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Assessment of Toluene Biodegradation Activity in Groundwater from a Shallow Bedrock Aquifer with Phytoremediation

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Background/Objectives. The storage of toluene in underground tanks for use as an organic solvent at a historical manufacturing facility (the Site) in Ontario, Canada led to contamination of the underlying shallow fractured bedrock aquifer system. Approximately 8 years ago a pilot phytoremediation system consisting of hybrid poplar trees (*Populus x Canadensis*) was installed to enhance attenuation of toluene mass within the source area. The Site represents an important field-based (in-situ) opportunity to quantitatively examine the active mechanisms of toluene attenuation relevant to multiple disciplines and to evaluate the efficacy of phytoremediation as an alternative remediation approach in urban environments. The Site provides an opportunity to examine the effects of a phytoremediation system on bacterial communities and activities within the shallow groundwater. The goal of this study is to apply advanced molecular tools to measure potential degradation activity relative to populations of bacterial toluene degraders across seasons and in relation to contaminant concentration, oxygen availability and proximity to the trees.

Approach/Activities. Seven multilevel wells were installed to provide lateral and vertical plume delineation and capture relevant groundwater quality parameters, including toluene concentrations and redox conditions, at high resolution. Groundwater sampling events occurred seasonally in November 2015, March 2016, June 2016 and November 2016 to capture seasonal variation in measured parameters including, dissolved oxygen and other redox indicators, toluene concentration, and compound stable isotope analyses (CSIA). Quantitative polymerase chain reaction (qPCR) targets the RNA transcripts, which are only produced by active bacteria, used to create functional toluene-degrading enzymes. We use qPCR to quantify toluene degrading activity by targeting benzylsuccinate synthase (*bssA*), an enzyme that completes the initial step of the only known anaerobic toluene degradation pathway. In addition, we will quantify methanogens to gain insight to the microbial communities that are active in groundwater from the site.

Results/Lessons Learned. Results to date indicate that *bssA* gene copies could be quantified in groundwater from all wells and all sampling dates between November 2015 and November 2016; however, presence and levels varied seasonally and spatially across the site. In some samples we were able to quantify *bssA* transcripts indicating that the populations were actively expressing these genes. Between the fall and spring sampling periods, toluene levels decreased in the majority of the ports sampled. Preliminary CSIA data suggest microbial degradation of toluene is occurring in some of the groundwater samples from the site. Ongoing analysis will examine relationships between toluene concentrations, gene expression, and isotopic signatures. By combining these analyses we aim to contribute to the understanding of toluene degradation by bacterial communities occurring on the Site.