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Enumeration of Toluene-Degrading Microorganisms in Combination with Vegetation Hydrocarbon Phytoscreening to Assess Phytoremediation of Toluene in a Shallow Fractured Bedrock Aquifer

Michael Ben-Israel (mbenisra@uoguelph.ca), Beth L. Parker, and Kari E. Dunfield (dunfield@uoguelph.ca) (University of Guelph, Guelph, ON, Canada)
Ramon Aravena (University of Waterloo, Waterloo, ON, Canada)
Elizabeth A. Haack (Advisian WorleyParsons Group, Markham, ON, Canada)
David T. Tsao (BP Corporation North America, Inc., Naperville, IL, USA)
Joel G. Burken (Missouri University of Science and Technology, Rolla, MO, USA)

Background/Objectives. Hybrid poplar trees (*Populus* × *canadensis*) are being used in a phytoremediation strategy to address a persistent toluene plume within a shallow fractured bedrock aquifer at an urban-industrial site in Ontario, Canada. Fifty-one poplar whips were planted in 2008 within a 220 m^2 area in nutrient-amended boreholes to promote deep rooting and rapid onset of phytoremediation activity. The system is now mature; however, from a contaminant mass-balance perspective, assessing phytoremediation efficacy is difficult due to the dynamic nature of the system and number of possible subsurface and plant-related processes for toluene uptake and attenuation. Novel methods are presented here to assess the phytoremediation performance as a subset of the mechanisms operative on the residual mass attenuation of the toluene plume. The objectives of this study are (i) to enumerate and characterize the activity of poplar-associated toluene-degrading microbial communities, and (ii) to assess toluene uptake and mass removal by the poplar stand.

Approach/Activities. Bulk and root-adherent soil samples were collected at various depths in November 2016 from trenches excavated across a gradient of toluene concentrations, outside of and within the planted area, to evaluate biodegradation. Toluene biodegradation is initiated in aerobic pathways with ring/side-chain hydroxylation, while anaerobic pathways begin with side-chain fumarate addition. Quantitative PCR was used to enumerate gene (DNA) and transcript (RNA) copies of enzymes responsible for these reactions in soil samples to elucidate degradative potential, activity, and favored pathways. Additionally, a phytoscreening approach assessing toluene uptake and translocation by the poplar stand was conducted by measuring toluene quantities in tissue samples and within transpiration streams. Branch clippings and trunk cores were collected and analyzed in September 2016, while passive samplers were inserted into trunk core holes and analyzed after two 2-week periods.

Results/Lessons Learned. Of the biodegradation enzymes targeted, only phenol hydroxylase (PH) genes and transcripts were detected at quantifiable levels. PH usually performs secondary oxidation reactions, but has significant homology to some toluene mono-oxygenases; thus, detected enzymes could be responsible for both initial and subsequent hydroxylation reactions. These results indicate that aerobic degraders are present and active at this site. No toluene was detected in tree tissue samples analyzed; however, it was quantifiable in the passive samplers, with highest concentrations detected proximal to the source zone. Superior detection limits afforded by passive sampling techniques enabled confirmation that toluene phytoextraction is occurring, however subsequent fate – phytodegradation or phytovolatilization – is unclear. It is also unclear if poplars are accessing impacted groundwater, vapour phase toluene infiltrating the vadose zone, or both. In order to better evaluate phytoremediation mechanisms active at this site, future studies will measure vapour phase toluene in the vadose zone, determine if poplars are transpiring groundwater (isotope analysis), and assess toluene uptake and translocation in relation to seasonal hydrology.