Root and Shoot Biomass 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

Using plants to enhance remediation of soil contaminated with crude oil is a viable remediation strategy. Rapid vegetative growth forms a plant canopy that acts to contain the contaminated soil by reducing surface runoff and erosion. Root growth in these soils stimulates microbial activity in the rhizosphere, which may accelerate bioremediation. The object of this study was to identify plants with the potential of enhancing bioremediation by measuring root and shoot biomass. In a greenhouse experiment, seeds were sown into a Windthorst sandy clay loam soil contaminated with 0 (control), 0.5, 5, or 10% unweathered north central Texas Crude oil. Experimental units consisted of seed flats with dimensions of 55 x 28 x 3.2 cm (L x W x H) with 20 individual rows. Seeds were sown in separate rows at the rate of ten seeds per row. Soil moisture was maintained near –30 kPa pressure by using subsurface irrigation. Treatments were conducted in triplicate in a completely randomized design. Plants were grown for 28 days after seeding. On day 28, root and shoot biomass was measured and the presence of nodules was noted. Averaged across all nineteenspecies, soil crude oil concentration of 0.5, 5, and 10% decreased shoot biomass by 75.8, 96.7, and 99.3% and root biomass decreased by 72.1, 96.1, and 99.5% relative to the control treatments. Lablab (Lablab purpureus) had the greatest shoot and root biomass production in the treatment with 0.5% crude oil. In the treatment with 5% crude oil Kenaf#2 (Hisbiscus cannabinus var. tainvng #2) had the greatest shoot and root production and in the 10% crude oil treatment Kenaf #3 (Hisbiscus cannabinus var. sf 459) had the greatest shoot and root production. The presence of unweathered crude oil inhibited nodule formation on all legumes. Results indicate that the species with the greatest potential to enhance phytoremediation are Lablab for soil with approximately 0.5% crude oil, Kenaf # 2 for soil contaminated with approximately 5% crude oil and Kenaf #3 for soils contaminated with 10% crude oil. Due to the drastic reduction in shoot and root biomass in crude oil contaminated soil, over-seeding, transplanting healthy plants, and delaying seeding to allow time for volatile phytotoxic-compounds to volatilize may decrease the time for a plant canopy establishment, encourage greater root biomass, and enhance phytoremediation.

KEYWORDS: Root biomass, Shoot biomass, Bioremediation
Phytoremediation, Remediation, Petroleum hydrocarbons

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INTRODUCTION

Oil contaminated soils are a concern because they are unsuitable for agricultural, industrial, or recreational uses and are potential sources for surface and ground water contamination that can endanger humans and wildlife. Phytoremediation, the use of plants to enhance remediation of soil *in situ* is an economic alternative to traditional methods of excavation and landfilling or incineration (Schnoor, 1997). With *in situ* remediation techniques it is vital to contain the contaminant to prevent its spread. A plant canopy established during phytoremediation can reduce contaminant spread by reducing surface runoff and erosion.

Using phytoremediation to enhance degradation of contaminants relies upon an extensive root system. Roots enhance remediation via several mechanisms including sequestering contaminants (Cunningham et al., 1995), improving soil aeration (Nye and Tinker, 1977; Schnoor, 1993) and reducing movement of contaminants in soil (Topp et al., 1986; Nair et al., 1993; Vlamis et al., 1985; Schnoor, 1993). Perhaps the greatest contribution of roots during phytoremediation of soil contaminated with crude oil is increasing the activity of soil microorganisms. Root growth stimulates microbial activity in the rhizosphere by 10-100 times that of non-rhizosphere soil (Pierzynski, et al., 2000). The increased microbial activity in the rhizosphere may increase the rate of biodegradation of crude oil into less toxic products such as CO , H O, cell biomass, and energy (Aprill and Sims, 1990; Nair, et al., 1993; McFarlane et al.,[2] 1990; and Sims and Overcash, 1983).

The object of this study was to identify plants with the potential of enhancing bioremediation by measuring root and shoot biomass.

MATERIALS AND METHODS

A Windthorst sandy clay loam soil (Fine, mixed, thermic Udic Paleustalfs) with no known history of contamination from petroleum hydrocarbons was selected for this study (Table 1). This soil was collected from the Tarleton State University Hunewell Ranch located 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 using a glass electrode and pH meter from a 1:1 soil to water solution (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 H_2O

Experiments were conducted in a greenhouse. Experimental units consisted of 20-row seed flats (55 x 28 x 3.2 cm) filled with soil in every other row. Soil was contaminated with 0 (control), 0.5, 5, or 10% unweathered north central Texas Crude oil that was 80.7% total petroleum hydrocarbons by weight. Nineteen plant species 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 at a depth appropriate for the

size of the seed. One species was seeded per row. Soils were kept near field capacity (-30 kPa pressure) by subsurface irrigation. Treatments were completely randomized and conducted in triplicate. On day 28, living plant biomass was removed from seed trays. Soil was rinsed from roots under a gentle stream of water. Roots and shoots were separated at the crown. Roots of legumes were examined for the presence of nodules. Biomass was oven-dried at 60 °C for at least 48 hours and final biomass of shoots and roots was recorded on a dry weight basis. Means of shoot and root biomass were separated using Duncan's multiple range test with means considered significantly different at $\alpha = 0.05$.

Table 2. Species screened to identify vegetative and root biomass production in soil contaminated with 0.5, 5, and 10% unweathered crude oil.

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

RESULTS

Production of shoot biomass was reduced as soil crude oil content increased from 0% (control) to 10% (Table 3). Averaging across all nineteen plant species and comparing to the controls, shoot biomass decreased by 75.8, 96.7 and 99.3% in soil contaminated with 0.5, 5, and 10% crude oil respectively. Of the all species tested, a majority was severely affected by the presence of crude oil in soil. The addition of 0.5% unweathered crude oil prevented growth of 10 of the 19 species. As the amount of unweathered crude oil increased to 5 and 10%, the number of species surviving after 28 days was reduce to 6 and 2 respectively.

Table 3. Influence of crude oil on shoot biomass production (dry weight basis) 28 days after seeding and the calculated slope and correlation coefficient from linear regression analysis correlating crude oil content vs. biomass.

Plant Species	Crude Oil (%)				Regression[a]		
	0	0.5	5	10	$ß_0$	$ß_1$	r^2
	Biomass (mg)[b]						
Kenaf #3	131.4 ef	136.8c	45.0b	21.1a	130.6	-12.1	0.91
Kenaf #2	173.1 e	273.7b	106.2a	17.0a	224.0	-21.0	0.82
Sunflower macro	429.1 d	44.6d	17.7c	0.0b	229.5	-27.5	0.39
Sorghum triumph	668.6 c	0.0d	11.1c	0.0b	323.2	-39.6	0.31
Rose Clover	7.3 f	00.d	1.0c	0.0b	3.69	-0.42	0.31
Johnsongrass	17.8 ef	2.2d	0.5c	0.0b	9.63	-1.16	0.41
Lablab	1233.1 a	548.9a	0.0c	0.0b	839.7	-101.7	0.66
Sunflower mammoth	955.5 b	160.0c	0.0c	0.0b	531.1	-65.1	0.44
Laredo soybean	930.8 b	156.4c	0.0c	0.0b	517.6	-63.4	0.44
Tall Jose wheatgrass	70.4 ef	6.3d	0.0c	0.0b	36.7	-4.51	0.38
Morning glory	616.2 c	0.0d	0.0c	0.0b	296.0	-36.6	0.31
Buffalograss	5.2 f	2.1d	0.0c	0.0b	3.44	-0.42	0.63
Illinois bundle flower	108.6 ef	0.0d	0.0c	0.0b	52.2	-6.46	0.31
Madrid yellow clover	39.5 ef	0.0d	0.0c	0.0b	19.0	-2.35	0.31
Hairy vetch	38.7 ef	0.0d	0.0c	0.0b	18.6	-2.30	0.31
Armadillo burr medic	23.3 ef	0.0d	0.0c	0.0b	11.2	-1.39	0.31
Hubam sweet clover	22.5 ef	0.0d	0.0c	0.0b	10.8	-1.34	0.31
Sunflower max	13.7 ef	0.0d	0.0c	0.0b	6.58	-0.81	0.31
Ragweed	4.6 f	0.0d	0.0c	0.0b	2.21	-0.27	0.31
Average	288.9	70.0	9.6	2.0	171.8	-20.4	0.50
Average decrease in height relative to control		75.8	96.7	99.3			

[a]Constant ($ß_0$), slope ($ß_1$), and correlation coefficient (r^2) of fitted first-order linear regression curves

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

Among the soil treatments with 0.5% crude oil, Lablab produced the most shoot biomass followed by Kenaf #2 and the two were significantly different. Sunflower mammoth, Laredo soybean, and Kenaf #3 had the next greatest amount of shoot biomass production, but were not significantly different from each other.

In the soils treated with 5% crude oil, Lablab emerged and grew well through 14 days. However, it was not able to survive at this level of crude oil content and died by day 21 of the experiment. Thus, after 28 days, shoot biomass was greatest from Kenaf #2 followed by Kenaf #3 and the two were significantly different. Measurable shoot biomass was also recorded from Sunflower macro, Sorghum triumph, Rose clover, and Johnsongrass.The remaining 13 species did not emerge.

In the soils treated with 10% crude oil, shoot biomass was produced only from Kenaf #3 and Kenaf #2, which were not statistically different. The remaining 17 plant species did notemerge.

Table 4. Influence of crude oil on root biomass production (dry weight basis) 28 days after seeding and the calculated slope and correlation coefficient from linear regression analysis correlating crude oil content vs. biomass.

Plant Species	Crude Oil (%)				Regression[a]		
	0	0.5	5	10	$ß_0$	$ß_1$	r^2
	Biomass (mg)[b]						
Kenaf #3	35.1 d	45.6 cd	24.4 b	8.2 a	40.9	-3.25	0.90
Kenaf#2	62.4 d	134.7 b	38.5 a	3.8 b	97.3	-9.66	0.66
Sorghum triumph	655.0 a	0.0 d	13.1 c	0.0 c	317.0	-38.71	0.31
Sunflower macro	191.1 c	31.7 cd	12.0 c	0.0 c	108.3	-12.79	0.45
Johnsongrass	8.7 d	1.3 d	1.3 d	0.0 c	5.00	-0.56	0.43
Rose clover	0.9 d	0.0 d	0.9 d	0.0 c	-0.60	-0.04	0.13
Lablab	243.5 bc	232.5 a	0.0 d	0.0 c	221.7	-26.51	0.81
Sunflower mammoth	345.8 b	137.8 b	0.0 d	0.0 c	228.2	-27.69	0.62
Laredo soybean	331.6 b	57.8 c	0.0 d	0.0 c	185.3	-22.70	0.45
Tall Jose wheatgrass	27.5 bc	6.4 d	0.0 d	0.0 c	16.1	-1.97	0.49
Morning glory	286.5 d	0.0 d	0.0 d	0.0 c	137.6	-17.03	0.31
Buffalograss	19.3 d	4.4 d	0.0 d	0.0 c	11.3	-1.38	0.49
Madrid yellow clover	44.0 d	0.0 d	0.0 d	0.0 c	21.1	-2.62	0.31
Hairy vetch	38.6 d	0.0 d	0.0 d	0.0 c	18.5	-2.29	0.31
Hubam sweet clover	19.7 d	0.0 d	0.0 d	0.0 c	9.46	-1.17	0.31
Illinois bundle flower	18.0 d	0.0 d	0.0 d	0.0 c	8.64	-1.07	0.31
Armadillo burr medic	8.8 d	0.0 d	0.0 d	0.0 c	4.22	-0.52	0.31
Sunflower max	2.0 d	0.0 d	0.0 d	0.0 c	0.96	-0.12	0.31
Ragweed	1.0 d	0.0 d	0.0 d	0.0c	0.83	-0.10	0.31
Average	123.1	34.3	4.7	0.6	75.4	-8.96	0.54
Average decrease in height relative to control		72.1	96.1	99.5			

[a]Constant ($ß_0$), slope ($ß_1$), and correlation coefficient (r^2) of fitted first-order linear regression curves

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

A fitted regression analysis, plotting crude oil content vs. shoot biomass, showed good correlation for Kenaf #3 ($r^2 = 0.91$) and Kenaf #2 ($r^2 = 0.82$), but showed a poor correlation for most other plant species (Table 3). For example, many of the species with little or not vegetative biomass had a correlation coefficient of 0.31.

Root biomass was significantly reduced in soil with increasing crude oil content (Table 4). Averaging across all nineteen plant species and comparing to the controls, root biomass decreased by 72.1, 96.1 and 99.5% % in soil contaminated with 0.5, 5, and 10%

crude oil respectively. In addition to decreased root biomass, nodules were not observed on legumes grown in soil containing unweathered crude oil.

In the treatment with 0.5% crude oil, the greatest amount of root biomass was produced by Lablab followed by Kenaf #2 and their differences were significant. Laredo soybean, Kenaf #3 and Sunflower macro had the next greatest amount of root biomass production, but were not significantly different from each other.

In soil treated with 5% crude oil, root biomass was produced by six species. Kenaf #2 produced the most root biomass followed by Kenaf #3, which was significantly lower. Crude oil contamination decreased the amount of root biomass by 38% for Kenaf #2 and 30% for Kenaf #3 when compared to controls. Sorghum triumph and Sunflower macro had the next greatest amount of root biomass production. Root biomass was also observed in Johnsongrass and Rose clover.

Root biomass production in soils treated with 10% crude oil was statistically greatest for Kenaf #3 followed by Kenaf #2. Root biomass production was drastically reduced compared to the controls for these two Kenaf varieties. Relative to the root biomass in the control soil, there was a 77 and 99% reduction in root biomass for Kenaf #3 and Kenaf #2 respectively. None of the remaining 17 plant species emerged in soil with 10% unweathered crude oil.

Fitted linear regression correlating crude oil content vs. root biomass was 0.54 for the average of all 19 species (Table 4). The correlation coefficients were higher for the species with the greatest amount of root biomass production in contaminated soils. For example, the correlation coefficient's for Kenaf #3, Kenaf #2, and Lablab were 0.90, 0.66, and 0.81 respectively.

DISCUSSION

Increasing vegetative biomass may act to contain contaminated soil and prevent the spread of crude oil into surface and subsurface water. Closing the plant canopy reduces the impact of raindrops and decreases the possibility of detachment and transport of contaminated soils. Although Kenaf #2 and Kenaf #3 were grown in soil with 10% crude oil, shoot biomass was reduced by 90% for Kenaf #2 and 84% for Kenaf #3 relative to shoot production in uncontaminated soil. Thus, higher soil contamination increases the time required to develop an effective canopy, which will likely increase the risk of surface runoff and erosion.

Plants identified in this study with the greatest root biomass production are recommended for further study to determine their impact on enhancing bioremediation of crude oil contaminated soils. Increasing root biomass may increase phytoremediation in several ways. The carbonaceous exudates from plant roots are readily metabolized by microorganisms in the soil, which increases total microbial populations in the rhizosphere. Increased populations leads to an increase in activity in the rhizosphere, which may increase metabolic and cometabolic transformations of contaminants to less toxic products (Aprill and Sims, 1990; Nair, 1993; McFarlane et al., 1990; and Sims and Overcash, 1983). Crude oil degrades most rapidly in aerobic environments (Atlas, 1981; Bossert and Bartha, 1984; Leahy and Colwell, 1990). Roots improve soil aeration by directly giving off oxygen to the root zone and allow improved entry of oxygen into the soil by diffusion along old root channels (Nye and Tinker, 1977; Schnoor, 1993). Plants may sequester, absorb, and translocate crude oil to plant tissues, thereby removing these hydrocarbons from the soil environment (Cunningham et al., 1995). Additionally, roots can reduce downward movement of contaminants through the soil profile by extracting excess water during transpiration, thus reversing the vertical hydraulic gradient (Topp et al., 1986; Nair et al., 1993; Vlamis et al., 1985; Schnoor, 1993).

Because legumes did not form nodules when growing in soil containing unweathered crude oil, N -fixation will not occur and these plants will likely require nitrogen fertilizer to optimize their growth.

Because both shoot and root biomass production were drastically reduced in contaminated soil, further research is needed to find ways to increase biomass production in these soils. Increasing the seeding rate beyond that typically recommended or transplanting actively growing plants into contaminated soil could enhance bioremediation and reduce the time required to form a plant canopy.

Allowing time for volatilization prior to seeding may reduce the number of toxic compounds in soil and thus increase shoot and root biomass. Volatile compounds associated with unweathered crude oil, like the type used in this study, are usually phytotoxic and may cause seed damage, reduce germination, and decrease plant biomass production. Because the majority of volatile compounds volatilize from soil within 24-48 hours (Rhykerd 1998), delaying seeding following spills may increase production of shoot and root biomass and enhance the potential for phytoremediation.

CONCLUSIONS

Of the plants examined, Lablab produced the most biomass in soils contaminated with 0.5% crude oil while, Kenaf #2, and Kenaf #3 produced the most biomass in soils contaminated with 5 and 10% crude oil. These species show the greatest promise to enhance remediation of oil contaminated soils.

Possible strategies to enhance shoot and root biomass production include increasing the seeding rate above that recommended, transplanting actively growing plants into the contaminated soil, and delay seeding immediately following a spill to allow volatilization may remove phytotoxic compounds from the soil.

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