5%, 5% and 10% crude oil respectively. Plant species with the greatest emergence and plant height in soil with crude oil were Kenaf #2 (*Hisbiscus cannabinus* var. *tainvng* #2) and Kenaf #3 (*Hisbiscus cannabinus* var. *sf 459*), which were the only seedlings that emerged in the treatment with 10% crude oil. These two varieties are recommended for use on crude oil spills of 10% or less (soil dry weight basis) in north central Texas. Delaying seeding for a few days following a spill and tilling the contaminated soil may remove toxic volatile components of the crude oil from soil and improve seedling emergence, plant growth, and enhance phytoremediation.

KEYWORDS: Germination, Survival, Bioremediation, Phytoremediation, Petroleum Hydrocarbons

INTRODUCTION

Texas leads the United States in crude oil production. According to the year 2000 statistics, Texas has approximately 160,000 oil wells that annually produce over 400,000,000 barrels of oil. (Railroad Commission of Texas, 2001). Due the high level of production in the state, there is an increased risk of accidental contamination of soil from production and shipping. Contaminated soils are an environmental concern because it is a potential

We thank Mr. Michael Faries for providing crude oil, Turner Seed Co. of Breckenridge, TX and Mr. Jeff Goodwin for providing seed, and Ms. Teddy Gawrys for technical assistance. This research was funded by the Tarleton State University Faculty Development Grant Program.

source for surface and ground water contamination that can endanger humans, wildlife, and area vegetation. The soils in areas of contamination require remediation to return them to a non-hazardous state. Remediation often involves excavation and transporting contaminated soil to an incinerator or landfill. However these options are relatively expensive. The cost to remediate one ton of soil by incineration is \$200-1500 while landfilling ranges from \$100 – 400 (Schnoor, 1997). Another remediation option is to treat the contaminated soil on-site. This remediation technique is much less expensive and typically cost between \$10-35 per ton (Schnoor, 1997). One type of on-site remediation uses plants, which is called phytoremediation, and has been described as an economic, potentially effective, low-maintenance approach to treating soils contaminated with crude oil (Banks et al., 2000).

Phytoremediation is still a relatively new technology, but has been shown to enhance the disappearance of crude oil from soil. A study in Germany found ryegrass significantly reduced the concentration of petroleum hydrocarbons compared to an unvegetated control in a laboratory study (Günther, 1996) and in a field study phytoremediation significantly accelerated the disappearance of petroleum hydrocarbons compared to unvegetated controls (Schwab and Banks, 1999). However, phytoremediation is not always successful. In field lysimeters, phytoremediation using Johnsongrass, ryegrass, and a Johnsongrass-ryegrass rotation did not enhance the disappearance of a mixture of hydrocarbons from soil (Corapcioglu et al., 1999). A limitation of phytoremediation is that plant emergence and growth can be inhibited by contaminants. For example germination of tall fescue decreased from approximately 85% in uncontaminated soil to 35% in a nutrient solution containing 60 mg TNT L⁻¹ while shoot growth decreased from 4.4 mm d⁻¹ to approximately 0.2 mm d⁻¹ (Peterson et al., 1996). For phytoremediation to be successful there is a need to identify plants that are capable of growing in crude oil contaminated soil.

The objective of this study was to identify plants that are native, naturalized, or that have been successfully introduced to north central Texas which will emerge and grow in crude oil contaminated soil.

MATERIALS AND METHODS

A Windthorst sandy clay loam soil (Fine, mixed, thermic Udic Paleustalfs) with no known history of crude oil contamination was selected for this study (Table 1). This soil was collected from the Tarleton State University Hunewell Ranch near Stephenville, TX. The soil was air dried and passed through a 2-mm sieve. Soil organic matter content was determined by the Walkley-Black method (Nelson and Sommers, 1982). Soil pH was measured from a 1:1 soil to distilled water solution using a glass electrode and pH meter (McLean, 1982). Particle size distribution was measured by the hydrometer method (Gee and Bauder, 1986).

Table 1. Physicochemical properties of a Windthorst sandy clay loam soil.

Sand ¹	Silt ¹	Clay ¹	OC ²	pH ³
%			%	
50	20	30	2.63	7.2

¹ Hydrometer method

² Organic carbon

³ pH meter using 1:1 soil:distilled H₂O ratio

Experiments were conducted in a greenhouse. Experimental units consisted of 20-row seed flats filled with contaminated soil in every other row. Soil was contaminated with 0 (control), 0.5, 5, or 10% north central Texas Crude oil that was unweathered. The total petroleum hydrocarbon content, considered the toxic portion of the crude oil was found to be 80.7% using EPA method 418.1 modified for soil (EPA, 1979). Plants that thrive in north

central Texas were selected for this study (Table 2). Seeds were obtained from Turner Seeds of Breckenridge, TX and from the Texas A&M University Stephenville Research and Extension Center. Plants were seeded at a rate of 10 seeds per row. One species was seeded per row. Seeds were sown from just below the soil surface to approximately 2 cm deep with the larger seeds being sown at the deepest depth. Soils were kept at near field capacity (-30 kPa) by subsurface irrigation. Treatments were completely randomized and conducted in triplicate. The number of emerged seed and plant height were measured on days 7, 14, 21, and 28. Means emergence and plant height were separated using Duncan's multiple range test with means considered significantly different at $\alpha = 0.05$.

Table 2. Species screened to identify emergence and growth as measured by plant height in crude oil contaminated soil

Scientific Name	Common Name
Armadillo burr medic	<i>Medicago polymorpha</i>
Buffalograss	<i>Buchloe dactyloides</i> var. <i>texoka</i>
Hairy vetch	<i>Vicia villosa</i>
Hubam sweet clover	<i>Melilotus albus</i>
Illinois bundle flower	<i>Desmanthus illinoensis</i>
Johnsongrass	<i>Sorghum halepense</i>
Kenaf #2	<i>Hibiscus cannabinus</i> var. <i>tainvng</i> #2
Kenaf #3	<i>Hibiscus cannabinus</i> var. <i>sf 459</i>
Lablab	<i>Lablab purpureus</i>
Laredo soybean	<i>Glycine max</i> var. <i>laredo</i>
Madrid yellow clover	<i>Melilotus</i> sp.
Morning glory	<i>Ipomoea</i> sp.
Rose Clover	<i>Trifolium hirtum</i>
Sorghum triumph	<i>Sorghum bicolor</i> var. <i>triumph</i>
Sunflower macro	<i>Helianthus annuus</i> var. <i>macrocarpus</i>
Sunflower mammoth	<i>Helianthus annuus</i> var. <i>mammoth</i>
Sunflower maximillian	<i>Helianthus maximiliani</i>
Tall Jose wheatgrass	<i>Agropyron elongatum</i>
Ragweed	<i>Ambrosia pilostachya</i>

RESULTS

Emergence

Increasing the soil's concentration of unweathered crude oil from 0 to 10% significantly decreased seedling emergence of the 19 plant species (Tables 3-6). Plant emergence was highly variable across plant species. For example, 76.7% of the Sorghum triumph seeds emerged and were still surviving 28 days after planting compared to 26.7% for Lablab in the controls (Table 6). In the 0.5% crude oil treatment emergence was reduced by approximately 80% relative to the control during the 28 days after planting and only 9 of the species emerged. The treatment with 5% crude oil decreased emergence by approximately 90% relative to the control and the number of species emerging was 6. In the 10% crude oil treatments, emergence decreased by approximately 98% relative to the control and only two species, Kenaf #2 and Kenaf #3, emerged.

In the 0.5% crude oil treatment, Kenaf #2 had the greatest percentage seedling emergence (Tables 3-6). Kenaf #2 emergence was 36.7% on day 7 and remained at that percentage during the remainder of the 28 day experiment. Kenaf #3 had the second greatest percentage emergence of 23.3%, which was observed at each time period. Lablab also had 23.3% germination on day 7 and 28, but was 20.0% on day 14 and 21

Table 3. Seedling emergence 7 days after seeding in crude oil contaminated soil.

Plant Species	Crude Oil (%)				Regression ^a		
	0	0.5	5	10	β_0	β_1	r^2
	Emergence (% of sown seeds) ^b						
Kenaf #2	20.0 cd	36.7a	13.3a	0.0	28.6	-2.86	0.76
Lablab	30.0 bcd	23.3b	13.3a	0.0	27.3	-2.76	0.97
Kenaf #3	26.7 bcd	23.3b	3.3ab	0.0	23.9	-2.74	0.88
Sunflower mammoth	26.7 bcd	16.6bc	3.3ab	0.0	20.9	-2.39	0.82
Laredo soybean	13.3 dfg	3.3d	3.3ab	0.0	8.48	-0.91	0.54
Tall Jose wheatgrass	60.0 a	6.7cd	0.0b	0.0	31.8	-3.91	0.39
Johnsongrass	6.7 gh	3.3d	0.0b	0.0	4.71	-0.57	0.69
Sorghum triumph	63.3 a	0.0d	0.0b	0.0	30.4	-3.76	0.31
Sunflower macro	35.0 bc	0.0d	0.0b	0.0	16.8	-2.08	0.31
Morning glory	40.0 b	0.0d	0.0b	0.0	19.2	-2.38	0.31
Hubam sweet clover	36.7 bc	0.0d	0.0b	0.0	17.6	-2.18	0.31
Illinois bundle flower	13.3 dfg	0.0d	0.0b	0.0	6.39	-0.79	0.31
Buffalograss	33.3 bc	0.0d	0.0b	0.0	16.0	-1.98	0.31
Hairy vetch	13.3 dfg	0.0d	0.0b	0.0	6.39	-0.79	0.31
Madrid yellow clover	13.3 dfg	0.0d	0.0b	0.0	6.39	-0.79	0.31
Armadillo burr medic	10.0 fgh	0.0d	0.0b	0.0	4.80	-0.59	0.31
Rose clover	6.7 gh	0.0d	0.0b	0.0	4.80	-0.40	0.31
Ragweed	3.3 gh	0.0d	0.0b	0.0	1.59	-0.20	0.31
Sunflower max	0.0 h	0.0d	0.0b	0.0	na	-0.00	na
Average	23.8	6.0	1.9	0.0	14.5	-1.69	0.53
Average decrease in emergence relative to control		74.9	91.9	100.0			

^aConstant (β_0), slope (β_1), and correlation coefficient (r^2) of fitted first-order linear regression curves

^bMeans within a column with the same letter are not statistically different from each other (Duncan's test, $p < 0.05$)

Table 4. Seedling emergence 14 days after seeding in crude oil contaminated soil.

Plant Species	Crude Oil (%)				Regression ^a		
	0	0.5	5	10	β_0	β_1	r^2
	Emergence (% of sown seeds) ^b						
Kenaf #3	26.7def	23.3b	10.0b	6.7a	24.4	-1.99	0.90
Kenaf #2	26.7def	36.7a	23.3a	0.0b	33.6	-3.09	0.86
Lablab	26.7def	20.0b	10.0b	0.0b	23.7	-2.45	0.96
Sunflower mammoth	26.7def	6.7cd	3.3c	0.0b	16.4	-1.88	0.53
Sorghum triumph	76.7a	0.0d	3.3c	0.0b	37.4	-4.50	0.31
Tall Jose wheatgrass	63.3ab	13.3bc	0.0c	0.0b	36.4	-4.45	0.48
Laredo soybean	53.3bc	6.7cd	0.0c	0.0b	28.6	-3.52	0.41
Sunflower macro	35.0de	3.3cd	0.0c	0.0b	18.3	-2.25	0.38
Johnsongrass	20.0efg	3.3cd	0.0c	0.0b	11.1	-1.36	0.44
Morning glory	40.0cd	0.0d	0.0c	0.0b	19.2	-2.38	0.31
Buffalograss	40.0cd	0.0d	0.0c	0.0b	19.2	-2.38	0.31
Hubam sweet clover	36.7cd	0.0d	0.0c	0.0b	17.6	-2.18	0.31
Hairy vetch	20.0efg	0.0d	0.0c	0.0b	9.61	-1.19	0.31
Madrid yellow clover	13.3fgh	0.0d	0.0c	0.0b	6.39	-0.79	0.31
Armadillo burr medic	13.3fgh	0.0d	0.0c	0.0b	6.39	-0.79	0.31
Illinois bundle flower	13.3fgh	0.0d	0.0c	0.0b	6.39	-0.79	0.31
Sunflower max	6.7gh	0.0d	0.0c	0.0b	6.39	-0.79	0.31
Rose clover	6.7gh	0.0d	0.0c	0.0b	3.22	-0.40	0.31
Ragweed	3.3h	0.0d	0.0c	0.0b	1.59	-0.20	0.31
Average	28.9	6.0	2.6	0.4	14.4	-1.69	0.48
Average decrease in emergence relative to control		79.3	90.9	98.8			

^aConstant (β_0), slope (β_1), and correlation coefficient (r^2) of fitted first-order linear regression curves

^bMeans within a column with the same letter are not statistically different from each other (Duncan's test, $p < 0.05$)

Table 5. Seedling emergence 21 days after seeding in crude oil contaminated soil.

Plant Species	Crude Oil (%)				Regression ^a		
	0	0.5	5	10	β_0	β_1	r^2
	Emergence (% of sown seeds) ^b						
Kenaf #2	26.7 def	36.7a	23.3a	3.3ab	33.3	-2.78	0.85
Kenaf #3	23.3 ef	23.3b	13.3b	6.7a	23.4	-1.73	0.98
Sunflower mammoth	26.7def	6.7cd	3.3c	0.0b	16.4	-1.88	0.53
Sunflower macro	40.0 cd	3.3cd	3.3c	0.0b	21.3	-2.49	0.38
Johnsongrass	26.7 def	3.3cd	3.3c	0.0b	14.9	-1.70	0.41
Sorghum triumph	76.7 a	0.0d	3.3c	0.0b	37.4	-4.50	0.31
Lablab	26.7 def	20.0b	0.0c	0.0b	21.8	-2.62	0.79
Tall Jose wheatgrass	63.3 ab	13.3bc	0.0c	0.0b	36.4	-4.45	0.48
Laredo soybean	53.3 bc	6.7cd	0.0c	0.0b	28.6	-3.51	0.40
Madrid yellow clover	36.7 de	3.3cd	0.0c	0.0b	19.1	-2.35	0.38
Buffalograss	70.0 a	0.0d	0.0c	0.0b	33.6	-4.16	0.31
Morning glory	40.0 cd	0.0d	0.0c	0.0b	19.2	-2.38	0.31
Armadillo burr medic	20.0 fg	0.0d	0.0c	0.0b	9.61	-1.19	0.31
Hairy vetch	16.7 fgh	0.0d	0.0c	0.0b	8.02	-0.99	0.31
Illinois bundle flower	13.3 fgh	0.0d	0.0c	0.0b	6.39	-0.79	0.31
Rose clover	6.7 gh	0.0d	0.0c	0.0b	3.22	-0.40	0.31
Sunflower max	6.7 gh	0.0d	0.0c	0.0b	3.22	-0.40	0.31
Ragweed	6.7 gh	0.0d	0.0c	0.0b	3.22	-0.40	0.31
Hubam sweet clover	3.4 h	0.0d	0.0c	0.0b	3.22	-0.40	0.31
Average	30.7	6.1	2.6	0.5	1.63	-0.20	0.47
Average decrease in emergence relative to control		80.0	91.5	98.3			

^aConstant (β_0), slope (β_1), and correlation coefficient (r^2) of fitted first-order linear regression curves

^bMeans within a column with the same letter are not statistically different from each other (Duncan's test, $p < 0.05$)

Table 6. Seedling emergence 28 days after seeding in crude oil contaminated soil.

Plant Species	Crude Oil (%)				Regression ^a		
	0	0.5	5	10	β_0	β_1	r^2
	Emergence (% of sown seeds) ^b						
Kenaf #2	26.7def	36.7a	30.0a	6.7a	34.1	-2.34	0.71
Kenaf #3	23.3ef	23.3b	10.0b	6.7a	22.7	-1.79	0.91
Rose clover	6.7g	0.0d	6.7bc	0.0b	4.4	-0.28	0.11
Johnsongrass	26.7def	6.7cd	3.3bc	0.0b	16.4	-1.88	0.53
Sunflower macro	40.0cd	3.3d	3.3bc	0.0b	21.3	-2.49	0.38
Sorghum triumph	76.7a	0.0d	3.3bc	0.0b	37.4	-4.50	0.31
Lablab	26.7def	23.3b	0.0c	0.0b	23.3	-2.79	0.81
Tall Jose wheatgrass	50.0c	13.3c	0.0c	0.0b	30.0	-3.66	0.52
Sunflower mammoth	26.7def	6.7cd	0.0c	0.0b	15.8	-1.93	0.51
Laredo soybean	53.3bc	3.3d	0.0c	0.0b	27.1	-3.34	0.35
Buffalograss	66.7ab	0.0d	0.0c	0.0b	32.0	-3.96	0.31
Morning glory	40.0cd	0.0d	0.0c	0.0b	19.2	-2.38	0.31
Madrid yellow clover	33.3de	0.0d	0.0c	0.0b	16.0	-1.98	0.31
Armadillo burr medic	20.0efg	0.0d	0.0c	0.0b	9.61	-1.19	0.31
Hairy vetch	16.7fg	0.0d	0.0c	0.0b	8.02	-0.99	0.31
Hubam sweet clover	15.0fg	0.0d	0.0c	0.0b	7.21	-0.89	0.31
Sunflower max	6.7g	0.0d	0.0c	0.0b	7.21	-0.89	0.31
Ragweed	6.7g	0.0d	0.0c	0.0b	3.22	-0.40	0.31
Illinois bundle flower	5.0g	0.0d	0.0c	0.0b	2.40	-0.30	0.31
Average	29.8	6.1	3.0	0.7	17.5	-1.97	0.47
Average decrease in emergence relative to control		79.4	90.0	97.6			

^aConstant (β_0), slope (β_1), and correlation coefficient (r^2) of fitted first-order linear regression curves

^bMeans within a column with the same letter are not statistically different from each other (Duncan's test, $p < 0.05$)

In the treatments with 5% crude oil, Kenaf #2 had the largest percentage germination during the 28 days after planting (Tables 3-6). Its germination percentage on days 7, 14, and 21 was 23.3%, which increased to 30.0% on day 28. Kenaf #3 had the second greatest percentage emergence on days 14, 21, and 28 with 10, 13.3, and 10 % emergence respectively. Lablab had the third highest percent emergence with 13.3 and 10 % on days 7 and 14 respectively. However, it was unable to survive in the 5% crude oil treatment and died by day 21.

Only the two Kenaf varieties emerged in the treatments with 10% crude oil (Tables 3-6). Their emergence was delayed relative to the control treatment as neither one had emerged on day 7. By day 14, 6.7% of Kenaf #3 had emerged where it remained for the duration of the experiment. Kenaf #2 first showed emergence on day 21 with 3.3%, which increased to 6.7% on day 28.

Linear regression was fit to the data to show correlations between soil crude oil concentration and emergence (Tables 3-6). In general, correlation (r^2) shows a good fit for treatments where emergence was observed in contaminated soil. For example, the correlation coefficient for Kenaf #2 and Kenaf #3 was 0.71 and 0.91 respectively on day 28 (Table 6). Correlation was poor for varieties that did not emerge in oil contaminated soil. Slopes (β) of the regression lines are shown in Tables 3-6 for each plant species and may be useful in predicting emergence of a given species in soil with a known crude oil content.

Plant Height

Plant height decreased as the crude oil content of soil increased (Tables 7-10). For example, when averaging plant height across all plant species and comparing soil treated with 0.5, 5 or 10% crude oil to controls, plant height decreased by 72.0, 75.0, and 96.2 % respectively.

Plant species with the greatest plant height varied during the 28 day trial (Tables 7-10). In the treatments with 0.5% crude oil, Lablab showed the greatest plant height followed by Kenaf #2 and Sunflower mammoth. On day 7, in treatments with 5% crude oil, plant height was greatest in Lablab followed by Kenaf #3 and Kenaf #2. On day 14, Lablab and Kenaf #2 had the tallest plant height followed by Kenaf #3. Although Lablab had the greatest height on day 14, it had only grown 3 mm during the previous 7 days and its leaves were becoming necrotic. Lablab died in the 5% treatment by day 21, resulting in Kenaf #2 having the greatest height followed by Kenaf #3 and Sorghum triumph. This order remained through day 28. Only the two Kenaf varieties were able to grow in the 10% crude oil treatment. By day 14, Kenaf #3 was the only species to grow in the 10% crude oil treatment, but by day 21 Kenaf #2 had also emerged and had the greatest plant height through day 28.

A fitted regression analysis, plotting crude oil content vs. plant height, showed good correlation for most species that germinated in crude oil contaminated soils. For example the correlation coefficient (r^2) for Kenaf #2 ranged from 0.68 (day 28) to 0.93 (day 14). Slopes (β) of the regression lines are shown in Tables 7-10 for each plant species and may be useful in predicting plant height of a given species growing in soil with a known crude oil content.

DISCUSSION

Because Texas law considers soils with 5% petroleum hydrocarbons or less no longer in need of remediation, some reduction in seedling emergence and plant height was expected, but not to the extent that was found. Other studies have observed more modest reductions in emergence (Salanitro et al., 1997, Günther et al., 1996, and Banks et al., 2000). A likely explanation for the low seedling emergence and plant height was the use of unweathered crude oil in this study. Commonly in lab studies, crude oil is heated to artificially weather the crude oil, which removes the more volatile fraction, including

Table 7. Influence of soil crude oil concentration on plant height 7 days after seeding.

Plant Species	Crude Oil (%)				Regression ^a		
	0	0.5	5	10	β_0	β_1	r^2
	PlantHeight (mm) ^b						
Lablab	41.8a	49.1a	45.0a	0.0	50.4	-4.25	0.75
Kenaf #3	20.7cde	20.1c	16.3b	0.0	22.0	-1.99	0.91
Kenaf #2	22.5bcd	39.5b	10.5b	0.0	30.5	-3.20	0.77
Sunflower mammoth	43.2a	32.3b	3.3c	0.0	35.9	-4.18	0.83
Tall Jose wheatgrass	28.3bcd	9.7d	0.0c	0.0	18.0	-2.18	0.58
Johnsongrass	9.3efg	7.0de	0.0c	0.0	7.62	-0.92	0.79
Laredo soybean	9.4efg	1.7de	0.0c	0.0	5.28	-0.65	0.45
Sorghum triumph	34.4ab	0.0e	0.0c	0.0	16.5	-2.04	0.31
Morning glory	31.8abc	0.0e	0.0c	0.0	15.3	-1.89	0.31
Sunflower macro	26.6bcd	0.0e	0.0c	0.0	12.8	-1.58	0.31
Hairy vetch	17.0def	0.0e	0.0c	0.0	8.17	-1.01	0.31
Hubam sweet clover	8.3efg	0.0e	0.0c	0.0	3.99	-0.49	0.31
Madrid yellow clover	7.1fg	0.0e	0.0c	0.0	3.41	-0.42	0.31
Illinois bundle flower	6.9fg	0.0e	0.0c	0.0	3.31	-0.41	0.31
Armadillo burr medic	5.9fg	0.0e	0.0c	0.0	2.83	-0.35	0.31
Buffalograss	5.0fg	0.0e	0.0c	0.0	2.40	-0.30	0.31
Ragweed	5.0fg	0.0e	0.0c	0.0	2.40	-0.30	0.31
Rose clover	3.8g	0.0e	0.0c	0.0	1.83	-0.23	0.31
Sunflower max	0.0g	0.0e	0.0c	0.0	na	na	na
Average	17.2	8.4	4.0	0.0			0.77
Average decrease in height relative to control	—	50.7	75.0	100.0			

^aConstant (β_0), slope (β_1), and correlation coefficient (r^2) of fitted first-order linear regression curves

^bMeans within a column with the same letter are not statistically different from each other (Duncan's test, $p < 0.05$)

Table 8. Influence of soil crude oil concentration on plant height 14 days after seeding.

Plant Species	Crude Oil (%)				Regression ^a		
	0	0.5	5	10	β_0	β_1	r^2
	PlantHeight (mm) ^b						
Kenaf #3	55.3de	28.6d	30.0b	4.8a	44.4	-3.80	0.74
Lablab	106.8b	90.8a	48.0a	0.0a	101.0	-10.2	0.99
Kenaf #2	61.0de	58.5b	46.1a	0.0a	64.1	-5.86	0.93
Sorghum triumph	148.6a	0.0f	10.7c	0.0a	73.3	-8.65	0.31
Sunflower mammoth	118.2b	54.8b	3.3d	0.0a	82.1	-9.81	0.68
Laredo soybean	80.3c	35.8cd	0.0d	0.0a	54.7	-6.63	0.66
Sunflower macro	111.8b	32.7d	0.0d	0.0a	68.4	-8.34	0.54
Tall Jose wheatgrass	65.7cd	17.0e	0.0d	0.0a	39.2	-4.79	0.52
Johnsongrass	30.4fg	11.7e	0.0d	0.0a	19.9	-2.41	0.61
Morning glory	78.3c	0.0f	0.0d	0.0a	37.6	-4.65	0.31
Hairy vetch	47.0ef	0.0f	0.0d	0.0a	22.6	-2.80	0.31
Illinois bundle flower	24.4gh	0.0f	0.0d	0.0a	11.7	-1.45	0.31
Madrid yellow clover	19.6ghi	0.0f	0.0d	0.0a	9.41	-1.17	0.31
Buffalograss	15.2ghi	0.0f	0.0d	0.0a	7.30	-0.90	0.31
Hubam sweet clover	15.2ghi	0.0f	0.0d	0.0a	7.30	-0.90	0.31
Armadillo burr medic	9.7hi	0.0f	0.0d	0.0a	4.66	-0.58	0.31
Ragweed	8.0hi	0.0f	0.0d	0.0a	3.84	-0.48	0.31
Rose clover	8.0hi	0.0f	0.0d	0.0a	3.84	-0.48	0.31
Sunflower max	5.7i	0.0f	0.0d	0.0a	2.74	-0.34	0.31
Average	53.1	17.4	7.3	0.3	34.6	-3.90	0.60
Average decrease in height relative to control	—	67.3	86.3	99.5			

^aConstant (β_0), slope (β_1), and correlation coefficient (r^2) of fitted first-order linear regression curves

^bMeans within a column with the same letter are not statistically different from each other (Duncan's test, $p < 0.05$)

Table 9. Influence of soil crude oil concentration on plant height 21 days after seeding.

Plant Species	Crude Oil (%)				Regression ^a		
	0	0.5	5	10	β_0	β_1	r^2
	PlantHeight (mm) ^b						
Kenaf #2	86.0d	81.2b	87.7a	22.7a	91.4	-5.67	0.72
Kenaf #3	80.3de	34.5cd	57.1b	20.3a	62.3	-3.67	0.42
Sorghum triumph	222.7a	0.0f	34.3c	0.0b	113.3	-12.6	0.30
Sunflower macro	167.4bc	43.7c	18.3d	0.0b	103.5	-11.9	0.54
Sunflower mammoth	169.0b	70.8b	6.0e	0.0b	114.2	-13.6	0.65
Johnsongrass	55.0ef	17.0df	6.0e	0.0b	35.2	-4.04	0.58
Lablab	144.3bc	115.3a	0.0f	0.0b	121.3	-14.5	0.80
Laredo soybean	157.1bc	71.7b	0.0f	0.0b	107.8	-13.1	0.66
Tall Jose wheatgrass	80.5de	19.1cdf	0.0f	0.0b	47.3	-5.78	0.50
Madrid yellow clover	25.5gh	3.0f	0.0f	0.0b	13.6	-1.67	0.40
Morning glory	146.6bc	0.0f	0.0f	0.0b	70.4	-8.71	0.31
Hairy vetch	137.3c	0.0f	0.0f	0.0b	66.0	-8.16	0.31
Illinois bundle flower	41.2fg	0.0f	0.0f	0.0b	19.8	-2.45	0.31
Buffalograss	27.6fgh	0.0f	0.0f	0.0b	13.3	-1.64	0.31
Hubam sweet clover	23.9gh	0.0f	0.0f	0.0b	11.5	-1.42	0.31
Ragweed	20.7gh	0.0f	0.0f	0.0b	9.94	-1.23	0.31
Sunflower max	12.7gh	0.0f	0.0f	0.0b	6.10	-0.75	0.31
Rose clover	12.5gh	0.0f	0.0f	0.0b	6.00	-0.74	0.31
Armadillo burr medic	10.4h	0.0f	0.0f	0.0b	5.00	-0.62	0.31
Average	85.3	24.0	11.0	2.3	53.4	-5.91	0.54
Average decrease in height relative to control	—	71.8	87.1	97.3			

^aConstant (β_0), slope (β_1), and correlation coefficient (r^2) of fitted first-order linear regression curves

^bMeans within a column with the same letter are not statistically different from each other (Duncan's test, $p < 0.05$)

Table 10. Influence of soil crude oil concentration on plant height 28 days after seeding.

Plant Species	Crude Oil (%)				Regression ^a		
	0	0.5	5	10	β_0	β_1	r^2
	PlantHeight (mm) ^b						
Kenaf #2	110.9d	100.9b	112.0a	46.3a	114.0	-5.53	0.68
Kenaf #3	111.8d	40.1cde	75.5b	28.5b	82.3	-4.74	0.34
Sorghum triumph	250.2a	0.0f	42.0c	0.0c	127.9	-14.1	0.30
Sunflower macro	215.0b	52.7	34.3c	0.0c	133.3	-14.9	0.53
Johnsongrass	84.9e	32.0de	9.7d	0.0c	57.0	-6.54	0.64
Rose clover	20.8f	0.0f	9.3d	0.0c	11.7	-1.08	0.26
Lablab	148.0c	174.7a	0.0d	0.0c	149.8	-17.8	0.79
Sunflower mammoth	208.2b	87.0b	0.0d	0.0c	139.2	-16.9	0.64
Laredo soybean	208.3b	43.3cd	0.0d	0.0c	119.6	-14.6	0.47
Tall Jose wheatgrass	105.5de	22.8e	0.0d	0.0c	61.0	-7.45	0.48
Morning glory	168.7c	0.0f	0.0d	0.0c	81.0	-10.0	0.31
Hairy vetch	148.8c	0.0f	0.0d	0.0c	71.5	-8.85	0.31
Buffalograss	37.2f	0.0f	0.0d	0.0c	17.9	-2.21	0.31
Illinois bundle flower	37.0f	0.0f	0.0d	0.0c	17.8	-2.20	0.31
Madrid yellow clover	35.5f	0.0f	0.0d	0.0c	17.1	-2.11	0.31
Sunflower max	27.7f	0.0f	0.0d	0.0c	13.3	-1.65	0.31
Ragweed	25.7f	0.0f	0.0d	0.0c	12.3	-1.53	0.31
Hubam sweet clover	19.8f	0.0f	0.0d	0.0c	9.51	-1.18	0.31
Armadillo burr medic	14.6f	0.0f	0.0d	0.0c	7.01	-0.86	0.31
Average	104.1	29.1	14.9	3.9	65.4	-7.07	0.53
Average decrease in height relative to control	—	72.0	85.7	96.2			

^aConstant (β_0), slope (β_1), and correlation coefficient (r^2) of fitted first-order linear regression curves

^bMeans within a column with the same letter are not statistically different from each other (Duncan's test, $p < 0.05$)

many of the phytotoxic compounds found in crude oil (Rhykerd et al., 1998, Rhykerd et al., 1999). By not weathering the crude oil used in this study, exposure of seeds to this volatile fraction of crude oil may have reduced emergence relative to other studies.

The crude oil contaminated soil in this study drastically reduced plant growth. This increases the time required to establish a plant canopy, which is an important component of successful phytoremediation. The plant canopy decreases the impact of raindrops on soil and reduces the potential of surface runoff and erosion that can contribute to surface water contamination (Cunningham et al., 1995).

Delaying seeding following crude oil spills and tilling contaminated soils may improve seedling emergence, plant growth, and enhance remediation. Delaying seeding may enhance seedling emergence because the majority of volatile compounds, many of which are phytotoxic, escape from soil within 24-48 hours following a spill. Additionally, tillage, which has been shown to increase bioremediation of crude oil contaminated soils (Rhykerd et al., 1999), may further enhance the removal of volatile compounds from soil exposing volatile compounds that had been trapped in the soil.

CONCLUSIONS

Vegetative cover on contaminated soil is necessary to promote phytoremediation and reduce surface runoff and erosion. However, this study found seedling emergence and plant height were significantly reduced in soils with increasing crude oil content. Of all the species tested in this study, Kenaf #2 (*Hisbiscus cannabinus* var. *tairvng* #2) and Kenaf #3 (*Hisbiscus cannabinus* var. *sf 459*) showed the greatest seedling emergence and plant height. These species are therefore recommended for use to establish vegetation and promote phytoremediation on soils with up to 10% crude oil contamination. To improve seedling emergence and plant growth, it is recommended that following a spill, seeding be delayed to allow for volatilization of a majority of the phytotoxic fraction of crude oil. Tilling the contaminated soil during this delay is further recommended to enhance the rate and extent of volatilization.

REFERENCES

- Banks, M.K., R.S. Govindaraju, A.P. Schwab, and P. Kulakow. 2000. Executive Summary of Demonstration. In S. Fiorenza, C.L. Oubre, and C.H. Ward (Eds) Phytoremediation of Hydrocarbon-Contaminated Soil. Lewis Publishers, Boca Raton, FL Pp. 3-6.
- Corapcioglu, Y.M., C.L. Munster, M.C. Drew, R.L. Rhykerd, K. Sung, and Y.Y. Chang. 1999. Phytoremediation and modeling of land contaminated by hydrocarbons. Proceedings, 5th International In Situ On-Site Bioremediation Symposium. San Diego, CA
- Cunningham, S.D., W.R. Berti, and J.W. Huang. 1995. Remediation of contaminated soils and sludges by green plants. In Hinchee et al. (eds.) Bioremediation of Inorganics. pp. 33-54. Third International In Situ and on site Bioreclamation Symposium, San Diego, CA.
- EPA Method 418.1. 1979. Petroleum hydrocarbons, total recoverable. In Methods for Chemical Analysis of Water and Wastes. U.S. EPA. Washington DC. EPA 600/4-79/020
- Gee, G.W., and J.W. Bauder. 1986. Particle size analysis. In Methods of Soil Analysis, Part 1, ed. A Klute. Am. Soc. Agron., Madison, WI, USA. Pp. 387-409.
- Günther, T., U. Dornberger, and W. Fritsche. 1996. Effects of ryegrass on biodegradation of hydrocarbons in soil. Chemosphere 33:203-215.
- McLean, E.O. 1982. Soil pH and lime requirement. In Methods of Soil Analysis. Part 2, ed. A.L. Page et al. Am. Soc. Agron., Madison, WI, USA. P. 199-224.

- Nelson, D. W., and L.E. Sommers. 1982. Total carbon, organic carbon, and organic matter. In *Methods of Soil Analysis. Part 2*, ed. A.L. Page et al. Am. Soc. Agron., Madison, WI, USA. P. 539-580.
- Peterson, M.M, G.L. Horst, P.J. Shea, S.D. Comfort, R.K. D. Peterson. 1996. TNT and 4-amino-2,6-dinitrotoluene influence on germination and early seedling development of tall fescue. *Environmental Pollution*. 93:57-62.
- Railroad Commission of Texas. 2001. Texas Monthly oil and gas production (1995-2001). <http://www.rrc.state.tx.us/divisions/og/information-data/stats/ogismcon.html>
- Rhykerd, R.L., B. Crews, K.J. McInnes, and R.W. Weaver. 1999. Impact of bulking agents, forced aeration, and tillage on remediation, of oil-contaminated soil. *Bioresource Technology* 67:279-285.
- Rhykerd, R.L. D. Sen, K.J. McInnes, and R.W. Weaver. 1998. Volatilization of crude oil from soil amended with bulking agents. *Soil Science* 163:87-92.
- Salanitro, J.P., P.B. Dorn, M.H. Huesemann, K.O. Moore, I.A. Rhodes, L.M. Rice-Jackson, T.E. Vipond, M.M. Western, and H.L. Wisniewski. 1997. Crude oil hydrocarbon bioremediation and soil ecotoxicity assessment. *Environ. Sci. Technol.* 31:1769-1776.
- Schnoor, J.L. 1997. *Phytoremediation*. Publication No. TE-98-01. Ground-Water Remediation Technology Analysis Center (GWRTAC), Pittsburgh, PA, available at http://www.gwrtac.org/html/tech_eval.html#PHYTO
- Schwab, P. and K. Banks. 1999. *Phytoremediation of petroleum-contaminated soils*. In *Bioremediation of Contaminated Soils*, ed. D.C. Adriano. American Society of Agronomy. Madison, WI. Pp. 783-795.