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## **Biodegradation and Toxicity of Hydrocarbons along Vertical Transects in a Groundwater Plume**

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Biodegradability of weathered hydrocarbons along vertical and horizontal transects was examined to determine the potential contribution of biodegradation to natural attenuation at a former oil field. The viscous crude oil extracted from this site was diluted with diesel-range-oil (DRO) to facilitate pumping. Leaks of this DRO at the site led to hydrocarbon contamination of both soil and groundwater. Critical source zones have been excavated, and natural attenuation is being evaluated as a polishing step. To provide the necessary lines of evidence for natural attenuation, it is important to demonstrate that the hydrocarbons continue to be biodegradable after partial biodegradation and weathering. Thus biodegradability was examined along transects to evaluate the sustainability of weathered hydrocarbon biodegradation. A previous study examined horizontal transects and showed that hydrocarbon biodegradability decreased with distance from source zones (and presumably with increased weathering), but that aerobic biodegradation was possible even for highly weathered hydrocarbons. In the current study vertical profiles were added to the study to allow for examination of biodegradability of hydrocarbons weathered under different redox conditions associated with sampling depth.

Groundwater samples were collected from 3 nested wells with sampling at 5 different depths. Biodegradability of hydrocarbons in each sample was determined for each sample in the laboratory by measuring 20-day aerobic biodegradation rate and respiration rate. Toxicity changes were evaluated by measuring Microtox<sup>®</sup> toxicity. Redox conditions were determined by measuring sulfate, dissolved Fe(II), dissolved methane and oxygen for each depth. The microbial community was characterized using terminal restriction fragment (TRF) analyses. Inorganic nutrients were also analyzed to check for potential nutrient limitations to biodegradation.

Results showed consistent biodegradability for all samples with initial total petroleum hydrocarbon (TPH) concentrations above 0.5 ppm. Observed biodegradation rates were consistent with previously observed first-order kinetics. For samples with initial TPH concentrations below 0.5 ppm, changes in TPH concentration over 20 days were not statistically observable in the laboratory study. Microtox<sup>®</sup> toxicity decreased significantly in 20 days for all samples that exhibited initial Microtox<sup>®</sup> toxicity above detection levels. These results suggest that hydrocarbon biodegradation is sustainable under a variety of conditions at this site, but that rates of biodegradation become slow at extremely low TPH concentration.