International Journal of Phytoremediation, 8:285–297, 2006

ISSN: 1522-6514 print / 1549-7879 online
DOI: 10.1080/15226510600992840

Taylor & Francis
Taylor & Francis Group

SELECTING PLANTS AND NITROGEN RATES TO VEGETATE CRUDE-OIL–CONTAMINATED SOIL

W. D. Kirkpatrick, P. M. White, Jr. , and D. C. Wolf
Department of Crop, Soil, and Environmental Sciences, University of Arkansas, Fayetteville, AR, USA

G. J. Thoma
Department of Chemical Engineering, University of Arkansas, Fayetteville, AR, USA

C. M. Reynolds
USACE Engineer Research and Development Center, Cold Regions Research and Engineering Laboratory, Hanover, NH, USA

Phytoremediation can be effective for remediating contaminated soils* in situ *and generally requires the addition of nitrogen (N) to increase plant growth. Our research objectives were to evaluate seedling emergence and survival of plant species and to determine the effects of N additions on plant growth in crude-oil–contaminated soil. From a preliminary survival study, three warm-season grasses—pearlmillet (*Pennisetum glaucum *[L.] R. Br.), sudangrass (*Sorghum sudanense *[Piper] Stapf [Piper]), and browntop millet (*Brachiaria ramosa *L.)—and one warm-season legume—jointvetch (*Aeschynomene americana *L.)—were chosen to determine the influence of the N application rate on plant growth in soil contaminated with weathered crude oil. Nitrogen was added based on total petroleum hydrocarbon-C:added N ratios (TPH-C:TN) ranging from 44:1 to 11:1. Plant species were grown for 7 wk. Root and shoot biomass were determined and root length and surface area were analyzed. Pearlmillet and sudangrass had higher shoot and root biomass when grown at a TPH-C:TN (inorganic) ratio of 11:1 and pearlmillet had higher root length and surface area when grown at 11:1 compared with the other species. By selecting appropriate plant species and determining optimum N application rates, increased plant root growth and an extended rhizosphere influence should lead to enhanced phytoremediation of crude-oil–contaminated soil.

KEY WORDS: biomass production, germination, phytoremediation, rhizosphere-enhanced remediation

INTRODUCTION

Oil pollution of soil is a common occurrence in areas where large amounts of oil are pumped, transported, refined, or stored. Traditional methods used to clean up crude-oil–contaminated soils such as landfilling and incineration can be labor intensive and costly. An alternative method is phytoremediation, the use of plants and the microbiota associated with the roots to remove, contain, or render harmless environmental contaminants. It can be a cost-effective, low-maintenance method of remediating crude-oil–contaminated soils on site (Cunningham *et al*., 1996).

Address correspondence to D. C. Wolf, Department of Crop, Soil, and Environmental Sciences, University of Arkansas, Plant Science 115, Fayetteville, AR 72701, USA. E-mail: dwolf@uark.edu

The rhizosphere associated with plant roots has been shown to increase the numbers, activity, and diversity of organic contaminant-degrading microorganisms compared to non-rhizosphere soil (Anderson and Coats, 1995; Banks, Mallede, and Rathbone, 2003; Hutchinson, Schwab, and Banks, 2003; Reynolds and Wolf, 1999). Before the phytoremediation of petroleum-contaminated soil can be successful, plants must be able to geminate, survive, and grow (Medina *et al*., 2003; Wenzel *et al*., 1999). Plant-species selection and soil-nutrient levels are critical considerations for implementing phytoremediation strategies (Adam and Duncan, 2002; Cunningham *et al*., 1996).

Grass species are excellent candidates for phytoremediation due to their extensive fibrous root systems, which allow for more interaction between the rhizosphere microbial community and the contaminant (Hutchinson, Schwab, and Banks, 2003). Because soils contaminated with high levels of crude oil are often nitrogen (N) limited, leguminous plants have also been used because of their N_2 fixation capability (Cunningham *et al*., 1996). Under certain climatic conditions, enhanced phytoremediation could result from combining warm- and cool-season plant species to extend the time of active plant growth and increase the duration of rhizosphere activity.

Soil nutrients generally considered to be most limiting in the phytoremediation of petroleum-contaminated soil are N and phosphorus (P). Active microbial degradation of crude-oil contaminants containing high levels of carbon (C) requires substantial N and P in addition to the nutrients required by the plant for survival and growth (Hutchinson *et al*., 2003). Often, nutrients are added at a C:N:P ratio of 100:10:1 based on the total elemental analysis of the contaminated soil. Such an approach can lead to an overestimation of the fertilizer application rate, because not all of the C is available for biodegradation. Excessive N and P application has the potential to result in an osmotic stress for the germinating seeds or plants growing in the contaminated soil and could cause surface and groundwater contamination if leaching or runoff occurs (Hutchinson *et al*., 2003). Matching plant species with adequate N rates at a given contamination level is an important component of phytoremediation, because increased plant production generally leads to increased root length and surface area. The increase in root growth allows for greater contact between the rhizosphere microbial community and the contaminant present in the soil and should accelerate degradation of the contaminant (Chang and Corapcioglu, 1998; Thoma, Lam, and Wolf, 2003a, 2003b).

The research objectives were to 1) determine seedling emergence and survival of 21 cool- and warm-season plant species grown in a crude-oil–contaminated soil and 2) determine the effects of N additions on shoot and root growth of four warm-season plant species in crude-oil–contaminated soil.

MATERIALS AND METHODS

Soils and Treatments

The two separate experiments, plant-selection and the N-rate greenhouse studies, were conducted using crude-oil–contaminated Sacul sandy loam (clayey, mixed, thermic, aquic Hapludult). The soils where collected from two sites near El Dorado, Arkansas, where an oil spill occurred 5 yr prior to sample collection. The soils were passed through a 2-mm sieve prior to initiating the studies.

For the plant selection study, 21 warm- and cool-season grasses and legumes were chosen for this study (Table 1). Seeds were planted at a rate of 10/pot in 8.5-cm × 7.5-cm

Table 1 Grasses and legumes evaluated for seedling emergence and survival in a weathered crude-oil–contaminated soil

Common name	Latin Binomial (variety/cultivar)
Warm Season	
Grasses	
Crabgrass	*Digitaria sanguinalis* (L.) Scop. (Red River)
Crabgrass	*D. sanguinalis* (L.) Scop. (Large)
Bahiagrass	*Paspalum notatum* Flüggé (Pensacola)
Dallisgrass	*P. dilatatum* Poir.
Pearlmillet	*Pennisetum glaucum* (L.) R. Br.
Centipedegrass	*Eremochloa ophiuroides* (Munro) Hack.
Browntop millet	*Brachiaria ramosa* L.
Weeping lovegrass	*Eragrostis curvula* (Schrad.) Nees
Sudangrass	*Sorghum sudanense* (Piper) Stapf (Piper)
Legumes	
Sericea lespedeza	*Lespedeza cuneata* (Dumont) G. Don.
Korean lespedeza	*L. stipulacea* Maxim.
Striate lespedeza	*L. striata* (Thunb.) H. & A.(Marion)
American jointvetch	*Aeschynomene americana* L.
Hairy vetch	V*icia villosa* Roth
Cool Season	
Grasses	
Ryegrass	*Lolium multiflorum* L. (Marshall)
Fescue	*Festuca arundinacea* Schreb. (KY31)
Wheat	*Triticum aestivum* L. (Roane)
Oat	*Avena sativa* L. (Chapman)
Legumes	
Crimson Clover	*Trifolium incarnatum* L. (Dixie)
Arrowleaf Clover	*T. vesiculosum* Savi (Yuchi)
Alfalfa	*Medicago sativa* L. (Riley)

plastic pots containing 300 g crude-oil–contaminated soil (dry weight equivalent) and that had a gas chromatography/flame ionization detector (GC/FID) total petroleum hydrocarbon (TPH) concentration of 2.93 $\pm$ 0.14%. The pots contained approximately 100 g of 1- to 2-cm diameter gravel in the bottom to allow drainage of excess soil water and prevent soil loss. Warm- and cool-season plant species were grown in separate growth chambers with temperature ranges of 25 to 28°C and 16 to 18°C, respectively. Pots were watered adequately to promote seed germination, seedling emergence, and plant growth and artificial lighting was provided at 12-h photoperiods and approximately 450 μmol of photons/m^2/s.

Based on their performance in the plant-selection study, three warm-season grasses, pearlmillet (*Pennisetum glaucum* [L.] R. Br.), browntop millet [*Brachiaria ramosa* L.], and sudangrass (*Sorghum sudanense* [Piper] Stapf), and one warm-season legume, American jointvetch (*Aeschynomene americana* L.) were chosen to determine their response to N addition in soil that had a TPH concentration of 1.66 $\pm$ 0.08%. Seeds were planted in 500-g dry soil equivalent at a rate of 10 seeds/pot in 25-cm $\times$ 6.4-cm Conetainers® and thinned to three plants/pot after 10 d. The plants were grown for 7 wk in a greenhouse with average maximum and minimum daily temperatures of 33°C and 25°C, respectively. A nonvegetated control was also evaluated. Approximately 100 g of gravel and 1 cm of glass wool were placed in the bottom of each Conetainer® to prevent soil loss.

In the N rate greenhouse study, NH_4Cl was added to the contaminated soil based on TPH-C: total added N (TPH-C:TN) at ratios of 44:1, 33:1, 22:1, and 11:1 that corresponded

to 320, 425, 640, and 1275 mg N/kg soil, respectively. The initial inorganic-N level of the soil was <10 mg N/kg. To determine if an organic N source had a different influence on plant growth than mineral N fertilizer, broiler litter at 44 g dry litter/kg soil or about 100 Mg/ha was added so available N was provided at a ratio equivalent to the 11:1 TPH-C:TN inorganic-N treatment. Available N in the broiler litter was calculated assuming that 90% of inorganic N in the litter was available and 60% of organic N in the litter was mineralized during the 7-wk study (Sims, 1987). The broiler litter contained 4.18% total N, and 1.20% NH_4-N + NO_3-N, 1.66% P, 2.75% K, and 34.15% C. A no-N control was also evaluated. Based on soil-test recommendations for the establishment of warm-season forages, $CaCO_3$ and K_2HPO_4 were added at rates of 2000 and 225 mg/kg, respectively (Chapman, 2000). The P addition was equivalent to 40 mg P/kg. No supplemental lighting was provided and pots were watered daily.

Soil Analysis

For soil-texture determination, organic C was removed from 40 g air-dried soil by adding 5 mL 30% H_2O_2 and heating for 1 h. The soil was dispersed in sodium hexametaphosphate and hydrometer readings were taken at 40 s and 2 hr to determine percentage sand and percentage clay, respectively (Gee and Bauder, 1986). Percentage silt was determined by the difference. Composite soil samples were collected and plant available nutrient content was determined by Mehlich 3 extraction and ICP analysis (Donohue, 1992). Total C and N were determined with a Leco CN 2000® (Leco Corp., St. Joseph, MI). The pH was measured in a 1:2 soil-to-distilled-water ratio. Selected properties of the soils contaminated with weathered crude oil are given in Table 2. The low soil pH reflects the typical acidic property of soils in the study area, where timber production is a major land use. The soil was not limed prior to conducting the plant-selection study, but was limed prior to conducting the N-rate study.

Composite soil samples were collected in duplicate. TPHs were extracted from the soil samples with acetone:methylene chloride (1:1 v/v) using the soxhlet extraction procedure following EPA Method 3540C (USEPA, 1998). The extracts were analyzed by injecting 1 μl of liquid sample to a gas chromatograph (Hewlett Packard 5890 Series II GC) with flame ionization detector following modified EPA Method 8015. The analysis was done at an injector temperature of 300°C and detector temperature of 330°C using cross-linked methyl siloxane (ZB-1), 30 m × 0.25 mm × 0.5 μm film thickness, capillary column. The oven temperature was programmed to increase from 40 to 300°C at a rate of 5°C/min. Ultra-pure helium was used as carrier gas at a flow rate of 50 mL/min. The hydrocarbon

Table 2 Selected chemical and physical properties of the crude-oil–contaminated soils prior to treatments

Mehlich 3 Extractable (mg/kg)								Total (%)			Particle Size TPH (%)
pH	P	K	Ca	Mg	Na	N	C	Sand	Silt	Clay	GC/FID
Plant-selection study											
4.6	2	13	146	17	112	0.05	5.83	81	10	9	2.93
N-rate greenhouse study											
4.7	3	20	208	28	71	0.04	3.32	80	10	10	1.66

components in the crude oil were quantified by integration with blank correction. Surrogate recovery of squalene averaged 103%.

Plant Analysis

In the plant-selection study, seed viability was assessed by determining germination. One hundred seeds were placed in petri dishes containing distilled water and the percentage of seeds exhibiting radicle formation within 9 d was recorded. Seedling emergence from the contaminated soil was determined as the percentage of seeds exhibiting visible aboveground plant material within 14 d of planting. Survival was determined as the percentage of seeds planted in the crude-oil–contaminated soil that emerged and were living 14 d after emergence (DAE) of the first seedling.

In the N-rate greenhouse study, shoot and root biomass were determined for each species and fertilizer-rate combination by cutting the shoots at the soil surface and separating roots from soil, rinsing with distilled water, and drying to a constant weight at 65°C. Prior to root biomass determination, roots were stained with a solution containing 0.1 g methylene blue/L 10% ethanol to determine root length and surface area by WinRHIZO® digital imagery. WinRHIZO® is a scanning system that acquires a digital image of the sample and scans the image to determine root length and an average root radius. Using the root length and average root radius, the root surface area was calculated.

Data Analysis

For the plant-selection study, the experimental design was a randomized complete block with four replications of each species. The study was blocked based on the location in the growth chambers. Due to several 100% values, heteroscedastic variances complicated the analysis of variance analysis (ANOVA) so means and coefficient of variation values for seedling emergence and survival for each species were calculated.

For the N-rate greenhouse study, the experimental design for shoot and root analyses was a randomized complete block with four replications of each plant species and N rate. Data were subjected to ANOVA and means separated by least significant differences (LSDs) at the 5% level with SAS software (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Plant-Selection Study

Warm-season grasses. For warm-season grasses, seed germination in water was >90% for sudangrass, browntop millet, pearlmillet, weeping lovegrass, and large crabgrass (Table 3). Percentages for germination in water and seedling emergence of sudangrass and browntop millet in soil with a TPH level of 2.93% were similar. The results indicate that soil conditions and compounds present in the weathered crude oil did not inhibit seedling emergence of sudangrass and browntop millet. There was a 30% decrease in seedling emergence in oil-contaminated soil compared to germination in water for pearlmillet, weeping lovegrass, and centipede grass. Compared to germination in water, seedling-emergence percentages were reduced by 50% for bahiagrass and large crabgrass species.

Seven of the warm-season grasses had >90% survival rates at 14 DAE. The weathered crude-oil contaminant in the soil did not have a detrimental effect on survival of the majority of the plant species evaluated. Weathered crude oil would typically have low levels of volatile organic compounds that are phytotoxic (Chaîneau, Morel, and Oudot,

Table 3 Germination in water and emergence and survival of plants in crude-oil–contaminated soil for warm-season species

Species	Germination in water %	Seedling emergence %	Survival 14 DAE[1] %
Grasses			
Sudangrass	92	90 (9.1)[2]	97 (6.2)
Browntop millet	91	83 (20.6)	91 (7.7)
Pearlmillet	95	65 (44.5)	100 (0)
Weeping Lovegrass	99	65 (36.6)	100 (0)
Crabgrass—large	97	43 (82.8)	77 (59.4)
Bahiagrass	62	28 (61.1)	73 (64.5)
Crabgrass—RR	66	18 (95.0)	100 (0)
Centipede grass	18	13 (100)	100 (0)
Dallisgrass	48	5 (100)	100 (0)
Legumes			
American jointvetch	87	90 (5.6)	97 (5.7)
Korean lespedeza	76	76 (13.2)	100 (0)
Striate lespedeza	85	73 (6.8)	100 (0)
Hairy vetch	77	73 (34.2)	100 (0)
Sericea lespedeza	14	8 (100)	33 (75.8)

[1]DAE = Days after emergence

[2]The coefficient of variation for each mean is given in parenthesis.

1997). Oil-contaminated soils have been shown to have water-repellent substances derived from petroleum residues (Roy, McGill, and Rawluk, 1999). The hydrophobic properties of the crude-oil contaminant may have reduced germination through the prevention or reduction of water and gas exchange to the seed (Amakiri and Onofeghara, 1984; Udo and Fayemi, 1975).

Warm-season legumes. American jointvetch, Korean and striate lespedezas, and hairy vetch had germination and seedling-emergence percentages of >70% in the crude-oil–contaminated soil (Table 3). Survival of these four species was ≥97%. Sericea lespedeza had low seed viability; therefore, seedling emergence was low in the contaminated soil with 8% of the seeds emerging. Survival of sericea lespedeza also was low, with only 33% of the seedlings surviving 14 DAE.

Cool-season grasses. Germination percentages were ≥87% for the four cool-season grasses studied (Table 4). Ryegrass, wheat, and fescue had ≥90% seedling emergence in the contaminated soil. All species exhibited ≥97% survival during the study. It appears that the soil contamination exerted only small effects on the performance of the species evaluated. Kulakow, Schwab, and Banks (2000) compared 29 plant species or varieties for growth in sediments contaminated with weathered petroleum hydrocarbons and reported that fescue had the top ranking for root-length density after 60 or 180 d growth.

Cool-season legumes. Crimson clover had the highest seedling emergence in oil-contaminated soil for the cool-season legumes evaluated with 100% of the seeds emerging; arrowleaf clover had the lowest with 60% of the seeds emerging (Table 4). Crimson clover also had the highest survival rate and alfalfa had the lowest survival rate. Differences in plant growth are common among genotypes of the same species. Wiltse *et al*. (1998) reported that agronomic performance was reduced and variability existed among alfalfa genotypes grown in a soil amended with 2% crude oil.

Table 4 Germination in water and emergence and survival of plants in crude-oil–contaminated soil for cool-season species

Species	Germination in water %	Seedling emergence %	Survival 14 DAE[1] %
Grasses			
Ryegrass	100	98 (4.8)[2]	97 (5.2)
Wheat	97	90 (0)	97 (5.7)
Fescue	94	90 (13.9)	100 (0)
Oat	87	70 (13.4)	100 (0)
Legumes			
Crimson clover	99	100 (0)	93 (5.4)
Alfalfa	81	83 (5.7)	36 (58.3)
Arrowleaf clover	86	60 (23.5)	50 (22.6)

[1]DAE = Days after emergence.
[2]The percent coefficient of variation for each mean is given in parenthesis.

Cool-season plant species had higher overall germination percentages than warm-season plants. Rogers *et al*. (1996) evaluated germination and survival of four legume and five nonlegume species in soil amended with a mixture of organic contaminants. They reported that germination and survival rates at 10°C were greater than rates at 25°C. Organic chemical concentrations of ≥4000 mg/kg caused an increase in seed mortality and a decrease in plant survival. The researchers hypothesized that the warmer temperature increased volatilization of compounds that penetrated cell membranes, injuring the seed embryo and plant tissue. Absorption of volatile compounds is increased when seed coats are cracked, scarred, or injured (Amakiri and Onofeghara, 1984). Seedlings are generally more sensitive to contaminants than mature plants.

If an arbitrary criteria is accepted that seedling emergence from contaminated soil must be ≥70% and survival must be ≥90%, the following plant species demonstrated potential for use in phytoremediation of the crude-oil–contaminated soil used in this study: Warm-season grasses— sudangrass and browntop millet; Warm-season legumes—American jointvetch, hairy vetch, Korean and striate lespedeza; Cool-season grasses—ryegrass, fescue, wheat, and oat; Cool-season legumes—crimson clover. A literature review by Frick, Farrell, and Germida (1999) indicated that fescue, ryegrass, and sudangrass demonstrated a potential to phytoremediate petroleum hydrocarbons while oat and wheat tolerated petroleum hydrocarbons. Summary information of plants that have potential for phytoremediation of petroleum hydrocarbon contaminants can also be found in the database PHYTOPET (McIntyre, 2003). By using a sequence of cool–warm–cool-season plants, the time that plant roots are actively growing in the contaminated soil would be extended, which could result in an increased contaminant degradation rate. Using a mathematical model, Thoma *et al*. (2003b) indicated that increasing root biomass or the rhizosphere thickness is equivalent for increasing contaminant degradation.

N-Rate Studies

The initial germination and survival study identified plant species that were suitable for vegetation establishment in crude-oil–contaminated soils. However, further studies were necessary to define N levels that would optimize plant growth in crude-oil–contaminated soil. Due to seasonal temperature levels in the greenhouse, only warm-season plant species

were evaluated for N response. The statistical analysis showed a significant N rate by plant species interaction for shoot and root biomass, root length, and surface area.

Shoot and root biomass. Pearlmillet, sudangrass, and browntop millet grown in soil containing 1.66% TPH and amended with either organic or inorganic N at TPH-C:TN ratios of 11:1 produced significantly higher shoot biomass than the same species when grown in soil with ratios of ≥22:1 (Table 5). Approximately twice as much shoot biomass was produced by pearlmillet and sudangrass when grown in soil with organic or inorganic ratios of 11:1 than when grown in soil amended with a ratio of 22:1. Pearlmillet and sudangrass produced higher shoot biomass than browntop millet and jointvetch when grown in soil amended with inorganic N at ratios of 11:1 and 22:1. Jointvetch exhibited no response to fertilizer additions. The maximum plant growth that resulted at the TPH-C:TN ratio of 11:1 indicated that plants growing in crude-oil–contaminated soil should have sufficient N to meet the N needed by the crude-oil–degrading microbial community plus N to maximize plant-growth requirements. Hutchinson *et al*. (2001) found that adding N at rates ranging from 1000 to 2500 mg/kg bimonthly over the course of a year resulted in significantly higher bermudagrass and fescue biomass than in soil amended with 0 or 120 mg N/kg in a petroleum-contaminated soil. They also reported that TPH degradation in fescue and bermudagrass treatments was significantly greater with N and P fertilizer additions, compared to no fertilizer addition.

In highly contaminated soils, adding enough N to achieve low C:N ratios may require adding large quantities of inorganic fertilizer salts, which may have implications with regard to microbial activity (Walworth *et al*., 1997), plant growth through osmotic stresses, and N contamination of ground and surface water. Results from the current study did not indicate the inhibition of plant growth at the highest N rate studied.

The ability of plants to produce higher levels of root biomass in crude-oil–contaminated soil is an important requirement for successful phytoremedation (Robson *et al*., 2003). Pearlmillet and sudangrass produced higher root biomass than jointvetch and browntop millet when the soil was amended with inorganic N at TPH-C:TN ratios of 22:1 and 11:1 (Table 6). Sudangrass produced higher root biomass than browntop millet and jointvetch when grown in soil amended with either organic or inorganic N at a ratio of 11:1.

Table 5 Influence of six N treatments on shoot biomass production of four warm-season plant species grown in a crude-oil–contaminated soil for 7 wk in a greenhouse study

		Shoot Biomass			
N Addition	TPH-C:TN[1]	Pearlmillet	Sudangrass	Browntop millet	Jointvetch
mg N/kg soil		g/plant			
0	—	0.01 g[3]	0.02 g	0.01 g	0.01 g
320	44	0.05 g	0.03 g	0.02 g	0.03 g
425	33	0.54 def	0.17 fg	0.09 fg	0.03 g
640	22	1.00 bcd	0.79 cde	0.33 fg	0.29 fg
1275	11	1.65 a	1.48 a	1.05 bc	0.42 efg
1275BL[2]	11	2.01 a	1.41 ab	0.98 bcd	0.33 fg

[1]TPH-C:TN is the ratio of total petroleum hydrocarbon C to added N.

[2]BL denotes N fertilizer added as broiler litter.

[3]There was a significant N rate by plant species interaction, so all means followed by the same lower case letter in the table are not significantly different ($P \leq 0.05$).

Table 6 Influence of six N treatments on root biomass production of four warm-season plant species grown in a crude-oil–contaminated soil for 7 wk in a greenhouse study

		Root Biomass			
N Addition	TPH-C:TN[1]	Pearlmillet	Sudangrass	Browntop millet	Jointvetch
mg N/kg soil		g/plant			
0	—	13 f[3]	16 f	5 f	12 f
320	44	39 f	15 f	6 f	19 f
425	33	173 de	59 f	16 f	17 f
640	22	344 abc	253 bcd	41 f	64 f
1275	11	465 a	460 a	197 cde	78 ef
1275BL[2]	11	350 abc	342 ab	141 cdef	84 def

[1]TPH-C:TN is the ratio of total petroleum hydrocarbon C to added N.

[2]BL denotes N fertilizer added as broiler litter.

[3]There was a significant N rate by plant species interaction, so all means followed by the same lower case letter in the table are not significantly different ($P \leq 0.05$).

Pearlmillet exhibited higher root biomass when grown at ratios of 11:1 and 22:1 (inorganic) than when grown in soil amended with other ratios, with approximately twice as much root biomass produced when comparing the 22:1 and 33:1 ratios. For inorganic N treatments, sudangrass produced higher root biomass when grown at a ratio of 11:1 than when grown at higher ratios. During the harvesting of roots, nodules were observed on jointvetch roots, especially in pots where no N was added. The nodules exhibited a reddish interior color characteristic of leghemoglobin, suggesting that active N_2 fixation was occurring.

At the 11:1 ratio, there was no difference in shoot or root biomass production for a given plant species between inorganic fertilizer and broiler litter (Tables 5 and 6). In addition to providing plant nutrients, broiler litter improved soil physical conditions by reducing soil bulk density and improving water-holding capacity (Sims and Wolf, 1994). White *et al*. (2003) reported that crude-oil–contaminated soil amended with broiler litter resulted in a significant reduction in TPH compared to the unamended control. The addition of poultry litter has also been shown to increase the amount of bacteria by 80% in gasoline-contaminated soil (Gupta and Tao, 1996). In laboratory and field studies, bioremediation of diesel-fuel–contaminated soil was increased by the addition of broiler litter (Williams, Grimes, and Mikkelsen, 1999) and the addition of dried caged-layer manure enhanced degradation of crude oil in contaminated soil (Ijah and Antai, 2003).

Root/shoot ratios of the four plant species were not different at a given N-addition rate. The highest root/shoot ratio was 0.95 for the no N addition treatment (data not shown). For the four plant species, the root/shoot ratio decreased as the N rate increased with correlation coefficients (R) of −0.806, −0.828, and −0.968 for N-addition rates of 0–1275 (inorganic), 0–1275 inorganic and broiler litter, and 0 to 640 mg N/kg soil, respectively. Even though the root/shoot ratio decreased with increased N addition, the total root biomass was greatest at the highest N rate (Table 6).

Root length and surface area. Pearlmillet yielded significantly higher root length than the other species when grown in soil amended with TPH-C:TN (inorganic) ratios of 22:1 and 11:1 (Table 7). Pearlmillet and sudangrass produced higher root length when grown in soil with a ratio of 22:1 than with a ratio of 33:1. Pearlmillet and browntop millet grown in soil amended with a 11:1 TPH-C:TN (inorganic) ratio produced significantly higher root length than in soil amended with ratios of ≥22:1. Broiler litter reduced the

Table 7 Influence of six N treatments on root length of four warm-season plant species grown in a crude-oil–contaminated soil for 7 wk in a greenhouse study

		Root Length			
N Addition	TPH-C:TN[1]	Pearlmillet	Sudangrass	Browntop millet	Jointvetch
mg N/kg soil		m/plant			
0	—	4.5 fg[3]	3.2 fg	0.6 g	2.3 g
320	44	11.2 ef	2.2 g	1.1 g	2.8 fg
425	33	42.4 cd	7.0 fg	4.4 fg	2.9 fg
640	22	81.7 b	32.7 de	10.2 fg	7.8 fg
1275	11	134.2 a	38.2 cd	36.5 cd	9.0 fg
1275BL[2]	11	67.9 bc	33.7 d	28.7 def	7.3 fg

[1]TPH-C:TN is the ratio of total petroleum hydrocarbon C to added N.

[2]BL denotes N fertilizer added as broiler litter.

[3]There was a significant N rate by plant species interaction, so all means followed by the same lower case letter in the table are not significantly different ($P \leq 0.05$).

root length and surface area of pearlmillet when compared to the 11:1 ratio of inorganic N. White *et al*. (2003) found that the germination of four plant species was inhibited in crude-oil–contaminated soil amended with broiler litter when compared to inorganic fertilizer. The researchers suggested that the uric acid in the broiler litter was sequentially hydrolyzed to urea and ammoniacal N. The hydrolysis results in a pH increase and NH_3 levels in microsites surrounding broiler litter solids could result in reduced root growth in broiler-litter–amended soil when compared to the inorganic-N amendment at the 11:1 TPH-C:TN ratio. Corn (*Zea mays*) root growth has been shown to be acutely reduced in soil contaminated with 1% crude oil (Baek *et al*., 2004).

The influence of N-addition rate on the root surface area was similar to the trend observed for root length. Pearlmillet produced a higher surface area than the other species evaluated when grown in soil amended with 22:1 and 11:1 TPH-C:TN inorganic-N ratios (Table 8). Pearlmillet had the highest root surface area when grown in soil amended with a

Table 8 Influence of six N treatments on root surface area of four warm-season plant species grown in a crude-oil–contaminated soil for 7 wk in a greenhouse study

		Root Surface Area			
N Addition	TPH-C:TN[1]	Pearlmillet	Sudangrass	Browntop millet	Jointvetch
mg N/kg soil		cm^2/plant			
0	—	28 fg[3]	25 fg	4 g	17 fg
320	44	85 efg	17 fg	7 g	22 fg
425	33	559 bc	66 fg	25 fg	23 fg
640	22	645 b	314 cd	69 fg	65 fg
1275	11	1017 a	391 cd	279 de	78 fg
1275BL[2]	11	601 bc	341 cd	207 def	65 fg

[1]TPH-C:TN is the ratio of total petroleum hydrocarbon C to added N.

[2]BL denotes N fertilizer added as broiler litter.

[3]There was a significant N rate by plant species interaction, so all means followed by the same lower case letter in the table are not significantly different ($P \leq 0.05$).

TPH-C:TN (inorganic) ratio of 11:1 than when grown in soil amended with other inorganic ratios or the 11:1 organic ratio. The highest root surface area produced by sudangrass occurred at ratios $\leq$22:1. Jointvetch did not exhibit a response in root surface area to N additions.

CONCLUSIONS

Several warm- and cool-season plant species have been identified as potential candidates to vegetate crude-oil–contaminated soil due to their high emergence and survival. Increasing N rates in soil contaminated with 1.66% weathered crude oil increased warm-season plant growth. Pearlmillet and sudangrass exhibited the highest shoot and root biomass production when grown in soil amended with a TPH-C:TN ratio of 11:1. Pearlmillet had the highest root length and surface area when grown in soil amended with a TPH-C:TN ratio of 11:1. Our results suggest that different plant species will require different amounts of N for successful plant growth. In the future, it would be beneficial to more specifically match N fertilizer rate with contaminant level and to the N needs of specific plants to optimize plant growth and potentially increase the efficacy of phytoremediation.

ACKNOWLEDGMENTS

The authors would like to thank K. J. Davis and Dr. E. E. Gbur for their assistance. This research was supported in part by the Integrated Petroleum Environmental Consortium (IPEC); U.S. Army Research Office (ARO) contract/grant DACA89-97-K-005/DAAG55-98-4-0379; and the Army Environmental Quality Technology Program, work unit EC-B06 BT25 "Biodegradation Processes of Explosives/Organics Using Cold Adapted Soil Systems."

REFERENCES

Adam, G. and Duncan, H. 2002. Influence of diesel fuel on seed germination. *Environ. Pollut.* **120**, 363–370.

Amakiri, J.O. and Onofeghara, F.A. 1984. Effects of crude oil pollution on the germination of *Zea mays* and *Capsicum frutescens*. *Environ. Pollut.* **35**, 159–167.

Anderson, T.A. and Coats, J.R. 1995. An overview of microbial degradation in the rhizosphere and its implications for bioremediation. In: *Bioremediation: Science and Application*, pp. 135–143. (Skipper, H. and Turco, R.F., Eds.). Soil Sci. Soc. Am. Spec. Pub. 43, Madison, WI, Soil Sci. Soc. Am.

Baek, K.-H., Kim, H.-S., Oh, H.-M., Yoon, B.-D., Kim, J., and Lee, I.-S. 2004. Effects of crude oil, oil components, and bioremediation on plant growth. *J. Environ. Sci. Health* **A39**, 2465–2472.

Banks, M.K., Mallede, H., and Rathbone, K. 2003. Rhizosphere microbial characterization in petroleum-contaminated soil. *Soil Sed. Contam.* **12**, 371–385.

Chaîneau, C.H., Morel, J.L., and Oudot, J. 1997. Phytotoxicity and plant uptake of fuel oil hydrocarbons. *J. Environ. Qual.* **26**, 1478–1483.

Chang, Y.-Y. and Corapcioglu, M.Y. 1998. Plant-enhanced subsurface bioremediation of nonvolatile hydrocarbons. *J. Environ. Engin.* **124**, 162–169.

Chapman, S.L. 2000. Soil test recommendation guide. Cooperative Extension Service and Agricultural Experiment Station, Fayettville, AR, University of Arkansas, United States Department of Agriculture, and Cooperating County Governments.

Cunningham, S.D., Anderson, T.A., Schwab, A.P., and Hsu, F.C. 1996. Phytoremediation of soils contaminated with organic pollutants. *Adv. Agron.* **56**, 55–114.

Donohue, S.J. 1992. Reference soil and media diagnostic procedures for the southern region of the United States. Bull. 374. Blacksburg, VA, VA Agric. Exp. Stn.

Frick, C.M., Farrell, R.E., and Germida, J.J. 1999. Assessment of phytoremediation as an in-situ technique for cleaning oil-contaminated sites. Petroleum Technology Alliance of Canada (PTAC), Calgary, AB, Canada.

Gee, G.W. and Bauder, J.W. 1986. Particle-size analysis. In: *Methods of soil analysis, Part 1. Physical and Mineralogical Methods*, pp. 383–411. (Klute, A., Ed.). Agron. Monogr. 9. Madison, WI, Soil Sci. Soc. Am.

Gupta, G. and Tao, J. 1996. Bioremediaiton of gasoline-contaminated soil using poultry litter. *J. Environ. Sci. Health* **A31**, 2395–2407.

Hutchinson, S.L., Banks, M.K., and Schwab, A.P. 2001. Phytoremediation of aged petroleum sludge: Effect of inorganic fertilizer. *J. Environ. Qual.* **30**, 395–403.

Hutchinson, S.L., Schwab, A.P., and Banks, M.K. 2003. Biodegradation of petroleum hycrocarbons in the rhiozosphere. In: *Phytoremediation: Transformation and Control of Contaminants*, pp. 355–386. (McCutcheon, S.C. and Schnoor, J.L., Eds.). Hoboken, NJ, John Wiley.

Ijah, U.J.J. and Antai, S.P. 2003. The potential use of chicken-drop microorganisms for oil spill remediation. *The Environmentalist* **23**, 89–95.

Kulakow, P.A., Schwab, A.P., and Banks, M.K. 2000. Screening plant species for growth on weathered, petroleum hydrocarbon-contaminated sediments. *Int. J. Phytoremed.* **2**, 297–317.

McIntyre, T.C. 2003. Databases and protocol for plant and microorganism selection: Hydrocarbons and metals. In: *Phytoremediation: Transformation and Control of Contaminants*, pp. 887–904. (McCutcheon, S.C. and Schnoor, J.L., Eds.). Hoboken, NJ, John Wiley.

Medina, V.F., Maestri, E., Marmiroli, M., Dietz, A.C., and McCutcheon, S.C. 2003. Plant tolerances to contaminants. In: *Phytoremediation: Transformation and Control of Contaminants*, pp. 189–232. (McCutcheon, S.C. and Schnoor, J.L., Eds.). Hoboken, NJ, John Wiley.

Reynolds, C.M., and Wolf, D.C. 1999. Microbial based strategies for assessing rhizosphere-enhanced phytoremediation. Environmental Technology Advancement Directorate (ETAD) of Environment Canada— Phytoremediation Technical Seminar Series. pp. 125–135. May 31–June 1, 1999. Calgary AB, Canada.

Robson, D.B., Knight, J.D., Farrell, R.E., and Germida, J.J. 2003. Ability of cold-tolerant plants to grow in hydrocarbon-contaminated soil. *Int. J. Phytoremed.* **5**, 105–123.

Rogers, H.B., Beyrouty, C.A., Nichols, T.D., Wolf, D.C., and Reynolds, C.M. 1996. Selection of cold-tolerant plants for growth in soils contaminated with organics. *J. Soil Contam.* **5**, 171–186.

Roy, J.L., McGill, W.B., and Rawluk, M.D. 1999. Petroleum residues as water-repellent substances in weathered nonwettable oil contaminated soils. *Can. J. Soil Sci.* **79**, 367–380.

Sims, J.T. 1987. Agronomic evaluation of poultry manure as a nitrogen source for conventional and no-tillage corn. *Agron. J.* **79**, 563–570.

Sims, J.T. and Wolf, D.C. 1994. Poultry waste management: Agricultural and environmental issues. *Adv. Agron.* **52**, 1–83.

Thoma, G.J., Lam, T.B., and Wolf, D.C. 2003a. Mathematical modeling of phytoremediation of oil-contaminated soil: Model development. *Int. J. Phytorem.* **5**, 41–55.

Thoma, G.J., Lam, T.B., and Wolf, D.C. 2003b. Mathematical modeling of phytoremediation of oil-contaminated soil: Sensitivity analysis. *Int. J. Phytorem.* **5**, 125–136.

Udo, E.J. and Fayemi, A.A.A. 1975. The effect of oil pollution of soil on germination, growth, and nutrient uptake of corn. *J. Environ. Qual.* **4**, 537–540.

USEPA (U.S. Environmental Protection Agency). 1998. SW-846 On-line test methods for evaluating solid wastes, physical/chemical methods. Available at http://www.epa.gov/epaoswer/hazwaste/test/main.htm (verified 10 October 2006).

Walworth, J.L., Woolard, C.R., Braddock, J.F., and Reynolds, C.M. 1997. Enhancement and inhibition of soil petroleum biodegradation through the use of fertilizer nitrogen: An approach to determining optimum levels. *J. Soil Contam.* **6**, 465–480.

Wenzel, W.W., Adriano, D.C., Salt, D., and Smith, R. 1999. Phytoremediation: A plant-microbe-based remediation system. In: *Bioremediation of Contaminated Soils*, pp. 457–508. (Adriano, D., Bollag, J.-M., Frankenberger, Jr., W.T., and Sims, R.C., Eds.). Soil Sci. Soc. Am. Agronomy Monograph 37. Madison, WI, Soil Sci. Soc. Am.

White, Jr., P.M., Wolf, D.C., Thoma, G.J., and Reynolds, C.M. 2003. Influence of organic and inorganic soil amendments on plant growth in crude oil-contaminated soil. *Int. J. Phytoremed.* **5**, 381–397.

Williams, C.M., Grimes, J.L., and Mikkelsen, R.L. 1999. The use of poultry litter as co-substrate and source of inorganic nutrients and microorganisms for the *ex situ* biodegradation of petroleum compounds. *Poultry Sci.* **78**, 956–964.

Wiltse, C.C., Rooney, W.L., Chen, Z., Schwab, A.P., and Banks, M.K. 1998. Greenhouse evaluation of agronomic and crude oil phytoremediation potential among alfalfa genotypes. *J. Environ. Qual.* **27**, 169–173.