

Sixth Quarter Progress Summary for the IPEC project titled,

“Using Plants to Remediate Petroleum-Contaminated Soil - Project Renewal”

EPA Grant Number: R827015-01-0

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Project Period: September 1, 2003 to August 31, 2004 *extended to May 31, 2005*

Project Amount: \$157,212

Research Category: Phytoremediation

This report covers the December 1, 2004 to February 28, 2005 period and summarizes our current IPEC phytoremediation studies that consist of an on-site field project in southern Arkansas, a laboratory study on microbial ecology, and a mathematical modeling project.

Progress Summary/Accomplishments:

Field Study

Materials and Methods

The field site in El Dorado, AR is located in a bermed crude oil storage/separation facility that was the site of an intentional spill in 1997 by vandals. The experimental plots consist of four replicates of the following treatments: (1) nonvegetated-nonfertilized control, (2) ryegrass (*Lolium multiflorum L.*) - fescue (*Festuca arundinacea Schreb.*) + fertilizer, and (3) bermudagrass (*Cynodon dactylon (L.) Pers.*) - fescue + fertilizer. Each field plot has 12 microplots (>soil socks=) that contain homogenized soil that allow monitoring of the field treatments, on a smaller scale, with less effect of field variability of the contaminant levels.

Sampling of the field site at El Dorado, AR occurred at 57 months after plot establishment and data for soil nutrient levels and soil TPH levels are being processed. Analyses for microbial, plant, and soil parameters for the field study sampling at 57 months are complete and statistical evaluation is underway.

Results and Discussion

Analysis of the soil samples collected 57 months after plot establishment show addition of fertilizer and lime resulted in the expected increase in P, K, Ca, and Mg levels. It has become apparent that a leak occurred from the brine separation tank adjacent to the research site, specifically next to the third replication of the fescue/ryegrass + fertilizer treatment (Plot C-1). During the 57 month sampling, it was noted that the majority of the plant material in this plot was dead. The soil test data analyzed has confirmed the brine leak with electrical conductivity values of 4500

umhos/cm for the impacted plot versus 77 umhos/cm for the corresponding plots of the same treatment. Due to this brine impact, data from this plot will not be considered in statistical analysis of treatment differences (Figs. 1 and 2).

Overall, additional fertilizer applications are warranted as a result of harvesting and removing substantial quantities of shoot biomass.

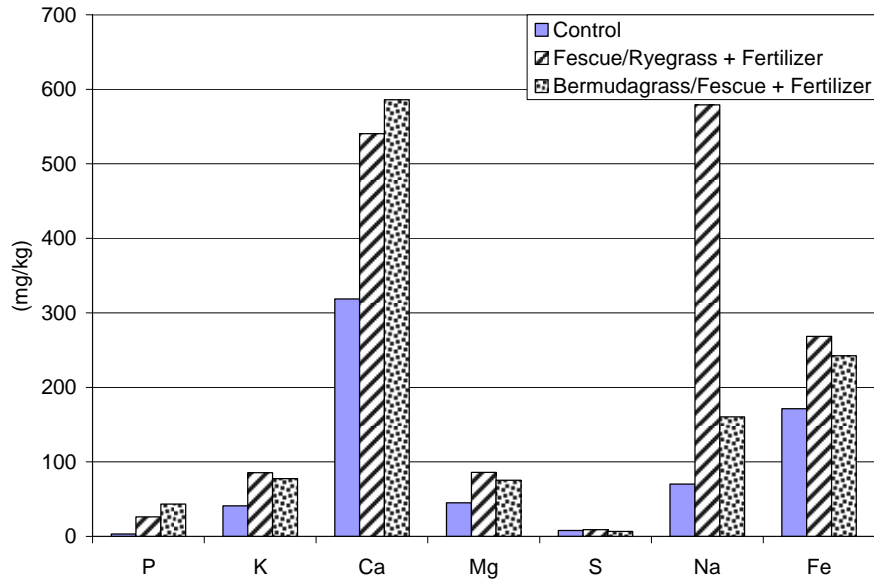


Figure 1. Selected soil chemical values for the three treatments at the El Dorado field site for samples collected 57 months after plot establishment. The brine impacted fescue/ryegrass + fertilizer plot was included in the averages presented.

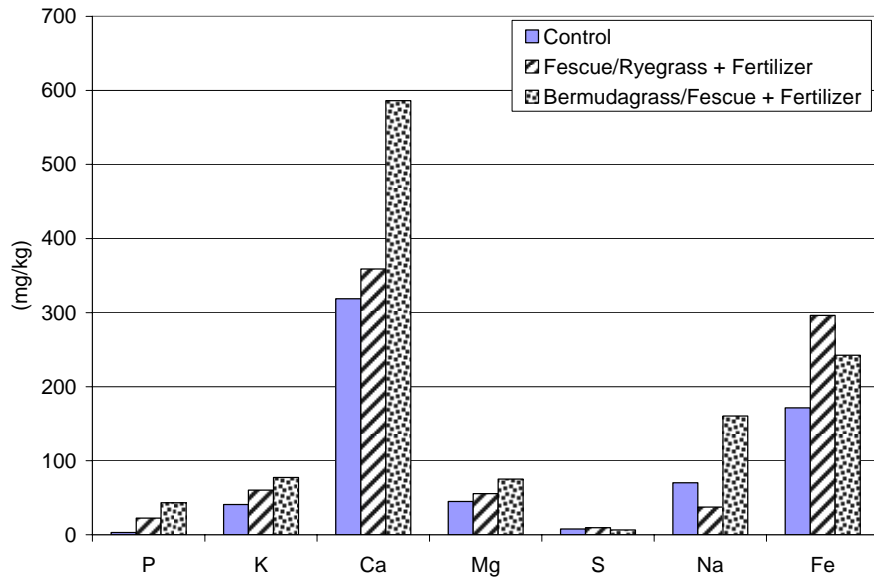


Figure 2. Selected soil chemical values for the three treatments at the El Dorado field site for samples collected 57 months after plot establishment. The brine impacted fescue/ryegrass + fertilizer plot was deleted from the averages presented.

Mini Rhizotron Studies:

We have developed a preliminary technique for quantification of the quantity of deposited pyrene or phenanthrene remaining on the quartz plate over the course of time. Pyrene was deposited on the quartz plate for varying lengths of time, resulting in different quantities of mass per unit area on the plate. Each of these plates was photographed under UV illumination and the fluorescent intensity recorded. A non-linear regression was used to correlate the intensity to the mass deposited. The calibration is shown in Figure 3 below. As expected, as the thickness increases the response

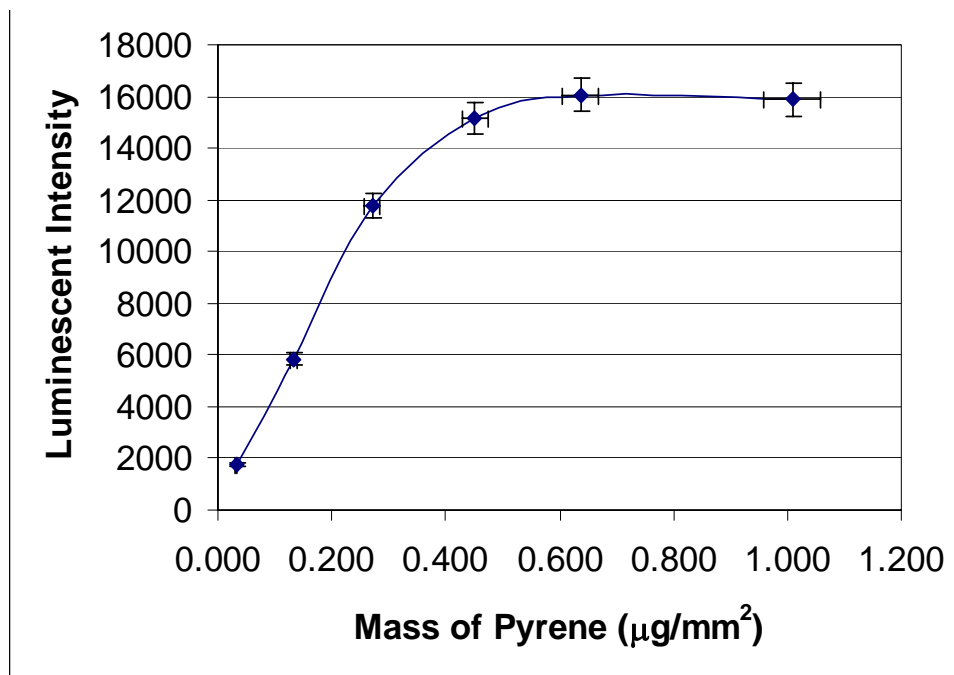


Figure 3. Non-linear correlation between mass deposited and luminescent intensity

becomes saturated, thus defining the allowable working range for the approach. We plan to use this with time series photographs to measure in-situ values for the rhizosphere degradation rate constant.

Publications/Presentations: December 1, 2004 to February 28, 2005

Abstracts and titles of poster or oral presentations:

None during this period.

Abstracts and titles that have been submitted for presentation as posters or presentations in the future include:

Savin, M.C., P.J. Tomlinson, K.J. Davis, S.E. Ziegler, G.J. Thoma, and D.C. Wolf. 2005. Using nematode diversity and maturity indices to assess ecosystem recovery during phytoremediation of a crude oil-contaminated soil. *In* Soil Ecology Society 10th Biennial International Conference. 22-25 May 2005. Argonne National Laboratory, Argonne, IL.

Thoma, G., T.B. Lam, S.E. Ziegler, and D.C. Wolf. 2005. Novel approaches to measurement of rhizosphere effects in phytoremediation of oil contaminated soils. *In* 3rd International

Phytotechnologies Conference. 20-22 April 2005. Atlanta, GA.

Manuscripts submitted:

Kirkpatrick, W.D., P.M. White, Jr., D.C. Wolf, G.J. Thoma, and C.M. Reynolds. 2005. Selecting plants and nitrogen rates to vegetate crude oil-contaminated soil. *Int. J. Phytorem.* (in review).

White, Jr., P.M., D.C. Wolf, G.J. Thoma, and C.M. Reynolds. 2005. Phytoremediation of alylated polycyclic aromatic hydrocarbons in a crude oil-contaminated soil. *Water Air Soil Pollut.* (in review).

Future Activities:

Our initial findings suggest that phytoremediation does reduce contaminant levels through the action of microbial communities associated with the rhizosphere. It is therefore important to develop successful agronomic management strategies that exploit this understanding. However, our detailed knowledge of the microbial ecology of the rhizosphere is lacking. We plan to use carbon-13 isotopic labeling of specific contaminants coupled with phospholipid fatty acid (PLFA) analysis to identify specifically which group of microbes are responsible for the degradation. We will continue to investigate the modes of action of a phytoremediation system; while keeping in mind that the ultimate goal remains site cleanup.

The mathematical model was extended to include climatic effects (specifically temperature and moisture level effects on kinetic degradation rate constants), so more site specific screening can be simulated.

Supplemental Keywords:

Rhizosphere; rhizodegradation; species selection; Arkansas; South Central United States

Relevant Web Sites:

Remediation Technologies Development Forum: www.rtdf.org; IPEC: ipec.utulsa.edu