Final Report Executive Summary

Period Covered by the Report: May 1, 2003 – April 30, 2004
Date of Report: September 19, 2005
EPA Grant Number: R83-0633-010
Title: A Continuation of Remediation of Brine Spills with Hay
Investigators: Laura P. Ford and Kerry L. Sublette
Institution: University of Tulsa
EPA Project Officer: Bala Krishnan
Project Period: May 1, 2003 – April 30, 2004
Project Amount: $35,314
Research Category: Soil Remediation

Objective(s) of the Research Project:

The overall objective of this project is to study the possible positive effect of organic matter in the remediation of brine-impacted soil. The efficacy of this treatment will be quantified in a field study conducted in the Tallgrass Prairie Preserve in Osage Co., Oklahoma. The study will also attempt to identify the mechanism or mechanisms of action of the organic matter, which possibly include: 1) a strictly physical effect on the texture of the soil; 2) an enhancement of the cation exchange capacity of the soil; 3) an enhancement of the water-stable aggregates formed from organic matter and soil mineral particles. Given that the all three of these mechanisms are affected by microbial action on the hay, the microbial populations will be determined both qualitatively and quantitatively and then correlated with the results of the field studies.

Summary of Findings:

First order rate constants for salt removal are shown in Table 1. For Gibbs 7, tilling with hay and fertilizers proved to be the best treatment for salt removal (80% confidence level, CL). For Gibbs 9, which is rockier than Gibbs 7, tilling was the best treatment for salt removal (79% CL). Our proposed treatment did not always improve the salt removal.

Table 1. First order rate constants for salt removal from the field sites

Treatment	Plot	Rate Constant (k) x 10^4, $days^{-1}$	
		Sodium	Chloride
Tilling	G7-2	-4	-8
	G9-4	-14	-13
Tilling and Fertilizer	G7-4	-6	-11
	G9-3	-6	-8
Tilling and Hay	G7-1	-7	-10
	G9-2	-7	-9
Tilling, Hay, and Fertilizer	G7-3	-8	-12
	G9-1	-7	-10

Data regarding our proposed mechanisms for both contaminated and control sites are presented in Table 2. Hay increased the soil's cation exchange capacity (99.9% CL), wet aggregate stability (99.0% CL), and soil moisture level (99.9% CL), but fertilizers decreased the cation exchange capacity and soil moisture levels (both at 95% CL). Although two of our proposed mechanisms,

cation exchange capacity and wet aggregate stability, were activated by hay, their improvement did not consistently translate into improved salt removal rates.

Table 2. Mean, standard deviation, and counts for in-lab analyses of soil samples from contaminated and control plots

Variable	Units	Tilling	Tilling and Fertilizer	Tilling and Hay	Tilling, Hay and Fertilizer
Soil Moisture	% dry weight	17.17 ± 5.10 (N = 39)	16.18 ± 4.48 (N = 39)	20.45 ± 3.63 (N = 39)	18.94 ± 4.81 (N = 39)
Wet Aggregate Stability	%	63.29 ± 12.69 (N = 15)	69.86 ± 13.42 (N = 15)	72.03 ± 11.29 (N = 15)	74.40 ± 12.32 (N = 15)
Cation Exchange Capacity	meq/100 g	14.27 ± 3.18 (N = 30)	14.09 ± 2.94 (N = 30)	16.95 ± 2.17 (N = 30)	14.84 ± 2.97 (N = 30)
Permeability	cm/hr	2.08 ± 1.75 (N = 16)	2.25 ± 1.29 (N = 15)	3.35 ± 3.30 (N = 17)	2.33 ± 1.55 (N = 15)

Since microbial action is important to our proposed mechanisms, we investigated the microbial populations in the soils with phospholipid fatty acid analysis (PLFA). The results of statistical analysis on the PLFA data are given in Table 3. Fertilizers increased the ratios of prokaryote and eukaryote populations in the contaminated sites to their populations in the treated controls, but hay increased only the ratio of the contaminated prokaryote population to the treated control population. There were also cross effects with treatment and time, but hay and fertilizer did not interact. Hay did increase the proportion of eukaryotes in the soil, thereby decreasing the Shannon's diversity index based on the soil microbial community. Fertilizers increased the microbial stress and decreased the growth rate of Gram-negative bacteria. Since fertilizers and hay brought the prokaryote and eukaryote populations closer to their treated control populations, they are considered to be restoring the ecosystem. DGGE samples from April 2004 did not show interesting band patterns by either treatment or site, so sequencing was not done.

Microcosms were made to study the effects of the treatments on the microbial population under controlled environmental conditions. Each of the field treatments was duplicated in a set of eight microcosms. PLFA data for the microcosms (Table 4) are similar to those for the field sites in some respects but not all. Fertilizer had no effects in the microcosms than in the field sites, hay had more significant effects in the microcosms, and there were no significant interactions in the microcosms. The addition of hay increased eukaryote and prokaryote PLFA concentrations. The community structure in all of the microcosms shifted toward the monoenoics and branched monoenoics over time, but the microcosms with hay shifted much more toward eukaryotes, primarily fungal, and away from mid-chain branched saturates, characteristic of actinomycetes, and the terminally branched saturates. Fertilizer did appear to somewhat decrease the diversity of the bacterial communities when examined using DGGE, although this was not apparent from the PLFA data. Small but statistically significant differences with treatment were seen in the metabolic status ratio and the environment stress for Gram-negative bacteria, but on the average the microcosms were in the stationary phase and relatively non-stressed. Shannon's diversity index increased with time and then decreased, and the diversity was lower with hay, due to the increased dominance of certain groups, primarily fungi. SEM images of hay from the microcosms appear to show bacteria and hyphae on the hay.

Table 3. MANOVA results showing the effects of time and treatment on microbial PLFA in the field sites

Variable	Time	Fertilizer	Hay	Time x Fertilizer	Time x Hay	Fertilizer x Hay	Time x Fertilizer x Hay
Prokaryotes (pmole)	***	-	-	-	-	-	-
Eukaryotes (pmole)	***	-	-	-	-	-	-
Prokaryote ratio to control	***	***	**	-	**	-	**
Eukaryote ratio to control	**	***	-	*	**	-	***
Metabolic Status Ratio	-	*	-	-	-	-	-
Environmental Stress Ratio	**	*	-	-	-	-	-
Shannon's Diversity Index	***	-	**	-	-	-	-

* = statistically significant at the 95% CL, ** = statistically significant at the 99.0% CL, *** = statistically significant at the 99.9% CL, - = not significant at the 95% CL

Table 4. MANOVA results showing the effects of time and treatment on microbial PLFA in the microcosms

Measurement	Time	Fertilizer	Hay	Time x Fertilizer	Time x Hay	Fertilizer x Hay	Time x Fertilizer x Hay
Prokaryotes (pmoles)	-	-	***	-	-	-	-
Eukaryotes (pmoles)	***	-	*	-	-	-	-
Metabolic Status Ratio	-	-	***	-	-	-	-
Environmental Stress Ratio	**	-	*	-	-	-	-
Shannon's Diversity Index	***	-	***	-	-	-	-

* = statistically significant at the 95% CL, ** = statistically significant at the 99% CL, *** = statistically significant at the 99.9% CL. - = not significant at the 95% CL.

As a bonus, a second microcosm study was done to compare leaching rates from contaminated soil treated with tilling alone and tilling, hay, and fertilizers. The microcosms that received hay and fertilizers had slower salt removal rates, but only at the 70% confidence level. Microcosms with hay and fertilizers had higher soil moisture levels and produced less leachate which was more concentrated with brine components.

We continue to recommend treatment of brine-contaminated sites with tilling, fertilizers, and hay. Tilling with hay and fertilizers was the best salt removal treatment in Gibbs 7 which

has a flat slope and non-rocky soil. Tilling alone may be the best treatment for salt removal in steep, rocky sites, but the fertilizers and hay improve the microbial community, which will improve the rest of the ecosystem over time.

Publications/ Presentations:

Remediation of Brine Spills With Hay, Continued, Laura Ford, Shailendra Singh, Kimberly Carter, and Kerry L. Sublette, University of Tulsa, Tulsa, OK; Kathleen Duncan, University of Oklahoma, Norman, OK, 10th Annual International Petroleum Environmental Conference, November 2003, Houston, TX.

Remediation of Brine-Impacted Soil with Organic Matter: Microcosms, Laura Ford, Shailendra Singh, Kimberly Carter, and Kerry L. Sublette, and Kathleen E. Duncan, 11th Annual International Petroleum Environmental Conference, October 2004, Albuquerque, NM.

Remediation of Brine Contaminated Soils with Hay, Shailendra Singh, Thesis, University of Tulsa, 2004.

Supplemental Keywords: land, precipitation, ecological effects, indicators, restoration, terrestrial, remediation, bioremediation, cleanup, public policy, cost benefit, environmental chemistry, biology, engineering, ecology, hydrology, south central, Oklahoma, OK, EPA Region 6, agriculture, Crude Petroleum and Natural Gas (SIC 1311), Crude Petroleum Pipelines (SIC 4612)

Relevant Web Sites:

International Petroleum Environmental Conferences: ipec.utulsa.edu/conference.htm