## Bioremediation of Petroleum Hydrocarbon-Contaminated Soils in Cold Climates: A Scaled-Up Field Experiment for the Feasibility of Extending Bioremediation beyond the Conventional Summer Treatment Season

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**Background/Objectives.** Vast regions in cold-climate countries including Canada are influenced by seasonal freezing, during which ambient air temperatures are very low (-20 °C or below). The bioremediation of petroleum hydrocarbon-contaminated sites in cold climates is very challenging, mainly due to short active treatment seasons (~4 months) and low temperatures. However, it has been reported that polar microorganisms adapted to cryoenvironments are metabolically active at sub-zero temperatures. Their habitable niches in frozen soils may be associated with unfrozen water films in accessible soil pores or with the interface between ice and unfrozen water. Yet, there is little information available about the onsite bioremediation potential of hydrocarbon-contaminated soils based on scaled-up field experiments conducted during an actual off-season, when the soils are subjected to seasonal freeze/thaw conditions. This field study aims at investigating the feasibility of enhancing indigenous hydrocarbon-degrading microorganisms for the bioremediation of petroleum hydrocarbon-contaminated soils during unconventional treatment periods at a cold-climate site.

**Approach/Activities.** Two pilot-scale biopiles (~3.2 metric tons per pile; 2 m x 2 m x 1.2 m) were installed at an outdoor landfarm facility in Saskatoon, Canada. The site soils (clayey soils) were treated with an optimized nutrient amendment and soil conditioning determined from the initial soil characterization. Two biopiles were constructed: one untreated control pile and one biopile treated at the end of October. The experiment continued until July of the following year. Soil and air temperatures and the soil water content were monitored in real time in both biopiles. O<sub>2</sub> and CO<sub>2</sub> concentrations in soil gas and the depth of snow cover were periodically measured. Hydrocarbons and biomarker analyses, as well as microbial assessments including microbial community analyses, were performed for soil samples that were periodically collected when the biopile soils were unfrozen, partially frozen, frozen, partially thawed and completely thawed.

**Results/Lessons Learned.** The field experiment indicated that enhanced hydrocarbon biodegradation occurred in the treated soils during seasonal freezing and thawing. Total petroleum hydrocarbon (TPH) quantities in the treated soils decreased by 33% between the end of October and early March under the natural seasonal freeze-thaw conditions that included sub-zero soil temperatures. TPH concentrations decreased further by 24% by July (26 °C), resulting in a total TPH removal of 57% over the experiment duration (p < 0.05, two-ANOVA). The removal of F3 (C16-C34) hydrocarbons was 39% during the winter months, and the final F3 concentration reached by July was 1,177 mg/kg, which is below the environmental standard. Biomarker analyses confirmed that *biological* hydrocarbon removal occurred in the treated biopiles during soil freezing. The treated soils held onto significant amounts of unfrozen water, which played a key role in sustaining the microbial activity in freezing and frozen soils. Soil respiration and microbial community shifts in response to seasonal changes in the thermal state of the treated soils supported the evidence of extended bioremediation during soil freezing. This study indicated the feasibility of bioremediation under seasonal freeze-thaw conditions in cold regions, suggesting a potential innovative strategy for bioremediation at cold-climate sites.