

# **A Continuation – Humate-Induced Remediation of Petroleum Contaminated Surface Soils – Post Project Period Report #2 Through December 31, 2004**

**Period Covered by Report:** June 4, 2004 to December 31, 2004

**Date of Report:** January 13, 2005

**EPA Grant Number:** 14-2-1201270-94843

**Title:** A Continuation: Humate-Induced Remediation of Petroleum Contaminated Surface Soils

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**EPA Project Officer:** Bala Krishnan

**Project Period:** September 4, 2002 to September 3, 2003

**Project Amount:** \$133,136

**Research Category:** Bioremediation of oil spills

## **Objectives of the Research Project:**

The objectives of this proposed research are: 1) to measure the biodegradation and bioavailability of <sup>14</sup>C-labeled heptadecane that is strongly adsorbed to humate, 2) to examine the adsorption of gasoline, gasoline oxygenates as a part of gasoline itself, and diesel fuel to humates, as well as their biodegradation in soil microcosms, and 3) to assess humate-induced remediation on a pilot-scale by conducting *ex-situ* tests of soils contaminated with crude oil, diesel fuel, and gasoline.

## **Progress Summary/Accomplishments:**

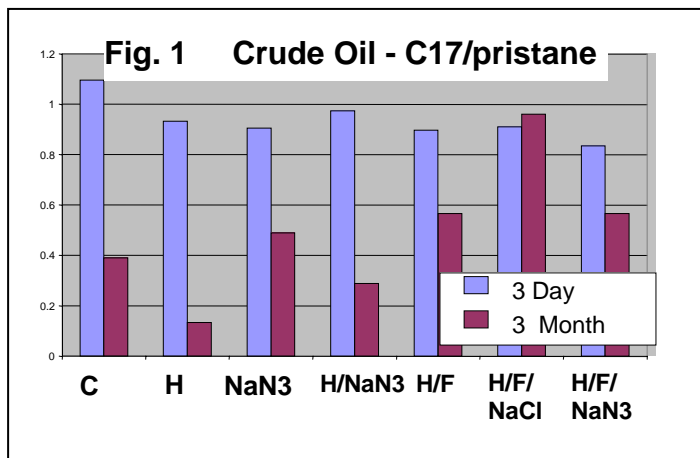
### **1. Analysis of Gasoline and Crude Oil Microcosm Samples (Day 3 and 3 Months)**

Crude oil and gasoline microcosms were sampled at day 3 and 3 months to examine humate's ability to enhance the biodegradation of gasoline and crude oil hydrocarbon components. The soil used in the microcosms originated from contaminated surface soils obtained from the field site near Electra, TX that was once a petroleum refinery. The humate used in these studies was obtained from Cuba, New Mexico (Earthgreen Menefee humate).

Analysis of the gasoline samples showed that the linear hydrocarbons typically present in gasoline are degraded relatively quickly in the control samples. The addition of humate, however, seemed to hinder this degradation. It is hypothesized that the sorption of these smaller linear alkanes to the humate decreased their availability to microbial degradation. An unexpected discovery was that the presence of humates enhanced the degradation of larger cyclic hydrocarbons, such as naphthalenes and phenanthrenes, present in gasoline. We don't have a solid explanation for this trend at the moment.

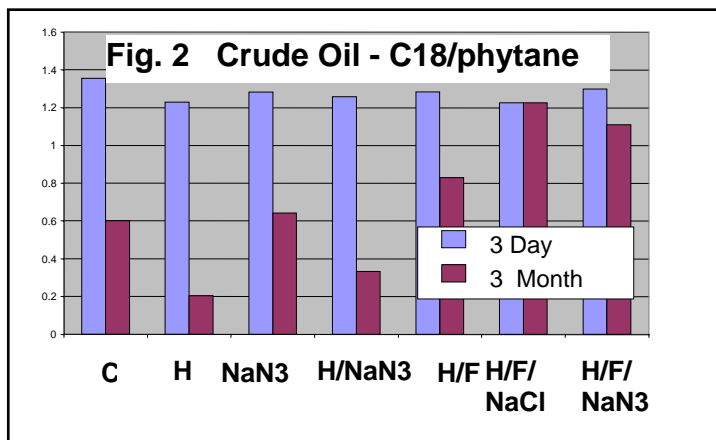
Figures 1 and 2 present the C<sub>17</sub>/pristine and C<sub>18</sub>/phytane ratios, simple indicators of initial biodegradation, for the crude oil microcosms. In both cases, humate enhanced biodegradation relative to the control microcosms. Figure 3 illustrates that the presence of humate resulted in a fairly equitable degradation of the linear alkanes regardless of length. Thus, this humate facilitates the biodegradation of linear alkanes up through C<sub>29</sub>. It is interesting to note that the presence of sodium azide (NaN<sub>3</sub>), used as a sterilizer, only moderately slowed biodegradation,

however the presence of sodium chloride (NaCl) prevented biodegradation. Likewise, just based upon the C<sub>17</sub>/pristine and C<sub>18</sub>/phytane ratios, it appears that fertilizer slows biodegradation, however, examination of the GC trace in Figure 4 shows that the presence of fertilizer and humate allowed for the degradation of linear alkanes and branched alkanes.



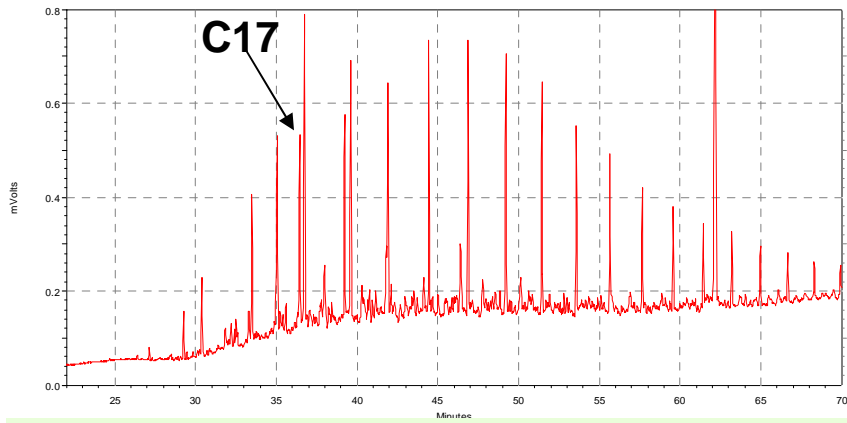
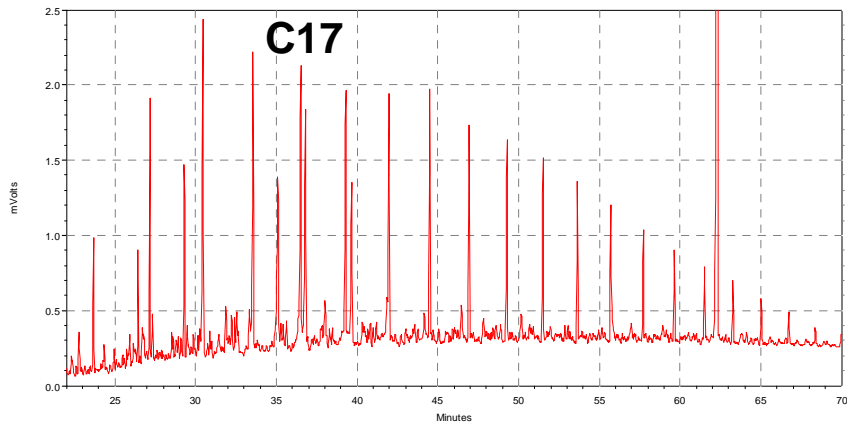
**Legend for Figures 1 & 2**

C = Control  
H = Humate  
NaN3 = sodium azide  
F = fertilizer  
NaCl = sodium chloride



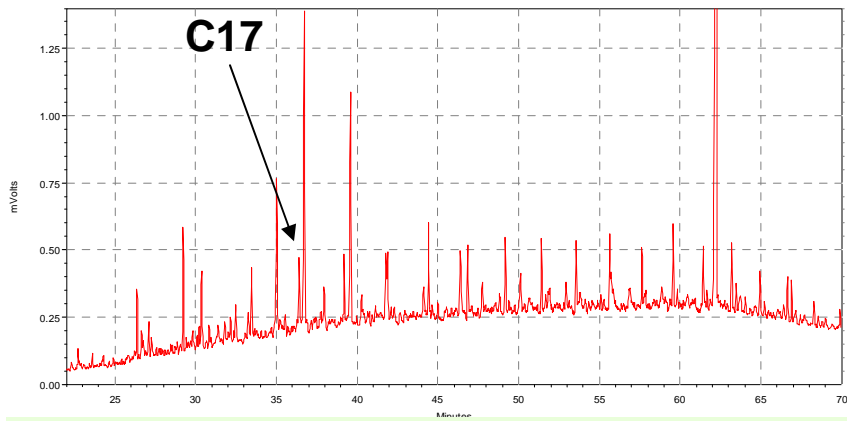
## Crude Oil: Control

3 Day



## Crude Oil: Control

3 Months

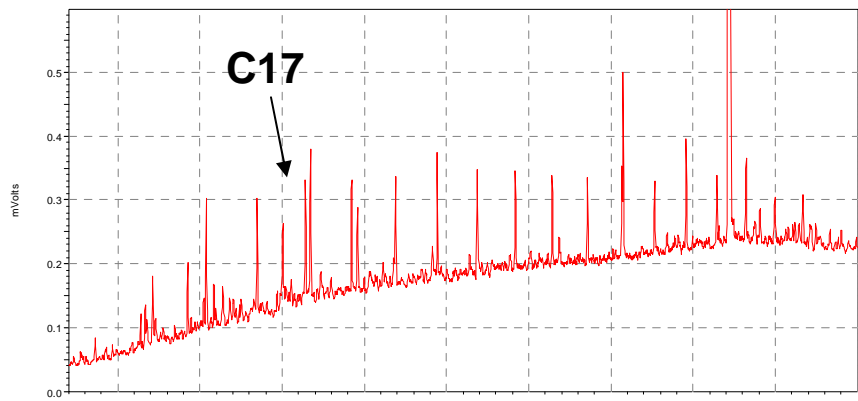
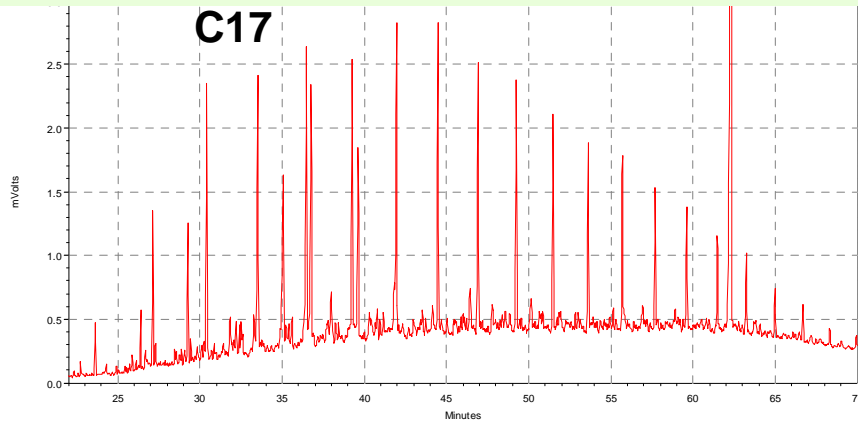


## Crude Oil: Humate

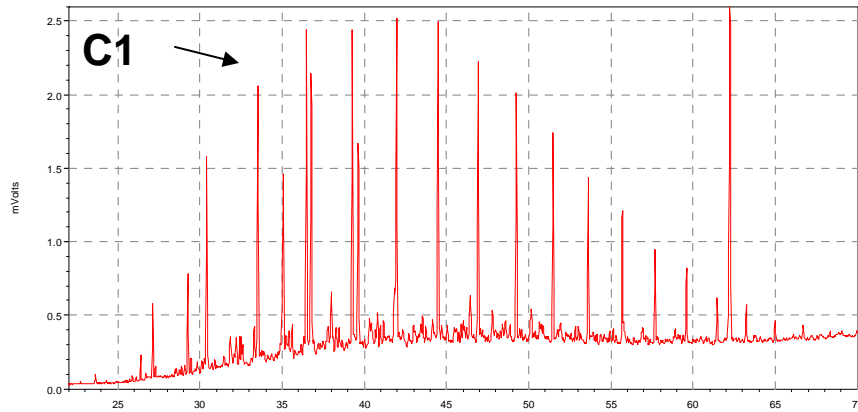
3 Months

Figure 3: GC traces of crude oil contaminated soil at Day 3 and 3 months, and with humate at 3 months.

## Crude Oil: Humate + Fertilizer 3 Day



## Crude Oil: Humate + Fertilizer 3 Months



## Crude Oil: Humate, Fert., NaCl 3 Months

Figure 4: GC traces of crude oil contaminated soil with fertilizer and humate at Day 3 and 3 months, and with humate, fertilizer, and sodium chloride at 3 months. Note that the presence of NaCl prevents biodegradation.

## 2. Analysis of Waggoner Field Site Samples

Windrows were created at the Waggoner Field Site as a small pilot-scale demonstration experiment. Unlike the microcosm studies, no attempt was made to control the moisture, temperature, or aeration. Unlike the microcosms that were aerated every 3 days, maintained at room temperature, and kept moist in amber jars, windrows were subject to drying and sunlight (and hence temperature variations). Examination of the  $C_{17}$ /pristine and  $C_{18}$ /phytane ratios (Figures 5 & 6) for the control versus humate addition for the crude oil contaminated soil (the same crude oil contaminated soil used in the microcosms) showed only moderate biodegradation of the linear alkanes over three months and no enhancement by the addition of humate. It is hypothesized that the drying and heating cycles resulted in limited biodegradation of the linear alkanes. If 12 month samples show limited biodegradation, efforts to minimize drying will be considered (such as covering windrows with a plastic tarp).

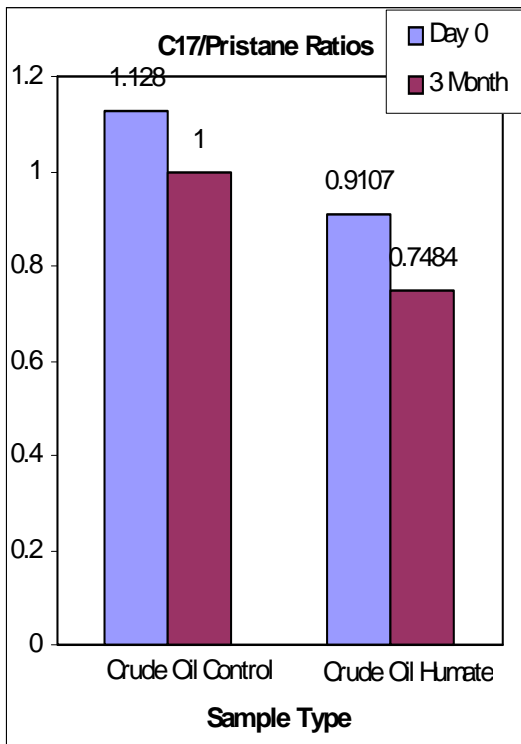


Figure 5 – C17/Pristane Ratios

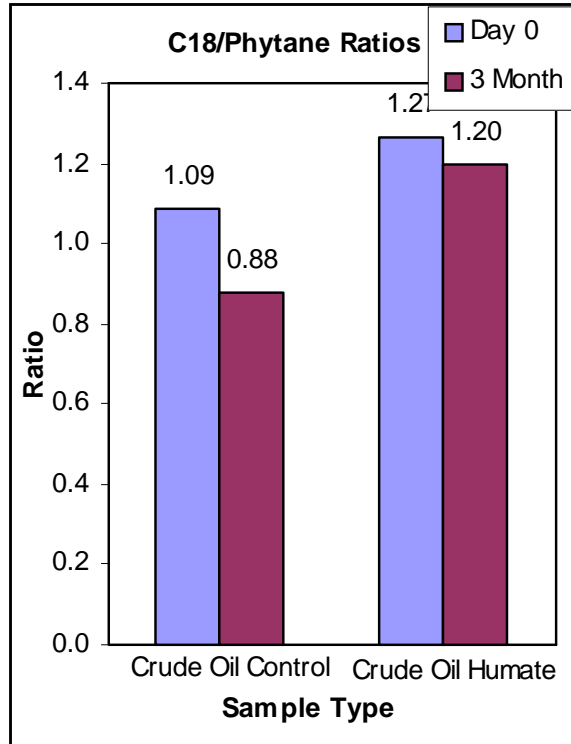


Figure 6 – C18/Phytane Ratio

## **Publications/Presentations:**

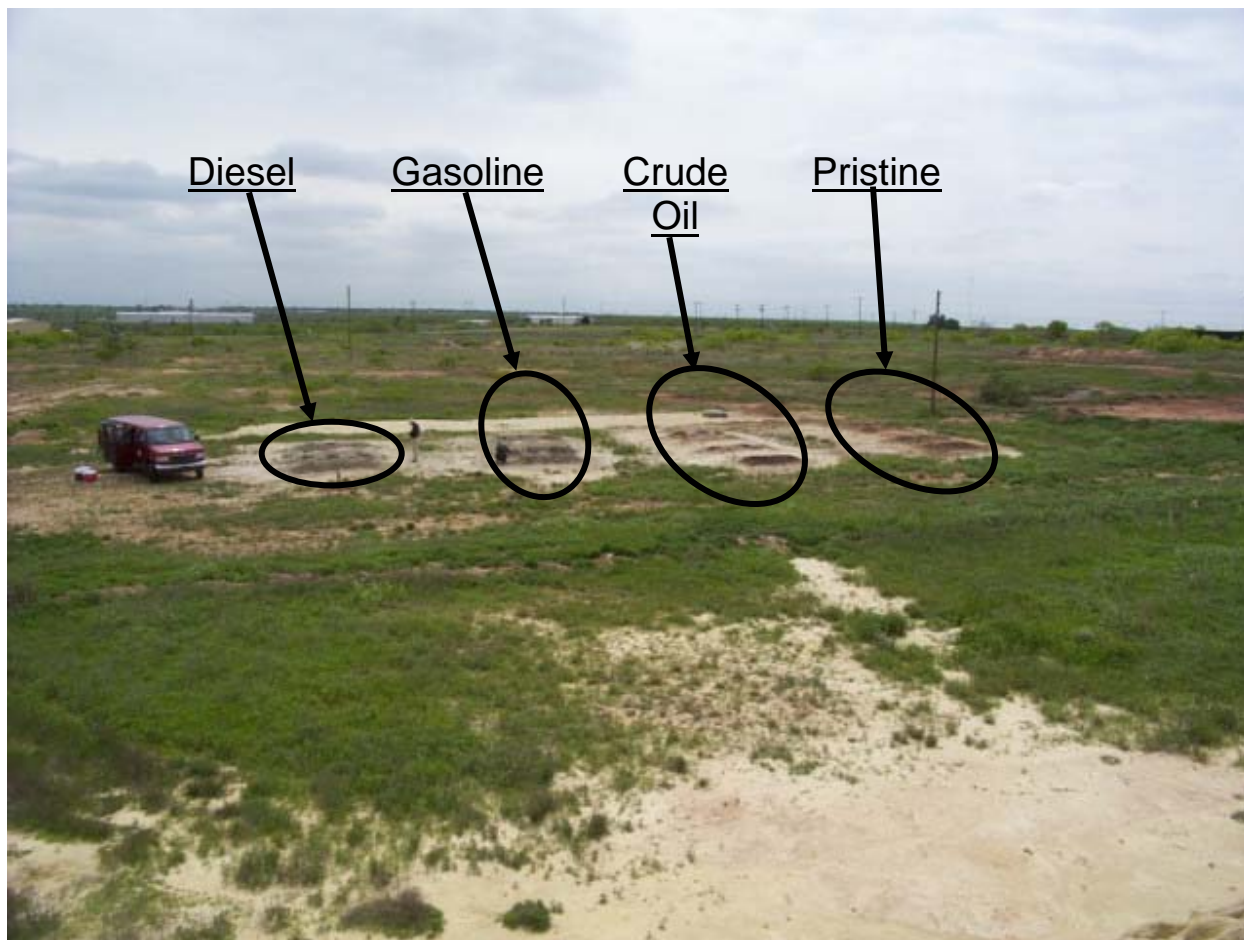
### **Research Presentation in Tomsk, Russia**

From August 12 – 15, 2004 Drs. Mark Nanny and Vlad Andrus visited Dr. Anatoly Golovko and his research group at the Institute for Petroleum Chemistry, Tomsk, Russia, and gave a research presentation on their IPEC-sponsored humate research. They discussed IPEC-sponsored humate research, as well as began to develop international research collaborations in this area. Travel funding was from the COBASE grants program. As a result, we are going to study the use of synthetic humates (prepared by the ozonation of asphaltenes and heavy oils; a method developed by Dr. Golovko's research group) for enhancing the remediation of surface soils.

### **Future Activities:**

- Continue sampling of pilot-scale tests consisting of 20 windrows of soils contaminated with gasoline, diesel fuel, and crude oil at Waggoner refinery site (Figure 7). We intend to continue sampling at the site for at least another year in order to fully assess long-term changes in the hydrocarbon composition as a function of humate treatment. Samples are being extracted, prepared, and analyzed by an undergraduate student supported by Dr. Nanny's SRI funds.

**Supplemental Keywords:** gasoline, diesel fuel, crude oil, microcosm, humate, adsorption



**Figure 7: View of Waggoner Field Site near Electra, TX looking east. Four rows consisting of five windrows each are shown. The five windrows are: control; humate amended; fertilizer amended; humate + fertilizer amended; and humate, fertilizer, and NaCl amended.**