

To: Mark Silbert  
From: Rachel Joan Dale *RJD*  
Date: April 28, 1990  
Subject: Effects of Oiling and Bioremediation of Cultural Remains

As requested in the Cultural Resources Working Group Meeting of April 27, 1990, I have attempted to formulate a series of questions that you could address to EPA and/or Exxon research personnel on our behalf. Our concerns are oriented to the effects of Prude Bay Crude and bioremediation agents on cultural remains that we would expect to find in the effect areas.

The cultural remains most likely found in sites in Prince William Sound and the Gulf of Alaska are:

- (1) charcoal
- (2) wood (softwoods)
- (3) bone (which can be differentiated on the relative degree of vascularity)
  - a. fish bone which is relatively avascular
  - b. land mammal bone which is relatively more vascular but has a relatively thick cortex with limited vascularity on the shaft
  - c. sea mammal bone which is much more vascular and therefore has more surface area to react chemically/physically with the surrounding environment
- (4) shell
- (5) siliceous materials
- (6) ivory (teeth)
- (7) woven grasses
- (8) metal objects (historic remains)

We are attempting to gain information on the direct and indirect effects that oil and bioremediation would have on cultural remains. Specifically we would like to find out:

- (1) what elements are present in crude oil. Would the elements present in crude oil effect the soil chemistry? If so, in what way (for example, would it change the pH of the soil). The soil pH is of interest because the majority of organic cultural remains are preserved despite the normally acidic forest soils because the large number of shells

increase the alkalinity of the soil and create an environment conducive to organic preservation;


(2) what are the microbes that bioremediation will enhance,

(3) what do these microbes eat? Would they effect the organic remains found at archaeological sites (would their presence adversely effect any or all of the items listed above)? What are the by-products of their metabolism of the hydrocarbons (and would those by-products effect organic remains/preservation)? Does the presence of these microbes change the soil chemistry? If so, how?

(4) what is the chemical composition of Inipol and other bioremediation agents, including their carriers? Would any of these chemicals effect the soil chemistry? Could these chemicals effect the cultural remains and in what way?

(5) From a short conversation with Roger Prince (Senior Staff Biochemist, Corporate Research-Science Laboratories, Exxon Research and Engineering Company), it is my understanding that the organisms which are enhanced by bioremediation do not metabolize wood, and that, the application of bioremediation agents (because they introduce nitrogen) actually inhibits the growth of the white rot fungi (which are the organisms that decompose wood). If my interpretation is correct, then would the application of bioremediation agents in the vicinity of wood actually act as a short-term preservational agent? Would this have any adverse long-term preservation effects?

May 1, 1990

TO: Rachel Joan Dale  
FROM: Dr. R. C. Prince   
SUBJECT: Effects of Dilling & Bioremediation on Cultural Remains

This memorandum hopes to address your concerns about potential deleterious effects on cultural remains, especially from oil, the biodegradation of that oil, or from fertilizers used to enhance biodegradation. We do not expect to see any deleterious effects by stimulating the natural process of bioremediation by adding nutrients to oiled sediments; herewith answers to the questions outlined in your letter.

1. Crude oil is essentially exclusively hydrocarbon, and as such has little effect on soil chemistry. It contains very few acidic groups, and is unlikely to change the pH of the sediments it encounters. Crude oil contains traces (ppm levels) of sulfur, vanadium and nickel, but at such low levels that they are unlikely to be significant.
2. The fundamental idea behind bioremediation is that the oil on the beaches provides a rich potential source of carbon and energy to those organisms capable of metabolizing it. These organisms were previously limited by the very low concentrations of oil in the natural environment, and hence made up only a tiny fraction (perhaps <0.01%) of the microbial community. With the influx of oil to the beaches, these organisms increased in number, but in doing so became limited by nitrogen and phosphorus. Bioremediation involves the addition of nitrogen and phosphorus to partially overcome the nitrogen and phosphorus limitation, and hence encourage the growth of the hydrocarbon degraders. It is important to note that the addition of the fertilizer is kept to a minimum because it could potentially stimulate the growth of the other organisms that have an unlimited carbon source, the seaweeds and algae; their carbon source, CO<sub>2</sub>, is essentially unlimited since it comes from the atmosphere.

The two fertilizers used in the bioremediation program are Inipol, an oleophillic fertilizer, and Customblen, a slow release fertilizer. Our winter monitoring program showed that the only organisms stimulated by the addition of fertilizer were oil-degrading microbes; there was no increase in the total bacterial population. No bacteria were added in the bioremediation process, so the organisms taking advantage of the fertilizer were all present before the spill. We have isolated and identified several oil-degrading microbes, listed on the enclosed sheet; all are common inhabitants of marine environments.

3. The indigenous bacteria are capable of metabolizing almost all the components of crude oil, with the possible exception of the asphaltene components which make up less than 2% of the oil. The products of biodegradation of crude oil are CO<sub>2</sub>, H<sub>2</sub>O and microbial biomass; no intermediates or by-products are produced in significant amounts, and so biodegradation of crude oil is unlikely to significantly affect soil chemistry or pH. The oil-degrading bacteria are unlikely to be degrading organic remains at archaeological sites, especially since the remains you have identified as present are not very biodegradable. One potentially readily degraded substrate, wood, is metabolized by bacteria, but this means that the most readily degraded components have probably already been removed. Wood's major component, lignin, is degraded by very few organisms. Indeed the only well characterized organisms that can metabolize lignin are the white rot fungi, which are very rare in marine environments, and their lignolytic activity is usually inhibited by the addition of nitrogenous fertilizer. Woven grasses might be quite biodegradable if in an aerobic environment, and I would expect their biodegradation to proceed regardless of additional fertilizer.
4. Inipol contains a saturated solution of urea as a microemulsion in oleic acid. This microemulsion is stabilized by laureth phosphate and butyl cellosolve; its pH is 5.5. Customblen is ammonium nitrate, calcium phosphate and ammonium phosphates encapsulated in polymerized vegetable oil. At the application rates being used, they are very unlikely to affect soil chemistry or pH, and since all the components of Inipol are readily biodegradable, any possible effects would be very short lived.
5. Although one might expect Inipol to inhibit the degradation of lignin, it might potentially stimulate the degradation of the cellulose component of the wood and woven grasses; I would expect no significant effect, but would not recommend the use of Inipol as a potential preservative.

I hope that these answers address your concerns, and would be happy to discuss the issue further if necessary.

RP:do:90517

Attachment

ACE 6906575  
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**HYDROCARBON OXIDIZING ORGANISMS  
FROM PRINCE WILLIAM SOUND**

Typically psychrotrophic Gram negative, non-fermentative, aerobic organisms, both motile and non-motile.

We have obtained many isolates, and these have been tentatively identified as:

- Pseudomonas* species, both fluorescent and non-fluorescent
- Acinetobacter calcoaceticus*
- Moraxella phenylpyruvica*
- Oceanospirillum* species
- Alcaligenes/Flavobacterium/Achromobacter* species
- Vibrio* species
- Arthrobacter/Brevibacterium* species

*Trichosporon* species

Most isolates grow well on tetradecane and hexadecane, grew well from 5-25°C, and grew well from 0-3% NaCl.