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Effect of Humic Acids on the Bioavailability of  
PAHs from weathered Soils

By:

S. Lesage, W. Li, K. Millar, D. Liu

NWRI Contribution # 99-201

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No. 99-  
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## EFFECT OF HUMIC ACIDS ON THE BIOAVAILABILITY OF PAHS FROM WEATHERED SOILS.

S. Lesage, W.C. Li, K. Millar and D. Liu.  
National Water Research Institute, Burlington, Ont., Canada. L7R 4A6

**ABSTRACT:** This is the last report on a comprehensive multi-year study on the effect of humic acids on the dissolution and biodegradation of aromatic hydrocarbons of petroleum origin. Early phases of the work showed that the addition of humic acids enhanced the dissolution of hydrocarbons from light fuels such as diesel, and thus indirectly helped the bioremediation, because dissolution is often rate limiting. The second phase of the work using radiolabelled phenanthrene, pyrene and benzo-a-pyrene showed that humic acid retarded the degradation of the pure PAHs spiked onto soil, but that this effect was reversed when the petroleum product was also added. This indicated that biodegradation was dependent on the relative sorption of PAHS onto soil, or humic acids. Sandy soils were predominantly used in these early studies. In the current work, humic acids were added to highly weathered clay soils. A subset also including freshly spiked PAHs was included as a control. The hypotheses to be tested were whether humic acids could displace the PAHs on the clay surface and enhance bioremediation and whether weathered PAHs were more difficult to degrade than freshly introduced contaminants. The results showed that humic acids had no noticeable effect on the biodegradation of either weathered or spiked soil. The most important factor was the presence of a microbial population capable of effecting the degradation.

### INTRODUCTION

In our previous studies, the addition of humic acids was found to increase the rate of dissolution of PAHs from diesel fuel in groundwater (Xu et al., 1994). The dissolved plume was also found to undergo biodegradation (Lesage et al., 1995). However, this initial experiment did not address the effect of different concentrations of humic acids on the biodegradation process itself and on higher molecular weight PAHs present in heavier fuels or crude oil. A separate study was undertaken in the laboratory, using microcosms, to look at the effect of humic acids on phenanthrene, pyrene and benzo-a-pyrene as representatives of the PAHs. In the first phase of the study (Lesage et al., 1996; 1997a), where the pure compounds were spiked to a sandy soil, the presence of high concentrations of humic acids was found to decrease the amount of  $^{14}\text{C}$  labelled phenanthrene that was mineralized. However, when crude oil was added to the mixture, this tendency was reversed. It was therefore postulated

that the lower amount of mineralization observed was due to sorption of either the parent compound or its metabolites onto the humic molecule.

In this last phase of the project, the effect of adding humic acid to a weathered clay soil was examined. Humic acids have been shown to bind strongly with clays (Filip and Alberts, 1994; Spark et al., 1997). Therefore, this experiment was designed to verify whether this binding would interfere with that of the PAHs, potentially displacing the aromatic molecules from the clay surface and increasing their bioavailability.

The sample came from a composite of soils from a decommissioned refinery. The levels of PAHs were relatively low, and most of the easily degraded hydrocarbons were absent from this fine clay soil. It was therefore decided to run two parallel sets of microcosms, one with the soil as received, and the other with an additional amount of PAHs spiked in. This would also show whether there was a significant difference in the bioavailability of spiked vs. weathered residues of PAHs. In addition to a mineral medium, a mixed bacterial consortium that has been found to degrade phenanthrene was added to ensure that at least some degradation would be observed. A non-inoculated control was also used. The degradation was followed by doing a solvent extraction and a GC/MS analysis of the parent compounds.

#### METHODS

The contaminated soil was given to us by Imperial Oil of Canada Ltd. and came from a decommissioned refinery undergoing remediation. The soil was a highly weathered clay from various areas on the refinery grounds, which had received various petroleum contaminants over a few decades. The soil was air-dried, ground and sieved through a 2-mm mesh sieve, then homogenised. A 1 kg portion of the soil was also spiked with acetone solutions containing phenanthrene (500 mg), pyrene (50 mg) and benzo-a-pyrene (50 mg).

The microcosms were made up in 500 mL Mason jars. Each contained 100 g dried soil which was mixed with 25 mL of mineral medium and sodium humate (Aldrich Chemicals, Milwaukee) at 0, 50, 500 or 2000 mg/L. A 10 mL bacterial inoculum (0.08 absorbance at 600 nm) of a consortium that has been selected for its ability for growing on phenanthrene was also added. The final soil moisture content was approximately 80% of field capacity. Abiotic controls were made by adding 0.02% sodium azide and by omitting bacterial inoculation. The samples labelled "control" were not inoculated and did not receive sodium azide.

The microcosms were opened and mixed on a weekly basis to enhance the oxygen availability. Periodic sampling consisted of removing 20 g of soil (wet wt.) and stopping the biodegradation by adding 5 mLs of a 2000 ppm solution of sodium azide. The samples were air-dried and ground before extraction. The

samples (10g dry wt.) were extracted by Soxhlet for 8 hours using a 1:1 mixture of hexane/acetone. The extracts were concentrated to 1 mL using a Snyder column and then made up to 20 mL with hexane. A 2 mL aliquot was cleaned up on a column of activated silica gel (11 x 300 mm). The aliphatic hydrocarbons were eluted with hexane and the PAHs with hexane containing 20% dichloromethane. Both fractions were analysed by GC/MS, using a 25 m x 0.2mm i.d. x 0.33µm film Ultra-2 (Hewlett-Packard) capillary column. The oven temperature was: 50°C to 180°C at 30°C/min, then to 300°C at 3°C/min - held for 20 min. d10-Anthracene was used as the internal standard.

#### RESULTS

The results of the degradation of phenanthrene in the spiked and unspiked soil are shown in Figure 1 a and b respectively. In the spiked sample, there was no loss of phenanthrene in the abiotic control or in the abiotic control to which humic acid had been added. The degradation was rapid, > 90% in two weeks, in all the other samples, regardless of the presence of humic acid. The degradation of the non-inoculated control lagged behind the other samples.

Similar results were obtained with the unspiked samples, although the difference between the inoculated samples and the control was not as dramatic, because of the much lower initial concentration of phenanthrene. The degradation was faster with all the inoculated samples compared to the non-inoculated control, except for the highest concentration of HA, which was similar to the control at two weeks. Overall, the most significant factor in the degradation of phenanthrene seems to be the addition of the acclimated bacteria. There was no significant difference in the half-life of phenanthrene between the spiked and weathered samples, although the residual concentration after 20 weeks was higher in the spiked samples (average 8 vs. 1 µg/g).

The results for pyrene are combined in one graph (Figure 2). The degradation of pyrene in the weathered soil was negligible in all samples within the 20 week period of the study, but there was a 50 percent reduction in concentration in the spiked samples within the same period. There was however no difference between humic acid treatments. Similar to what was observed with phenanthrene, the slowest degradation was in the non-inoculated control. The addition of a bacterial consortium, even if it wasn't of bacteria specifically acclimated to pyrene did have a positive effect, although it was much less important than with phenanthrene.

The degradation of benzo-a-pyrene (results not shown) was not significant in either the weathered or the spiked samples. This is not very surprising, because as was shown in previous work (Lesage et al., 1997b), benzo-a-pyrene has been found to be recalcitrant to bacterial degradation because of its combined low solubility and the difficulty to enter the cell. Fungal degradation has obtained much more success.

Figure 1a. Degradation of phenanthrene with/without HA in weathered clay spiked with 500 mg/kg phenanthrene.

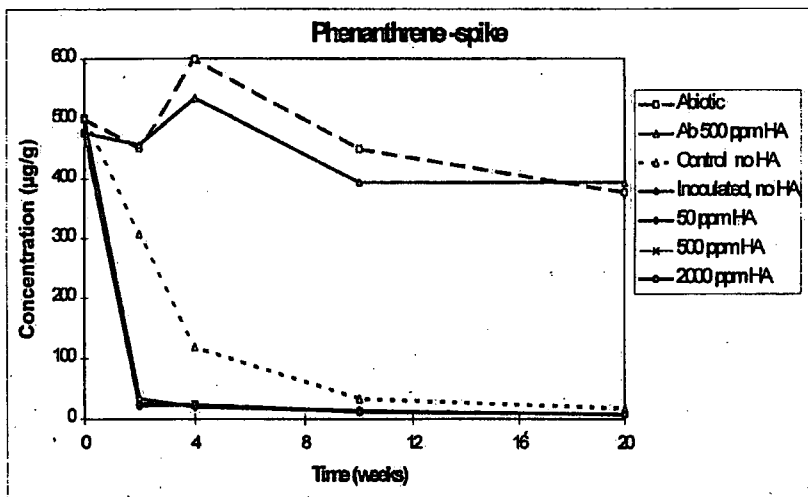


Figure 1b Degradation of phenanthrene with/without HA in weathered clay.

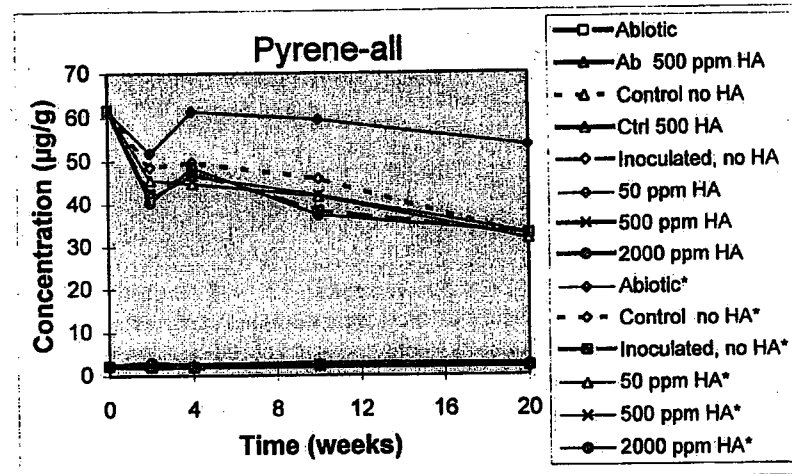
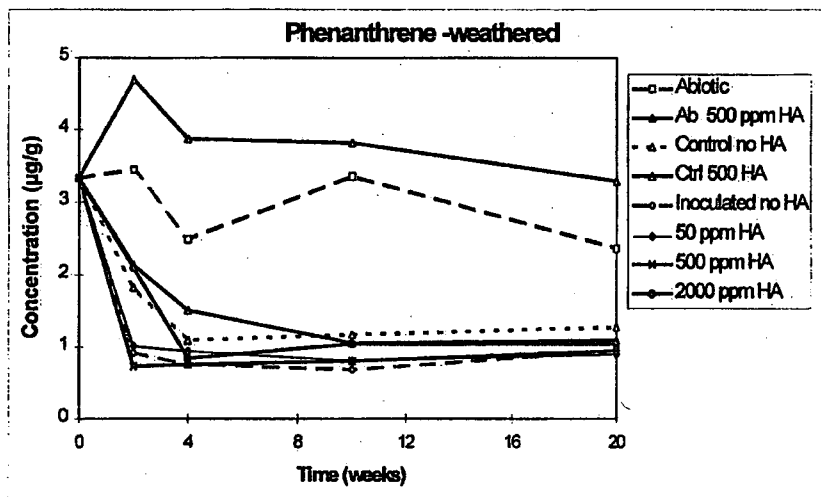


Figure 2. Degradation of pyrene in weathered and spiked\* samples in the presence of increasing concentrations of humic acid (HA).

## CONCLUSION

In summary, the addition of humic acid to a clay soil did not improve the bioavailability of the PAHs. The rate of degradation of individual compounds seems to be governed mostly by the presence of a bacterial population capable of degrading them. In the case of phenanthrene, the rate of degradation was not very different between the spiked and weathered sample. The degradation of pyrene was only significant in the spiked sample, and no significant degradation of benzo-a-pyrene was observed in the 20-week period. Apart from a slight decrease in the degradation of phenanthrene in the presence of 2000 ppm of humic acid in the weathered sample, humic acids had no discernible effect on the degradation of PAHs.

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## ACKNOWLEDGEMENTS

Funding for this project was received from the Panel on Energy Research and Development (PERD) of Natural Resources Canada and from Environment Canada. The authors are indebted to Imperial Oil of Canada Limited in Montreal, Canada, for providing the contaminated soil.

## IN SITU BIOREMEDIATION OF DEGRADABLE CONTAMINANTS FROM A FORMER WOOD TREATMENT FACILITY

Barry C. O'Melia, Randy Siegel, Gary Dupuy, Geoffrey C. Compeau, (University of Greiner Woodward-Clyde Seattle), RueAnn Thomas (International Paper, Memphis)

Tim Syverson (GeoSyntec Consultants)

**ABSTRACT.** A cleanup action using biosparging and bioventing at a former wood treatment site was implemented in October 1998. The objective was to remediate mobile waste components of creosote and petroleum hydrocarbons including monoaromatic and polycyclic aromatic hydrocarbons (PAHs). Two source areas subject to treatment are separated by a nearly contiguous silt layer. The groundwater in the lower system is anoxic under existing conditions. Sparged air is being injected in the lower saturated groundwater and upper system. Baseline field data suggest that populations of hydrocarbon-degrading microorganisms exist in most site areas. Additional operational data, including respiration and nutrient analysis, are being used to continually optimize and evaluate system effectiveness.

## INTRODUCTION

Cleanup of soil and groundwater impacted during past wood treatment operations requires an approach addressing a unique and complex mixture of recalcitrant and degradable organic contaminants. Carcinogenic polynuclear aromatic hydrocarbon (cPAH) compounds typically encountered at these sites are comprised of 4-7 condensed aromatic rings and are difficult to biodegrade. However, these contaminants are relatively insoluble with limited mobility in the environment. When exposure pathways to human and environmental receptors are mitigated or eliminated, impacted soil and groundwater can often be left in place. The key to this strategy is to effectively treat the more mobile PAHs and condensed aromatic rings at wood treatment sites, which are amenable to biodegradation.

**Background.** The site is a former wood treatment facility with creosote, pentachlorophenol and petroleum hydrocarbon impacted soil and groundwater. Investigations showed that groundwater migration from this site is extremely limited and containment of the soils and groundwater with a slurry wall surrounding the area covered with an engineered low permeability cover effectively eliminated migration pathways. In situ enhanced biodegradation using bioventing/biosparging is being implemented to further mitigate and reduce concentrations of contaminants of concern (COCs). Site COCs have varying physical properties affecting mobility (Table 1).

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