

Second 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: 11 October 2005 to 10 October 2006

Project Amount: \$133,030

Research Category: Phytoremediation

This report covers the 11 January 2006 to 10 April 2006 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.

The 2005 sampling of the field site at El Dorado, AR occurred at 65 and 69 months after plot establishment. Analyses for microbial, plant, and soil parameters were conducted using standard methods and appropriate quality assurance protocols were followed.

Results and Discussion

As part of the Quality Assurance Project Plan, we have summarized data for the oil degraders, root parameters, and soil nutrient levels for samples collected during the Spring and Fall 2005. Previous reports have summarized data prior to 2005 sampling. Precision was calculated as the Relative Percent Difference (RPD)=[(n1-n2)/mean]100. Range was calculated as (n1-n2) for a duplicate analysis of a given sample. Completeness was determined as the percent of the required samples actually collected and analyzed.

OIL DEGRADER NUMBERS

- A. Precision: A total of four samples were analyzed in duplicate for Alkane, Petroleum, and PAH degrader numbers and the precision estimates for one Alkane and one Petroleum degrader numbers exceeded the corrective action limit of 0.3 log (Fig. 1). The 0.3 log unit limit appeared to be too restrictive for the parameters analyzed. When the data were calculated as RPD, all degrader values were within the 20% limit (Fig 2). We have modified the Quality Assurance Project Plan to reflect this change in our method of estimating precision for this parameter.
- B. Bias: Not applicable for the analyses.
- C. Completeness: Experimental protocol required collection of 12 samples for each of the two sample times and a total of 24 samples were collected and analyzed (Table 1).

ROOT PARAMETERS

- A. Precision: Plant root length, surface area, diameter, and volume were measured in duplicate for 5 samples over the two sample times and all values were below the corrective action limit of 20% (Fig. 3).
- B. Bias: Not applicable for the analyses.
- D. Completeness: Experimental protocol required collection of 12 samples for each of the two sample times and a total of 24 samples were collected and analyzed (Table 1).

SOIL NUTRIENT LEVELS

- A. Precision: Soil chemical parameters were measured in duplicate for four samples over the two sample times. The calculated RPD for pH, electrical conductivity (EC), P, and K has a QC corrective action limit of 10% while Ca, Mg, Na, Zn, and Cu is 20%. For pH, all RPD values were below the 10% limit (Fig. 4). Two of the EC values exceeded the 10% limit by 0.5% and were not considered further. Due to the low extractable P and K concentrations in the soil, two of the four values exceeded the 10% level. All 4 of the Ca, Mg, Na, Cu RPD values fell below the 20% correction action limit (Fig. 5). Three of the four Zn values were within the QC limit. For the Zn, the very low levels resulted in the marginal RPD value for sample D3.
- B. Bias: Not applicable for the analyses.
- C. Completeness: Experimental protocol required collection of 12 samples for each of the two sample times and a total of 24 samples were collected and analyzed (Table 1).

RHIZOTRON IMAGE ANALYSIS

We have been refining the calibration procedures for the determination of the quantity of pyrene remaining in the root zone in the images acquired from the rhizotron apparatus. A feedback control loop has been coupled with pencil heaters embedded in the base of the deposition chamber that now allows the entire deposition chamber to be heated to the operating temperature within about 2 minutes compared to approximately 20 minutes using the hot plate in the previous design. This has led to more rapid turnaround in the preparation of the plates. We are investigating the effects of operating temperature and pressure and deposition time on the uniformity and density of the prepared films.

PUBLICATIONS/PRESENTATIONS: 11 January 2006 to 10 April Abstracts and titles of poster

or oral presentations given during this quarter include:
None during this period

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

- Thoma, G.J., K. Karim, D. Wolf, P. White, O. Alba, and K. Davis. 2006. A Five-Year Field Study to Evaluate Phytoremediation of a Crude Oil-Contaminated Soil. Am. Inst. Chem. Engin. San Francisco, CA, 12-17 November 2006.
- Thoma, G.J., T. Lam, P.-T. Hsu, K. Karim, D. Wolf, and S. Ziegler. 2006. In-Situ Measurement of Rhizosphere Degradation Kinetics. Am. Inst. Chem. Engin. San Francisco, CA, 12-17 November 2006.
- Thoma, G.J., T. Lam, P.-T. Hsu, K. Karim, and D. Wolf. 2006. In-Situ Measurement of Rhizosphere Degradation Kinetics. Am. Soc. Agronomy Abstracts. Indianapolis, IN, 12-16 November 2006.
- Karim, K., G.J. Thoma, P. White, K. Davis, and D. Wolf. 2006. A field study evaluating phytoremediation of a crude oil-contaminated soil. Am. Soc. Agronomy Abstracts. Indianapolis, IN, 12-16 November 2006.
- Kirkpatrick, W.D., P.M. White, Jr., K.J. Davis, D.C. Wolf, and G.J. Thoma. 2006. Petroleum Degradation Microbial Populations in Rhizosphere and Non-rhizosphere Crude Oil-contaminated Soil. Int. Petroleum Environ. Conf. San Antonio, TX, 17-20 October 2006.
- Karim, K., G.J. Thoma, K. J. Davis, E.E. Gbur, and D.C. Wolf. 2006. Influence of Sampling Variability in Assessing Phytoremediation Effectiveness of a Crude Oil-contaminated Soil. Int. Petroleum Environ. Conf. San Antonio, TX, 17-20 October 2006.
- Karim, K. G.J. Thoma, K. J. Davis, and D.C. Wolf. 2006. Using the Criteria Working Group Protocol to Determine Toxicity of Crude Oil-contaminated Soil in a Phytoremediation Field Study. Int. Petroleum Environ. Conf. San Antonio, TX, 17-20 October 2006.

Manuscripts published:
None during this period

Manuscripts submitted:
Kirkpatrick, W.D., P.M. White, Jr., D.C. Wolf, G.J. Thoma, and C.M. Reynolds. 2006. Selecting plants and nitrogen rates to vegetate crude oil-contaminated soil. Int. J. Phytoremed. 8:(in press).

Thesis completed:
None during this period

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.

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

Table 1. Number of samples collected and completeness of sample analyses for critical measurements.

	<u>Sample Time (months)</u>		Total
	65	69	
----- Field Study -----			
-			
<i>Samples Collected</i> -----			
-			
Oil Degradar Numbers	12	12	24
Root Parameters	12	12	24
Soil Nutrient Levels	12	12	24
<i>Samples Analyzed</i> -----			
-			
Oil Degradar Numbers	12	12	24
Root Parameters	12	12	24
Soil Nutrient Levels	12	12	24
<i>Completeness (%)</i> -----			
-			
Oil Degradar Numbers	100	100	100
Root Parameters	100	100	100
Soil Nutrient Levels	100	100	100

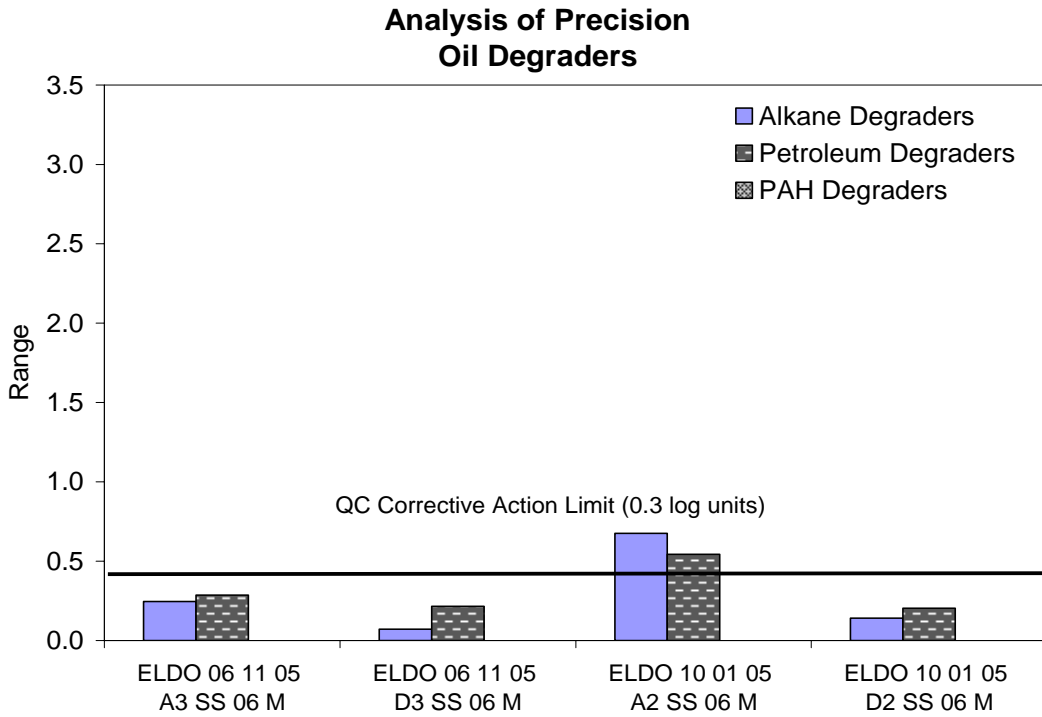


Fig. 1. Oil degrader precision results for four soil samples from the IPEC study at El Dorado, AR, during 2005.

Analysis of Precision Oil Degraders

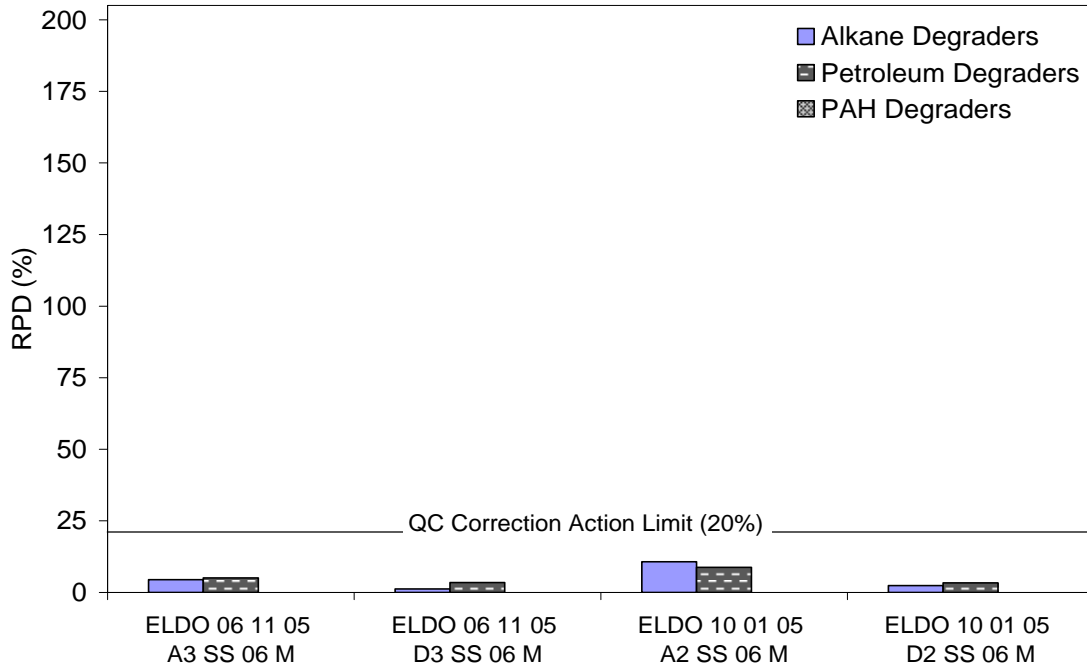


Fig. 2. Oil degrader precision results for four soil samples from the IPEC study at El Dorado, AR, during 2005.

Analysis of Precision Root Parameters

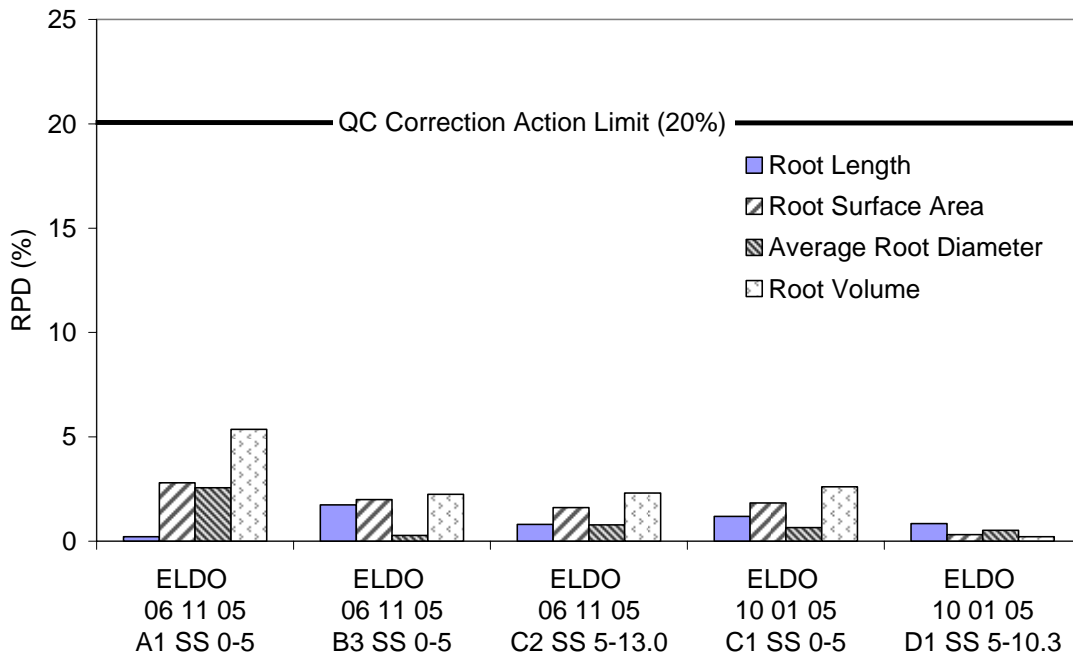


Fig. 3 The root parameter precision results for five soil samples from the IPEC study at El Dorado, AR, during 2005.

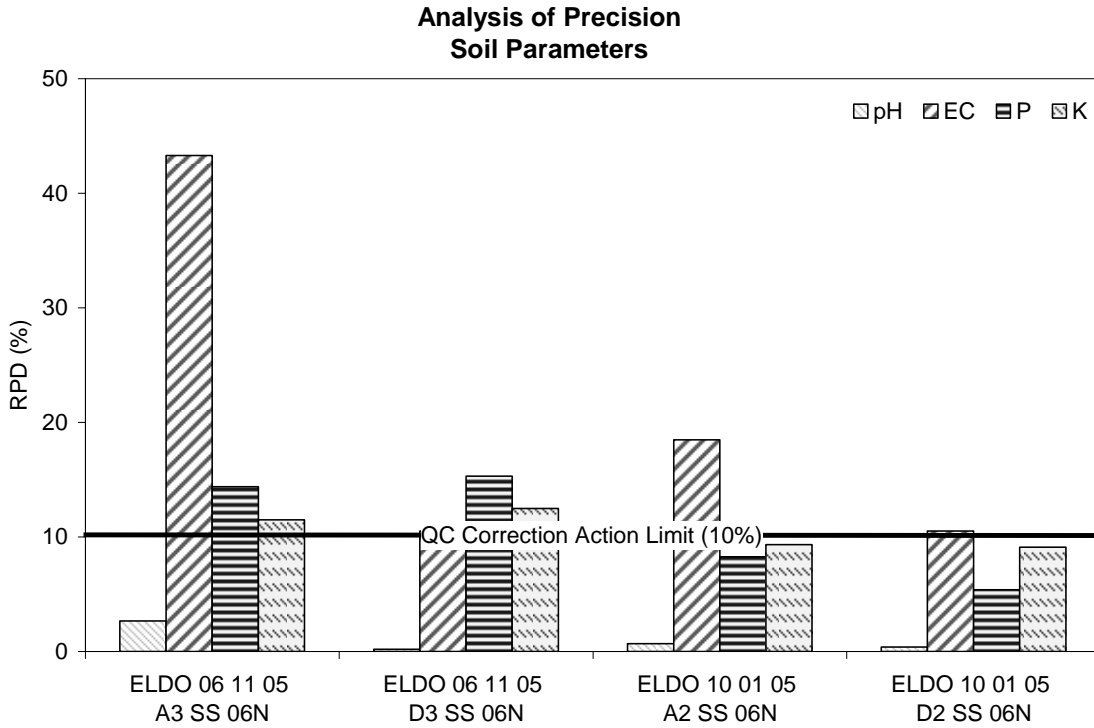


Fig. 4. Soil pH, electrical conductivity (EC), and Mehlich 3 extractable P and K precision results for four soil samples from the IPEC study at El Dorado, AR, during 2005.

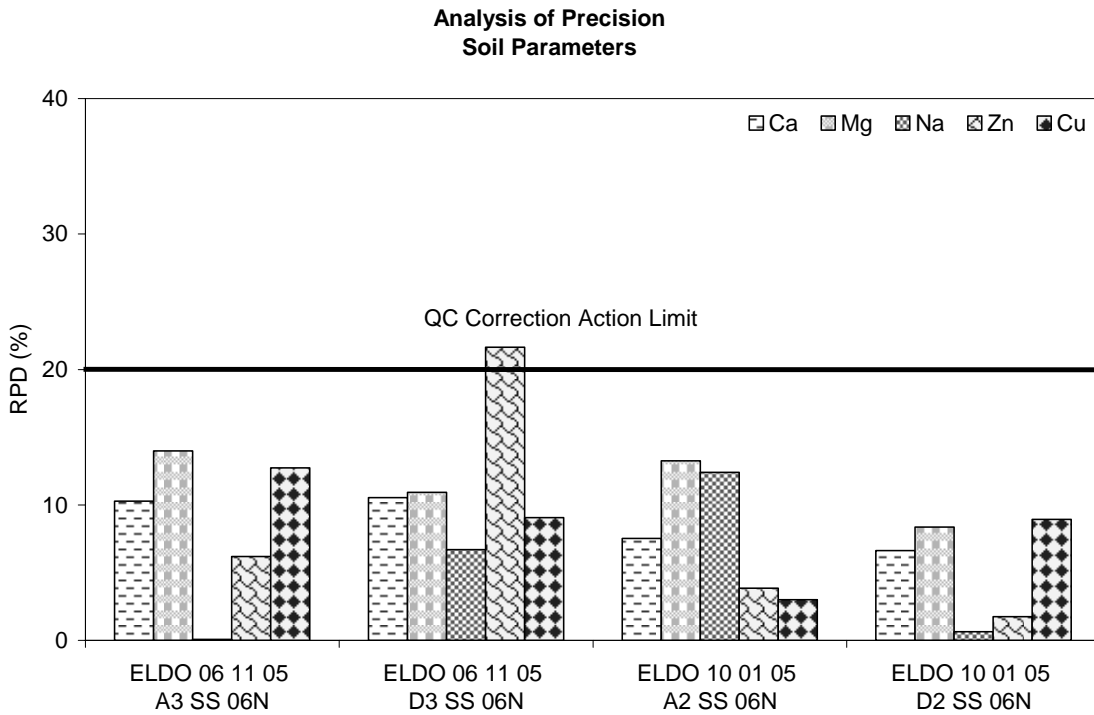


Fig. 5. Mehlich 3 extractable Ca, Mg, Na, Zn, and Cu levels in soil for four soil samples from the IPEC study at El Dorado, AR, during 2005.