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## **Biodegradation of Weathered Hydrocarbons in Laboratory Microcosms and Soil Columns Simulating Natural Attenuation Field Conditions**

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Controlled laboratory microcosms and soil columns were used to quantify biodegradation of weathered hydrocarbons in groundwater under conditions mimicking natural attenuation field conditions at a former oil field near Guadalupe, CA. Diesel range oil (DRO) was used as a diluent during oil production at this site from 1950-1991 to facilitate pumping the viscous crude oil. Leaking tanks and pipes resulted in hydrocarbon source zones and groundwater plumes. Following more aggressive treatments such as excavation, the feasibility of using natural attenuation to monitor the levels of diluent is currently being investigated using native microbial species. A laboratory study was undertaken using soil columns with soil and groundwater from the site and carboys with groundwater and no soil to ascertain the degradation kinetics and to ultimately evaluate the relative contributions of the local microbial species to bioremediate hydrocarbons under conditions more closely matched to site conditions than previous studies. Also of interest was the sustainability of biodegradation over long periods of time as well as changes in toxicity during biodegradation.

Laboratory soil columns were set up using three sets of three vertical soil columns in series filled with soil from the field site. Groundwater from the site with an initial total petroleum hydrocarbon (TPH) concentration of  $2.3 \pm 0.1$  mg/L was recirculated through the column with the same Darcy velocity as observed in the field. Microcosms were set up in duplicate 12-L carboys with no soil. The microcosms were filled with the same groundwater as collected for the soil columns and were stirred constantly and aerated. Biodegradation rates were determined by monitoring the (TPH) concentration during simultaneous 150-day experiments. Toxicity was monitored using Microtox<sup>®</sup> *Vibrio fischeri* bacteria. Dissolved oxygen, pH, nutrient levels ( $\text{SO}_4^{2-}$ ,  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{NH}_3$  &  $\text{PO}_4^-$ ) and total organic carbon (TOC) were also monitored.

TPH biodegradation was most rapid during the first 20 days. After 150 days TPH concentration decreased to  $0.76 \pm 0.0$  mg/L in the soil columns and to  $0.88 \pm 0.156$  mg/L in the carboy microcosms. The faster biodegradation in the soil columns was statistically significant with 95% confidence. The TPH concentration in an azide-inhibited control actually increased to  $4.6 \pm 0.0$  mg/L. Since TPH did not decrease in the azide-inhibited control adsorption onto soil particles is unlikely and observed TPH loss in the soil columns can be attributed to biodegradation by bacterial cells. Microtox<sup>®</sup> toxicity rapidly decreased to below detection limits within 30 days. These results suggest that natural attenuation is feasible at this site.