

## **Development of Relevant Ecological Screening Criteria (RESC) for Petroleum Hydrocarbon-Contaminated Exploration and Production Sites**

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**Title:** Development of Relevant Ecological Screening Criteria (RESC) for Petroleum Hydrocarbon-Contaminated Exploration and Production Sites

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**Research Category:** Ecorisk Assessment

### **Description:**

#### **Quarterly Progress Report covering the period from October - December 2000:**

The major accomplishments of this quarter were:

- a. Commencement of preliminary soil invertebrate toxicity tests with Enchytraeids
- b. Presentation of research outline and initial results at IPEC conference in Albuquerque, New Mexico
- c. Continued characterization of physical/chemical characteristics of test soils from the Tallgrass Prairie preserve

Research this quarter involved the physical chemical characterization of the hydrocarbon-contaminated soils collected from the Tallgrass Prairie Preserve in Pawhuska, OK. In addition to TPH (aromatic and aliphatic), parameters measured included electrical conductivity, pH, texture, moisture, total soluble salts, potassium adsorption ratio, sodium adsorption ratio, exchangeable potassium percentage, exchangeable sodium percentage, total organic carbon (dry combustion), buffer index, NO<sub>3</sub>-N, N, P, Na, K, Ca, Mg, and boron. All these parameters are summarized in Table 1 of the manuscript prepared for the proceedings of the IPEC conference held in Albuquerque, NM.

Data on bacterial numbers at the study sites was provided for presentation at the 2000 IPEC Conference. Soil samples were collected October 6, 2000 for enumeration of aerobic heterotrophic bacteria and naphthalene-degrading bacteria and for direct extraction of bacterial DNA for molecular analyses. Phenotypic and limited molecular characterization of bacteria isolated from soil samples collected July, 1999 suggested that different species of naphthalene-degrading bacteria dominated a site depending on whether the site had been contaminated with crude oil recently (6 months, "recent spill site") or eight years ago ("old spill site"). This indicates that certain species may have a selective advantage in the biodegradation of the more bioavailable hydrocarbons present at the recent spill site or may be better able to tolerate the environmental conditions imposed by the remediation treatments, and that a different set of species are favored in the old spill site, perhaps due to changes in the bioavailability of hydrocarbons and/or soil environmental conditions. Dominant naphthalene-degrading bacteria from an adjacent uncontaminated control site differed from those present at the two contaminated sites, suggesting that these bacteria can serve as sensitive indicators of crude oil contamination.

Data from soil invertebrate bioassays has been summarized and is presented in Figures 1 and 2 of the manuscript. A preliminary *Enchytraeus albidus* test has been completed and the six-week reproduction test is presently underway. Preliminary seed germination tests indicated low germination rates with seeds of the native prairie plants *Schizachyrium scoparium* (Little Bluestem) and *Andropogon gerardii* (Big Bluestem) with seeds purchased in the fall of 2000. Germination rates for the two species in Tallgrass Prairie reference soils were 45% and 35%, respectively. Tests with a new seed stock of these two species, as well as with lettuce *Lactuca sativa* and turnip *Brassica rapa* will be started in the current quarter. Tests will be conducted according to the ASTM Standard Guide for Conducting Terrestrial Plant Toxicity Tests (1998).

Plans for the current quarter include completion of the toxicity test with enchytraeids, preparation for toxicity tests with plant species, assembling existing data on the toxicity of hydrocarbons in soils, microbial assessment of soils, and further chemical analysis of soils.

Provided below is a paper prepared on the work conducted during this project and presented at the 7<sup>th</sup> Annual IPEC Conference, Nov. 7-10, Albuquerque, NM.

## **DEVELOPMENT OF RELEVANT ECOLOGICAL SCREENING CRITERIA (RESC) FOR PETROLEUM HYDROCARBON-CONTAMINATED EXPLORATION AND PRODUCTION SITES**

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### **ABSTRACT**

Faced with the task of assessing clean-up options based on ecological risk-based criteria at thousands of small upstream sites, oil and gas producers are in need of a streamlined rationale for assessing ecotoxicological risk at these sites. The development of a risk based corrective action (RBCA) approach for the protection of ecological resources provides a mechanism for site-specific ecological risk assessment (1). The proposed research will use crude oil-contaminated soil from the Tall Grass Prairie Preserve, Pawhuska, OK, that varies in hydrocarbon content to conduct laboratory-based toxicity tests with microbes, soil invertebrates, and plants. Chemical exposure for total petroleum hydrocarbons (TPH) has been determined by standard techniques (IR and GC/MS) ranging from below detect limits to 13,243 mg/kg TPH. Toxicity tests have been conducted with soil invertebrates (earthworm *Eisenia fetida*, potworm *Enchytraeus albidus*, and springtail *Folsomia candida*), plant species (*Lactuca sativa*), and soil microbial assessments. Preliminary results show that sublethal effects such as cocoon production have been observed in *E. fetida*.



# INTRODUCTION

Very few soil standards or criteria exist for the protection of ecological resources in hydrocarbon contaminated terrestrial environments. The American Society for Testing and Materials has developed a process for screening contaminated soils under the Risk Based Corrective Action (RBCA) framework. RBCA employs a tiered approach in which successively more sophisticated uncertainty reducing studies are undertaken to determine the most appropriate clean-up level. The RBCA framework was originally created for human health risk assessments, and is now being extended for the protection of ecological receptors. Tier 1 of the ecological version of RBCA proposed to define chemical specific Relevant Ecological Screening Criteria (RESC). RESC can be defined as numerical ecological criteria or guidelines that are applicable to relevant ecological receptors and habitats, exposure pathways, and site conditions (1). An example of a RESC would be a soil level of a petroleum product, defined by some chemical measure that would result in no effect on the growth and biomass of a defined plant species.

We propose to develop a test data set for screening petroleum-contaminated sites that includes the responses of an array of soil macro- and micro-organisms and plants as effects assessment endpoints and measures of bioavailable petroleum hydrocarbon as exposure measures. The Nature Conservancy's Tall Grass Prairie (TGP) Preserve near Pawhuska, OK, provides an ideal site to conduct the proposed research. The TGP contains dozens of private oil production wells and pipelines. One release of dewatered crude oil from a pipeline break in 1999 produced two zones of contamination, both approximately 0.25 hectares in surface area (North Lobe and South Lobe). The general experimental approach will be to collect soil from this release site and appropriate reference areas. Once the TPH gradient has been established, assess petroleum hydrocarbon availability with an array of standard and non-standard test organisms and measure chemical exposure by TPH and also with a passive sampling device, solid phase microextraction fibres (SPME, Supelco Inc., Bellefonte, PA).

Plants, invertebrates and SPMEs will be exposed to petroleum hydrocarbon contaminated and reference soils. Microbial activity will provide a measure of the bioavailability of hydrocarbons to microbes. Selected groups of bacteria (aerobic heterotrophs, naphthalene degrading (NAP), phenanthrene degrading (PHE)) will be enumerated by viable counts on selective agar media to determine if they are reflective of available TPH levels (2). Standard soil chemical (e.g., TPH) and physical (e.g., pH, total organic carbon) parameters will also be measured. The relationships between chemical analysis of soils, the biological responses of microbes, invertebrates and plants will be examined to develop RESC for the Tall Grass Prairie Preserve.

# METHODS

Soils were collected from a crude-oil pipeline release event that occurred in February 1999 at the Nature Conservancy's Tallgrass Prairie Preserve in Pawhuska, Oklahoma. Eight hydrocarbon contaminated soils, one tilled uncontaminated soil, and one untilled prairie reference soil were collected for analysis. All soils were collected in May of 2000 and stored at  $2 \pm 2^\circ\text{C}$  in plastic 5-gallon pails.

Each of the 5-gallon soil samples were individually sieved to remove rocks and vegetative debris and pushed through a 6-mm mesh screen. The soil was then mixed in a 30-gallon drum until soil was visually homogenous. The sieves, screen, and drum were cleaned between samples.

Soil physical and chemical parameters and hydrocarbon characteristics were determined on all contaminated and reference soils. The physical and chemical measurements were taken prior to conducting toxicity tests and include soil pH, electrical conductivity, soil texture and particle size, salinity (total soluble salts, sodium and potassium adsorption ratio), soil nutrients (i.e. NO<sub>3</sub>-N, P, K, B, Ca, Mg), and percent moisture (Table 1).

Hydrocarbon contamination was assessed by extracting soils using accelerated solvent extraction (ASE). Sample extracts were then concentrated and sent to Soil Analytical Services, Inc. for total petroleum hydrocarbon (TPH) analysis. Methods followed were EPA 418.1 and EPA 8015-B. TPH was broken down into aliphatic and aromatic fractions.

Chemical estimates of bioavailability were made on all soil samples using solid phase microextraction (SPME) technology. The method followed has been refined by Wells and Lanno (3) and is described in brief. The SPME fibre was coated with a 7- $\mu$ m thick coating of polydimethyl siloxane (PDMS). A 500 mg sample of soil (dry weight) was placed in a precleaned 21 mm by 70 mm amber vial, filled with 15 mL of deionized water. A stir bar was added and the vial was sealed with a Teflon® PTFE-faced silicone septum and phenolic cap with minimal headspace. The vial was then placed on a magnetic stirrer (~1000 rpm) and the SPME fibre was introduced through the septum. The sample was mixed until steady state was reached (96 hours). At the end of this period, the total moles of hydrocarbon associated with the SPME fibre was quantified by GC-FID.

Six of the eight contaminated soils were chosen for toxicity bioassays based on achieving a suitable concentration gradient for toxicity testing.

### *Invertebrate Bioassays*

Earthworm bioassays were conducted according to standard protocols for reproductive tests (4). Tests were conducted in 900 mL mason jars in replicates of four. Ten clitellate adult *Eisenia fetida* were obtained from laboratory cultures maintained at 24 $\pm$ 2°C. Testing was conducted in controlled environmental chambers at 25°C on a 24-hour light cycle. Continuous light was maintained to encourage the burrowing of worms into the soil. Test soil was weighed and deionized water was added to reach equal moisture content for all soils. In each jar, 500 g (wet weight) of soil was allowed to sit in environmental chambers for 24 hours before the worms were introduced. Worms were fed moistened horse manure on the surface of the soil on days 2, 7, and 14. Jars were checked for any mortalities on days 2, 4, 7, 14, 21, and 28. Any cocoons that were encountered were removed and kept in separate containers in the same environmental chamber. The location of cocoon deposition was noted. On day 28 all worms and cocoons were removed. Worms were wrapped in hexane-rinsed aluminum foil, then stored at -20°C. Cocoons were left to hatch on moistened filter paper until juveniles emerged.

Collembola bioassays were conducted according to a protocol described by Wiles and Krogh (5), with a few modifications. Tests were conducted in 100 mL mason jars with 20 g of soil (wet weight) smoothed to the bottom of the jar. Ten, 10-12-day-old *Folsomia candida* were placed in each mason jar using a fine paint brush. All *F. candida* were collected from our laboratory culture maintained at 22 $\pm$ 2°C. Testing was conducted in controlled environmental conditions at 20°C with a 12:12 light dark cycle. *F. candida* were allowed to acclimate to test conditions for 24 hours prior to placement in jars. *F. candida* were fed a drop of liquid Brewers yeast on a filter paper disk every 4 days. On day 28 all jars were flooded with deionized water dyed dark green (food colouring). This improved the visibility of the white *F. candida* against the dark green background. Each jar was digitally photographed. All photographs were manipulated in Adobe Photoshop using the 'threshold' function. All pixels darker than a set amount

(50) were converted to black. The remaining white spots are counted as individual organisms. Each of the 6 replicates were photographed 3 times to account for the *F. candida* bunching together on the surface of the water.

Enchytraeid bioassays were conducted according to modified protocols of Rundgren and Augustsson (6). Tests were conducted in 4 cm by 5 cm glass jars in replicates of 3. All enchytraeids used in the test were *Enchytraeus albidus* Henle 1837 obtained from our laboratory cultures maintained at  $22\pm 2^{\circ}\text{C}$ . Tests were conducted under controlled environmental conditions at  $20^{\circ}\text{C}$  in darkened chambers. Ten clitellate *E. albidus* over 1 cm in length were placed in test jars. Each jar was filled with 10 g (wet weight) of soil. *E. albidus* were fed 1 mg of dried ground oats on day 4 and 14. On day 21 all worms were removed and the soils were returned to environmental chambers for 3 more weeks. Then all worms were preserved in amber tubes and stored at  $-20^{\circ}\text{C}$ .

### *Plant Assays*

Plant assays were conducted according to procedures outlined by Saterbak (7). Seeds were obtained from the Holman Seed Company (Colinsville, OK). After soils were brought to a similar moisture content, 100 g was placed in 9-mm diameter petri dishes. The soil surface was smoothed and 10 seeds were gently pressed into the soil surface of each petri dish. Seeds of similar size and color were selected for the test. Petri dishes were covered and kept in darkened environmental chambers at  $20^{\circ}\text{C}$  for 4 days. At the end of the test, seeds containing visible roots were considered germinated. Root lengths were measured to the nearest mm. All germinated and ungerminated seeds were then stored in amber tubes at  $-20^{\circ}\text{C}$ . To date, the test has been conducted with Lettuce (*Lactuca sativa*, Iceburg). The test has been conducted twice, both with replicates of five for each soil type.

### *Data Analysis*

$\text{EC}_{50}\text{s}$  for growth or reproduction were analysed by ANOVA with a subsequent *a posteriori* Tukey's Honestly Significant Difference test ( $\alpha=0.05$ ).

# RESULTS & DISCUSSION

## *Soil Physical – Chemical Characteristics*

Soil pH values ranged from 5.5 to 7.0 (Table 1). Soil electrical conductivity for 9N1 was higher (963 umhos/cm) than in all other samples. 9N1 was collected closest to the road, and may be road affected. High sodium readings were found in ES1 (Na:178, SAR: 9, ESP: 10). Plant macro and micronutrients were found to be similar between samples. Nitrate-nitrogen in 6N1 was somewhat higher than other samples.

The TPH results in the release areas show a gradient of hydrocarbon concentration from below detect limits to 13,243 mg/kg. The aliphatic fraction was greater than the aromatic fraction in all oil affected soils, except 7N1. There was a large amount of heterogeneity between duplicate samples sent for TPH analysis. The TPH profile revealed that soils sampled closest to the pipeline break were not necessarily the highest in TPH concentration. The highest level of contamination was found in the north lobe of the spill area.

## *Soil Invertebrate and Plant Analysis*

No mortalities were observed throughout the *E. fetida* toxicity test. Cocoon production was compared over the four-week period (Figure 1). A statistically significant difference exists with cocoon production between the different levels of TPH in soil ( $\alpha=0.05$ ,  $n=35$ ,  $p<0.05$ ). Juvenile production will be reviewed separately. Multifactorial analysis was performed on cocoon production, Na, ESP, and SAR. No interaction was found with these parameters.

Collembola reproduction does not seem to be related to increased TPH in these TGP soils (Figure 2). A high amount of variability existed within and between concentrations. Juveniles began to emerge in all containers by the third week of the test.

Preliminary results for the *E. albidus* bioassay indicate minimal lethality. Sublethal measures of cocoon production will be monitored.

Lettuce germination assays show no apparent effect on germination success in different soil TPH concentrations. Sometimes, the addition of petroleum hydrocarbons enhances the growth of corn (8), so multiple species tests are needed to complete the plant database before RESC can be developed.

A collection of petroleum ecotoxicity benchmarks summarized by Efroymson (9) found a wide range of toxicity benchmarks for plants and soil invertebrates (between 17 mg/kg and 20 000 mg/kg). Because the availability of petroleum is sensitive to changes in soil organic matter, soil pH, etc., a more thorough review of the site-specific data is needed to create appropriate RESC for Tallgrass Prairie soil. Future work includes toxicity assays with native plant species (i.e. Big Bluestem), a more complete SPME analysis and a rigorous data review.

# ACKNOWLEDGEMENTS

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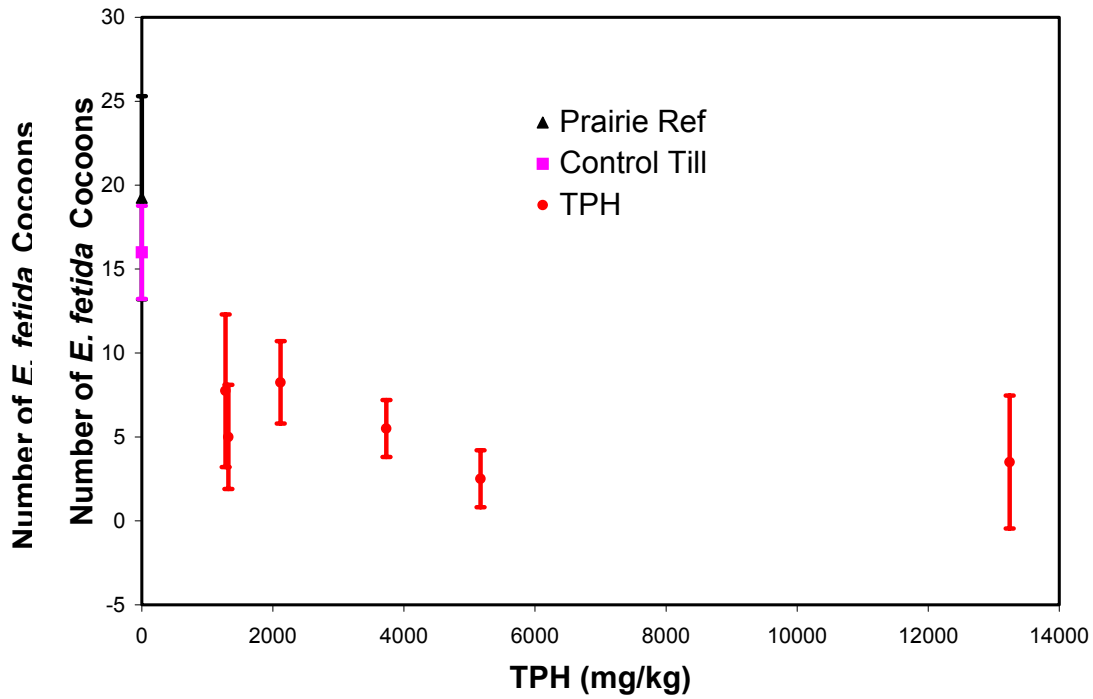


Figure 1. Effects of TPH on cocoon production of *Eisenia fetida* in a standard 28-day reproduction test. Values are mean (n=4) cocoons produced by day 28 with 95% CI.

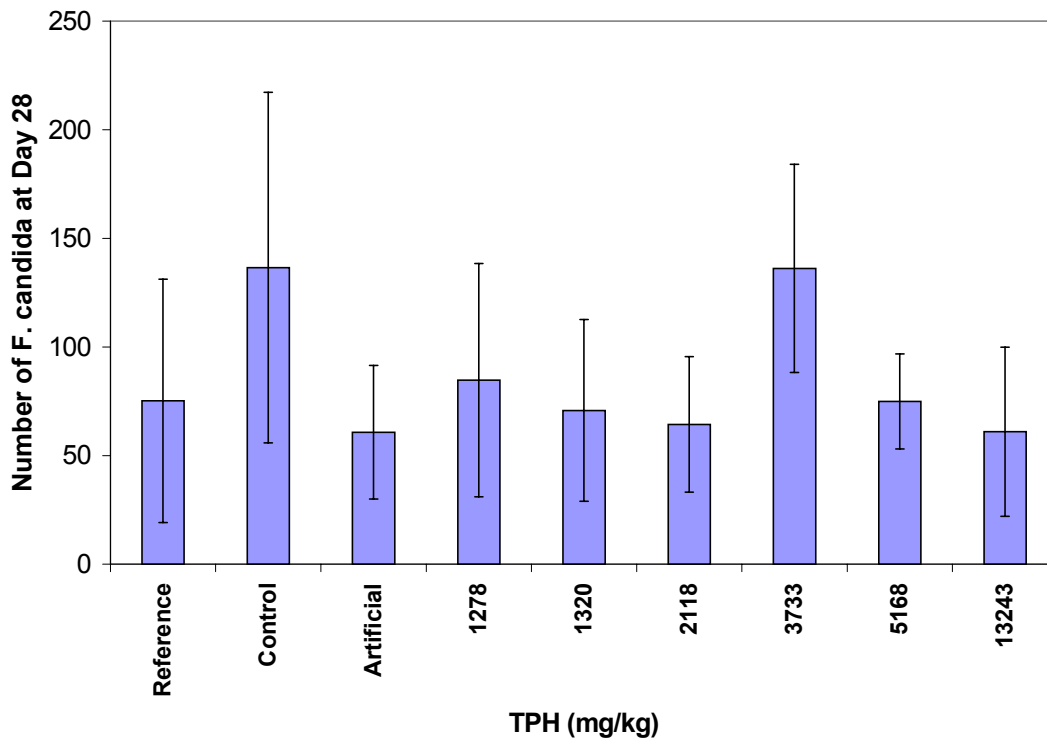


Figure 2. Effects of TPH on reproduction of *Folsomia candida* of TPH contaminated field soil in a 28-day reproduction test. Bars represent mean (n=6) number of *F. candida*  $\pm$ SD.

Table 1. Physical-chemical characteristics of Tallgrass Prairie Soil.

Physical-Chemical Test	Soil Sample					
	Reference	Control	6N1	7N1	8N1	9N1
aliphatic (mg/kg)	0	0	1086.3	6184.2	3316.7	1356.3
aromatic (mg/kg)	0	0	233.8	7058.3	1851.7	761.3
pH (units)	5.5	5.9	5.8	6.2	6.2	7.0
EC1 (umhos/cm)	591	861	870	291	461	963
Texture	medium-coarse	fine-medium	fine-medium	fine-medium	fine-medium	fine-medium
Na (ppm)	26.9	29.7	74.1	53.7	21.3	177.0
K (ppm)	6.5	11.1	8.3	5.6	4.6	5.6
Ca (ppm)	33.4	67.9	54.6	37.9	32.3	52.9
Mg (ppm)	10.6	17.8	26.2	14.5	12.2	11.7
Boron (ppm)	0.1	0.1	0.1	0.2	0.1	0.1
TSS2 (ppm)	390.1	568.3	574.2	192.1	304.3	635.6
PAR3	0.15	0.18	0.14	0.11	0.10	0.11
SAR4	1.04	0.82	2.06	1.87	0.81	5.74
EPP5	4.89	5.23	4.80	4.59	4.47	4.50
ESP6	0.27	0.30	1.74	1.46	0.26	6.68
TOC7 (%)	1.88	2.31	2.89	2.29	3.46	2.29
Buffer Index (units)	6.67	6.80	6.77	7.00	6.83	
NO3-N (ppm)	5.17	11.83	27.17	1.83	1.67	1.50
P	7.33	7.00	7.67	5.67	5.67	10.00
K	135.0	232.0	190.0	215.0	185.3	152.0
percent moisture	10.0	18.4	21.8	23.0	22.5	19.6

1 = Electrical

Conductivity

2 = Total Soluble Salts

3 = Potassium

Adsorption Ratio

4 = Sodium Adsorption

Ratio

5 = Exchangeable Potassium

Percentage

6 = Exchangeable Sodium

Percentage

7 = Total Organic Carbon determined by dry combustion using a LECO 2000CN