

Using Plants to Remediate Petroleum-Contaminated Soil - Project Continuation – Annual Report

Principal Investigators: Greg Thoma,

Period Covered by the Report: July 1, 2001 through June 30, 2002

Date of Report: 7/31/2002

EPA Grant Number: R827015-01-0

Title: Using Plants to Remediate Petroleum-Contaminated Soil - Project Continuation

Investigators: Greg Thoma, Duane Wolf, Susan Ziegler

Institution: University of Arkansas

EPA Project Officer: Bala Krishnan

Project Period: July 1, 2001 to June 30, 2002 (N/C extension till May 15, 2003)

Project Amount: \$145,298

Research Category: Phytoremediation

Objective(s) of the Research Project:

This report covers the period from July 1, 2001 through June 30, 2002. Our goal continues to be to evaluate phytoremediation for clean up of petroleum-contaminated soil through field, greenhouse and modeling studies. To successfully implement phytoremediation in crude oil-contaminated soils, it is necessary to: (i) identify plants that will germinate and survive, (ii) select plants with associated rhizosphere microbial populations to enhance biodegradation of the oil, and (iii) define agronomic management strategies to enhance plant growth and microbial degrader activity.

Field study objectives: Our initial design for the field study included a sufficient number of plots for 6 years of semiannual sampling. We will continue implementation of the field study at the Langley site in Union County, AR that was designed to evaluate appropriate plant species and management systems to enhance phytoremediation of petroleum-contaminated sites. Data from the field study will be used to evaluate the following hypotheses: 1) Significant differences in the remediation associated with each experimental treatment will be strongly correlated to a combination of root biomass and microbial community structure; and 2) Biomass production and microbial community structure can be manipulated through agronomic practices to accelerate site cleanup.

Laboratory study objectives: Our studies with soil amendments have shown increased plant growth, but that the rate of application is problematic. The objectives of the reported research were to: i) determine the effect of N rates on the growth of three warm-season grass species and one warm-season legume, and ii) evaluate the influence of plants on alkane, TPH, and PAH degraders in a crude oil-contaminated soil.

Modeling objectives: The modeling efforts will expand our existing phytoremediation model to incorporate the effects of variable degradation rates in the rhizosphere. In addition, improved models of root turnover for perennial and annual grasses will be included to estimate the influence of roots on the total soil volume during the remediation process. Our working hypothesis is that root turnover and biomass significantly affect the fate of relatively immobile contaminants like weathered crude oil.

MATERIALS AND METHODS

Warm-season Plant N Rate Study

Three warm season grasses, pearl millet (*Pennisetum glaucum* (L.) R. Br.), browntop millet (*Brachiaria ramosa* L. Stapf), sudangrass (*Sorghum sudanense* (Piper) Stapf), and one legume, American jointvetch (*Aeschynomene americana* L.) were grown for 7 weeks in a soil contaminated with 3% by weight weathered crude oil. A non-vegetated control was also evaluated.

Ammonium chloride was added to the contaminated soil based on total petroleum hydrocarbon-C : total added N (TPH-C:TN) at ratios of 80:1, 60:1, 40:1, and 20:1 that corresponded to 320, 425, 640, and 1275 mg N/kg soil, respectively. A nitrogen-free control was also evaluated. Seeds were planted in 500 g dry soil equivalent at a rate of ten seeds/pot and thinned to three plants/pot after 10 days.

Shoot biomass production was determined by clipping the shoots at the soil surface, washing in distilled water, and drying to a constant weight at 60°C. Root biomass was removed from soil, washed with distilled water, and blotted with a paper towel. The roots were digitized and analyzed for root length, surface area, and volume using the WinRHIZO[®] image analysis system.

Field Study

Shoot biomass production was determined by clipping all vegetation 2.5 cm above the soil surface from a 0.5-m² area centered over the microplot in the vegetated plots. Shoot biomass was measured as described previously. Root biomass was sampled by collecting a 5-cm diameter by 15-cm deep core from the microplot. Roots were collected and analyzed as described above.

For soil biological analyses, representative soil samples from the microplots were aseptically collected, place in sterile containers, and transported on ice to the laboratory. Ten-fold serial dilutions were prepared using MPP buffer. Total bacterial numbers were assessed using 0.1 strength Tryptic Soy Agar and fungal levels were determined using Martins Medium. The Most Probable Number (MPN) of alkane, TPH, and PAH degraders were measured using a 96-well microtiter plate procedure.

Soil samples collected from microplots were extracted with hexane:acetone (1:1) by accelerated soxhlet following modified EPA Method 3541 and analyzed for TPH by GC/FID following modified EPA Method 8015 and by gravimetric measurement. Subsamples were extracted with pentane and analyzed for TPH by GC/FID following the TPH criteria working group method.

RESULTS AND DISCUSSION

Warm-season Plant N Rate Study

Pearlmillet produced an average shoot biomass of 0.8 g/plant which was significantly higher than that of browntop millet and jointvetch, which produced 0.5 and 0.2 g/plant, respectively, across all N rates. Sudangrass shoot biomass was 0.6 g/plant and not significantly different from pearlmillet. For root parameters evaluated, pearlmillet yielded significantly higher root length than the other species when grown in soil amended with TPH-C:TN ratios of 60:1, 40:1, and 20:1 (Fig. 1). Pearlmillet grown in soil amended with a 20:1 TPH-C:TN ratio had significantly higher root length than when grown in a soil amended with a ratio of 60:1 with

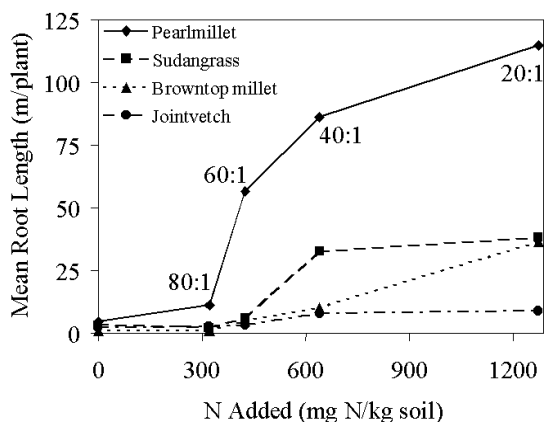


Figure 1. Root length values measured for four plant species following a 7 week greenhouse experiment. The TPH-C:TN ratios are indicated.

approximately twice as much root length being produced. Pearlmillet produced higher surface area than the other species evaluated when grown in soil amended with 60:1, 40:1, and 20:1 TPH-C:TN ratios. Pearlmillet had significantly higher root surface area when grown in soil amended with a TPH-C:TN ratio of 20:1 than when grown in soil amended with ratios of 40:1 and 60:1. If degradation in the rhizosphere is important, these results suggest management approaches that increase the root biomass should be effective at improving phytoremediation.

Microbial analysis revealed that significantly higher populations of total hydrocarbon, alkane, and PAH degraders were present in the rhizosphere of sudangrass and jointvetch than in bulk soil across all N rates. A similar trend was observed in alkane degraders from jointvetch vegetated pots. Although there was not an increase in total PAH degraders associated with jointvetch and decreasing

TPH-C:TN ratios, there was an increase in the contribution to total PAH degraders from the rhizosphere of jointvetch (data not shown). Additional research is needed to elucidate the plant mechanisms that stimulate specific contaminant degrading microbial populations.

Field Study

Initial vegetation establishment was successful at the field site. Subsequent plant growth was reduced due to moisture stress, but continuing and substantial plant growth was evident. Root surface area, and length values were higher at T = 17 months compared to T = 6 months (Table 1).

Table 1. Root growth parameters at T=6 and T=17 months after field plot establishment.

Time Months	Surface Area cm ² /m ³	Length km/m ³
6	20.6 b*	28.4 b
17	108.2 a	125.3 a

* Means in a column with the same letter are not significantly different at the 10% level.

Results from microbial samples at T = 6 and 17 months showed significantly higher bacterial and fungal numbers in the vegetated fertilized plots than in the non-vegetated non-fertilized plots. Bacterial and fungal numbers for bermudagrass-fescue and ryegrass-fescue plots were not different. Alkane and TPH degrader levels were not significantly different across treatments with values of 6.05 and 5.47 log MPN/g dry soil, respectively.

Increasing the number of PAH degraders is essential to achieving more complete levels of crude oil decomposition. Results from T = 6 and 17 months showed a significant increase in PAH degraders in the vegetated fertilized plots compared to the non-vegetated non-fertilized plots. Bermudagrass-fescue + fertilizer and ryegrass-fescue + fertilizer had 2.7 and 2.3 times the number of PAH degraders in the non-vegetated non-fertilized plots, respectively. The increased PAH degrader levels may indicate that more easily degraded compounds have been degraded or are not bioavailable.

Paramount to successful phytoremediation is contaminant reduction to acceptable levels. Gravimetric total petroleum hydrocarbon levels were significantly lowered for bermudagrass-fescue + fertilizer plots at T = 6 months as compared to the non-vegetated non-fertilized plots. The relative degree of crude oil degradation can be assessed by evaluating the resolved peaks (RP) on a chromatogram by baseline skimming. Fresh petroleum products have a much larger number of RPs than more degraded products. Vegetated fertilized treatments had significantly lower RP TPH (by GC/FID) than non-vegetated non-fertilized plots at T = 6 months (Fig. 2). Separation of TPH into aliphatic and aromatic fractions allows a more detailed characterization of the remaining contaminant. At T = 6 months, both aliphatic and aromatic TPHCWG fractions had significantly lower levels in vegetated fertilized plots than the non-vegetated non-fertilized plots (Fig. 2). Contaminant levels at T = 17 months are currently being determined.

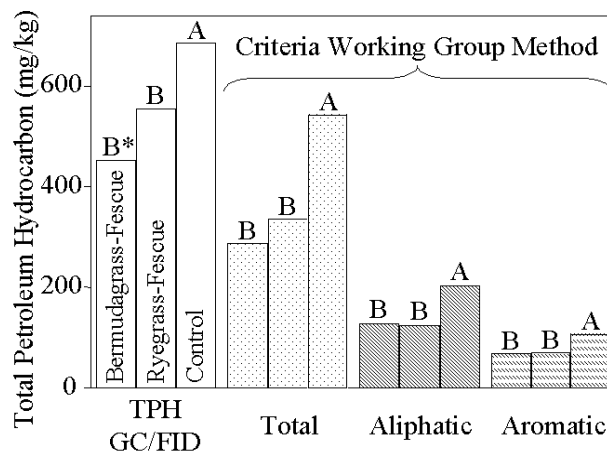


Figure 2. Resolved peak TPH (GC/FID), TPHCWG total, TPHCWG aliphatic, and TPHCWG aromatic levels at T = 6 months. *Bars with the same letter for a given fraction are not significantly different at the 10% level.

Mathematical Modeling

Sensitivity studies of the effect of different plant growth patterns throughout the year were performed after modification of the model equations to include two separate grass species. Simulations in which the average annual biomass was 0.2% (v/v) and 0.45% (v/v) were completed for scenarios in which the root turnover rate

was varied, separately for each species, between 10% and 98% per year. Figure 3 presents a summary of the simulations performed over a range of rooting patterns. These results suggest that the most significant effects of phytoremediation are associated with root biomass expressed as length, and the rate of root turnover.

The simulation results suggest that the high turnover, but lower biomass system is approximately equivalent to the low turnover, high biomass system. This is apparent with the light purple color in the upper left of the lower surface and the dark blue in the lower right of the upper surface.

CONCLUSIONS

In a greenhouse study of four warm-season plants, increasing N application rates in soil contaminated with 3% weathered crude oil increased plant growth. Pearl millet exhibited the highest shoot biomass production and root length, surface area, and volume when grown in soil amended with TPH-C:TN ratios of < 60:1. Petroleum degrading microorganism populations were significantly greater in the rhizosphere of sudangrass and American jointvetch when compared to bulk soil across all N amendment rates.

Vegetation was successfully established at a field site contaminated with 2.5% weathered crude oil. Significant reductions in TPH and TPHCWG concentrations were observed in vegetated fertilized plots as compared to non-vegetated non-fertilized plots at T = 6 months. Analysis of the T = 17 months sampling should allow for more complete evaluation of the phytoremediation treatments. Total bacterial, fungal, and PAH degrader levels were significantly higher in vegetated fertilized plots than in non-vegetated non-fertilized plots. Increased plant growth with the subsequent increase in degrader numbers in the rhizosphere should enhance the overall effectiveness of phytoremediation.

Mathematical modeling of the system suggests that root turnover and root biomass have approximately equal importance in terms of system management for enhancing the rate of contaminant removal.

Technology Transfer

Abstracts and titles of poster or oral presentations given during this quarter include:

Kirkpatrick, W.D., P.M. White, G.J. Thoma, D.C. Wolf, C.M. Reynolds, and E.E. Gbur. 2001. Plant response to N addition in petroleum-contaminated soil. *In Annual Meetings Abstracts [CD-ROM]*. ASA, CSSA, SSSA, Madison, WI.

Thoma, G.J., T.B. Lam, S. Ziegler, D.C. Wolf, R.M. Reynolds. 2001. A mathematical model of phytoremediation of crude oil contaminated soils. *In 8th Annual International Petroleum Environmental Conference*. 6-9 November 2001. Houston, TX.

Thoma, G.J., T.B. Lam, S. Ziegler, D.C. Wolf, R.M. Reynolds. 2001. A mathematical model of phytoremediation of crude oil contaminated soils. *Presented at the Battelle On-site and In-situ bioremediation conference*. 3-5 June 2001. San Diego, CA.

White, Jr., P.M., G.J. Thoma, D.C. Wolf, and E.E. Gbur. 2002. Field evaluation of crude oil-contaminated soil phytoremediation. *In 2002 Southern Branch Agronomy abstracts*. ASA, Madison, WI.

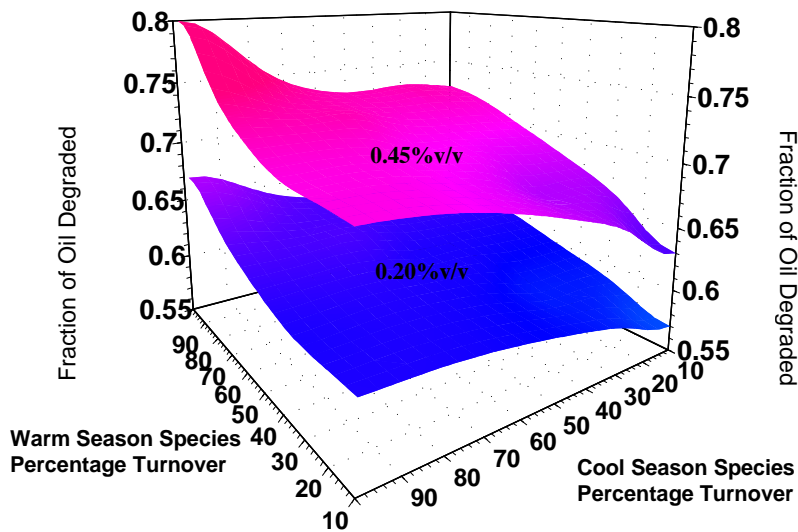


Figure 3. Summary of simulations testing the effects of root biomass and turnover of the efficacy of weathered diesel removal. The upper surface is for a system with 0.45% (v/v) average annual biomass; the lower surface for 0.2% (v/v).

- White, Jr., P.M., G.J. Thoma, D.C. Wolf, and E.E. Gbur. 2002. Field evaluation of crude oil-contaminated soil phytoremediation. *In* 2002 Southern Branch Agronomy abstracts. ASA, Madison, WI. 3-5 Feb. 2002. Orlando, FL. (*Selected as first place winner in M.S. Graduate Soil Science Student Competition*)
- White, Jr., P.M., G.J. Thoma, D.C. Wolf, and E.E. Gbur. 2002. Field scale phytoremediation Phytoremediation of crude oil-contaminated soil. *In* 8th Annual International Petroleum Environmental Conference. 6-9 November 2001. Houston, TX.
- White, P.M., W.D. Kirkpatrick, G.J. Thoma, D.C. Wolf, C.M. Reynolds, and E.E. Gbur. 2001. Field study to evaluate phytoremediation of petroleum-contaminated soil. *In* Annual Meetings Abstracts [CD-ROM]. ASA, CSSA, SSSA, Madison, WI.
- Thoma, G.J., T.B. Lam, and D.C. Wolf. 2002. Modeling phytoremediation for petroleum contaminated soil: Model development. *Int. J. Phytoremed. accepted with minor revisions*
- Thoma, G.J., T.B. Lam, S.E. Ziegler, and D.C. Wolf. 2002. A mathematical model of phytoremediation for petroleum contaminated soil: sensitivity analysis. *Int. J. Phytoremed. accepted with minor revisions*

Supplemental Keywords:

Arkansas (AR), petroleum, phytoremediation, EPA Region 6, rhizosphere

Relevant Websites:

Remediation Development Technologies Forum, www.rtdf.org

Integrated Petroleum Environmental Consortium: ipec.utulsa.edu