USGS Harpeth River Sampling and Bio-degradation Study

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Introduction - Remediating groundwater contamination in karst landscapes is often hindered by complex hydrology and unique variables at each site. A wide range of variables, such as water residence time, microbial community, geochemistry, and rate of biodegradation, must be assessed to determine if bioremediation is a viable attenuation strategy. A study was conducted to assess these variables at the Egyptian Lacquer Manufacturing Company (ELMCO) site in Franklin, Tennessee contaminated with mono-aromatic hydrocarbons (benzene, ethylbenzene, toluene, xylene; BTEX). The study had three components: (1) a seepage run along the Harpeth River to quantify contaminants entering the stream, (2) in situ monitoring of water level, temperature, dissolved oxygen and specific conductance in two wells screened in the bedrock aquifer, (3) a laboratory experiment to evaluate natural and supplement-enhanced biodegradation of the BTEX compounds found in water samples collected at the site.

Site description – The site is located in a karst terrain in Franklin, TN (fig. 1). Karst terrain is dominated by carbonate bedrock that can have small or large dissolution openings (fig. 2). This complicates the hydrology and makes it difficult to predict the exact flow path. Also, every site has different karst features that make them unique.

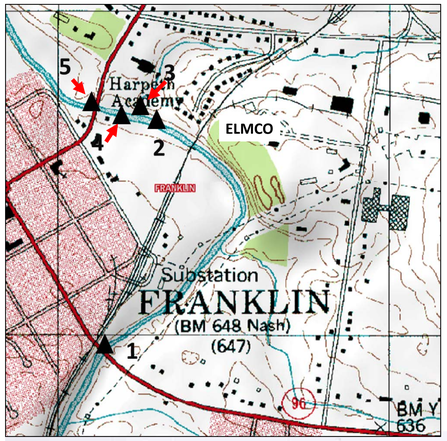


Figure 1. (left) Locations along Harpeth River and Liberty Creek where discharge was measured and samples collected. (site located at "ELMCO", seepage measurements at sites 1 – 5)

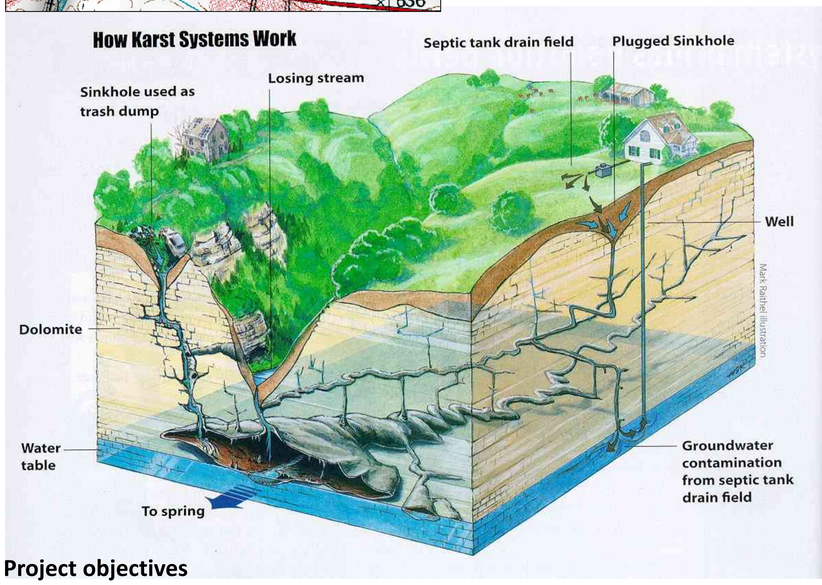


Figure 2. (below) Example of karst hydrology. The site in Franklin, TN is unique and different from this image, but the complexity of the hydrology is real. Image credit: Mark Raithel, Missouri Dept. of Conservation.

Project objectives

1. Calculate contaminant loads if BTEX compounds were detected in water samples from the seepage run sites at Liberty Creek and the Harpeth River.
2. Use in situ monitoring data to characterize geochemistry and water levels in response to weather events, especially rain; use groundwater samples from two contaminated bedrock wells to characterize diversity of microbial populations in the karst aquifer.
3. Evaluate the potential for natural and supplement-enhanced biodegradation of the BTEX

Methods and Results

Results Seepage Run in October 2014 - Seepage was measured using the acoustic Doppler current profiler method to determine gains or losses in discharge between four sites on the Harpeth River and a Parshall flume was used at one site on Liberty Creek in the fall of 2014 (table 1), during low baseflow conditions. River water samples were also collected according to standard USGS protocols and sent to the TDEC-DOR lab for analysis of BTEX compounds (lab report TDEC-DOR N00012413).

Disclaimer - It was beyond the scope of this project to determine if the bioremediation-enhancing supplements could successfully be injected into the aquifer at this site.

Map number	Site	Discharge 10 / 9 / 2014
1	Harpeth R. at Hwy 96	31.0 ft^3/sec
2	Harpeth R. above Liberty Creek	31.0 ft^3/sec
3	Liberty Creek at mouth	0.06 ft^3/sec
4	Harpeth R. below Liberty Creek	31.2 ft^3/sec
5	Harpeth R. at US-31	31.4 ft^3/sec

Table 1. Seepage run locations and instantaneous discharge measured in October 2014.

Figure 3 (right). USGS scientists collecting discharge data using the ADCP method. Photo credit: USGS stock photos

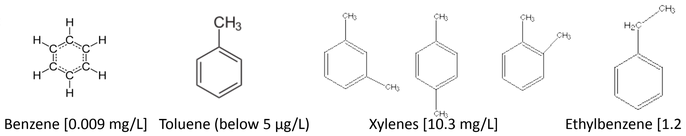
Main Findings for objective 1: This study showed that the Harpeth River gained approximately 0.2 ft^3/second (approx. 90 gallons / minute) in discharge as it flowed past the ELMCO site. Concentrations of BTEX compounds were below method reporting limits (< 5 µg/L method quantitation limit) for all the sites, thus loads could not be calculated.

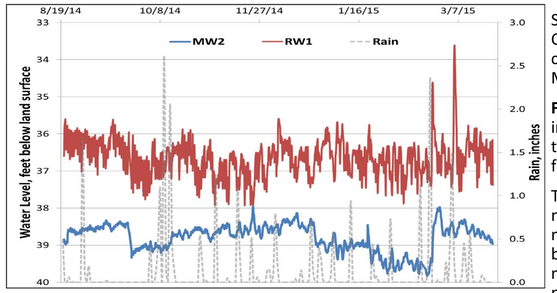
In Situ Monitoring and Geochemical Sampling of Two Contaminated Wells

Monitoring instruments were placed in two contaminated wells (MW-2 and RW-1; fig. 4). Water level (fig. 6), temperature, specific conductance (fig. 7), and dissolved oxygen were monitored continuously over 8 months from fall 2014 through the spring of 2015. This data can tell us something about residence time of groundwater in the wells and what affect that has on geochemical conditions in the wells. Water samples were collected from each well using a dedicated bladder pump. Dissolved oxygen, pH, and specific conductance were recorded at the time of sampling, and the water samples were analyzed for sulfide, sulfate, ammonia, nitrate, iron (Fe^{2+}), phosphate, and BTEX compounds at the laboratory (figs. 7 and 8). The remaining collected water was stored in clean, sterile glass bottles and chilled in a refrigerator until used for the biodegradation study in the lab. Biological Activity Reaction Tests (BART™) were used to identify and quantify sulfur-reducing bacteria (SRB), iron-reducing bacteria (IRB), and slime-producing Pseudomonad bacteria (SLYM) present in the well water (fig. 9). It should be noted that toluene was not detected in these waters during two sampling events, but benzene, ethylbenzene and xylene were detected (2015 TDEC-DOR report). The lack of toluene may be due to preferential biodegradation of toluene by indigenous bacteria (Byl and others, 2014, *Journal of Cave and Karst Studies*, 76: 104–113). It is common for a preferred substrate to be removed at a faster rate than some of the other compounds

Figure 4. The wells used in this study were designated MW-2 and RW-1. They were in the far west corner of the ELMCO site, near the source of the contamination. Concentration of BTEX in the well water are shown below.

Figure 5. Benzene was found in MW-2, and, xylenes and ethylbenzene in RW-1, Sept. 23, 2014.





Sampling by TriAD Environmental Consultants, Inc. confirmed mono-aromatic compounds BTEX were present in wells MW-2 and RW-1 during the study period.

Figure 6. Water levels varied by +/- 1 foot in MW-2, and by +/- 2 feet in RW-1 over the 8 month monitoring period. (Rain data from Nashville international airport)

The monitors placed in the wells showed no oxygen in either well over the entire monitoring period. (Data not shown because they were both flat lines at 0.0 milligrams per liter over the 8 month period)

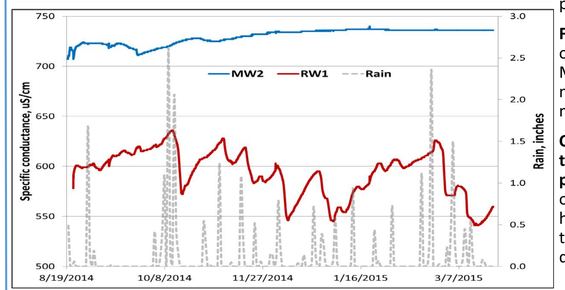


Figure 7. Specific conductance (a measure of dissolved minerals) was very stable in MW-2, and varied by less than 100 microSiemens/centimeter over the 8 month monitoring period.

CONCLUSION from well monitoring – the two wells are relatively stagnant with poor hydraulic conductivity. Compared to other karst wells (Byl and Williams, 2000, http://pubs.water.usgs.gov/wri994285), these constituents were very stabile and did not fluctuate much.

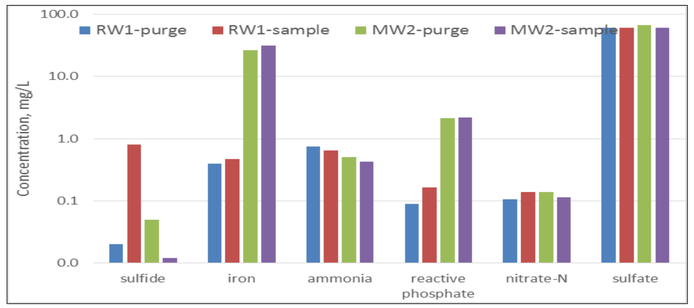


Figure 8. Geochemistry of purge waters and sample waters from wells RW-1 and MW-2, Sept. 23, 2014. The pH of the water ranged from 6.5-7.4 based on field readings during sampling (data not shown).

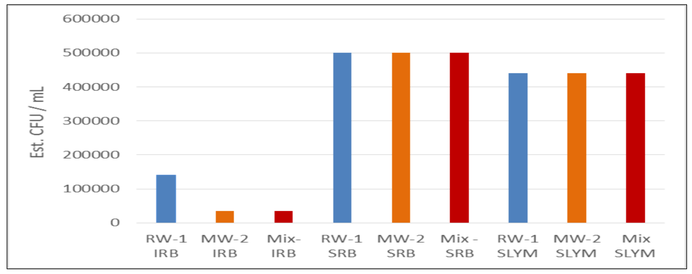
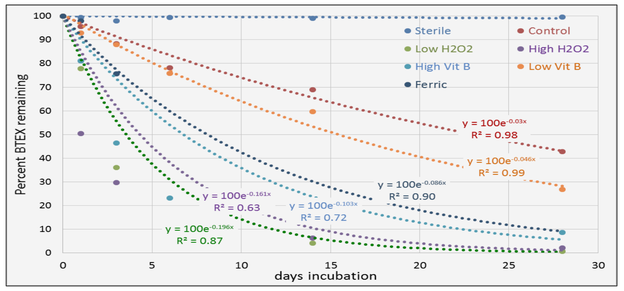


Figure 9. Microbial community based on BART™ assays. [Est. CFU/mL = estimated colony forming units per milliliter; Mix = mix of RW-1 and MW-2 water; *IRB = iron reducing bacteria, SRB = sulfur reducing bacteria, SLYM= Pseudomonad bacteria*].

Stable water levels and specific conductance values indicate that water in the two wells is relatively stagnant, and hydraulic conductivity within the screened portion of the aquifer is poor. The presence of sulfide, dissolved iron, and ammonia and lack of dissolved oxygen in the groundwater support the conclusion that the contaminated wells intersect anaerobic sections of the aquifer. The bacteria communities in both wells were dominated by sulfur-reducing bacteria and Pseudomonads, well documented BTEX compound degraders.

Figure 10. Microcosms after 2 weeks incubation. A 1:1 volume of RW-1 & MW-2 waters were placed into 300 mL bottles, 3 replicates per treatment. Seven treatments were evaluated for rate of mono-aromatic removal from the water [fluorometer used to measure BTEX hydrocarbons. Initial conc ~6 mg/L]. Treatments = sterile control, live control, 15 mg/L O_2, 75 mg/L O_2, high & low conc vitamin B complex, 0.33 mg/L Fe^{3+}.



Treatment	Half-life = $T_{1/2}$(days)
sterile	= 6930
live control	= 23
ferric iron	= 8*
low Vit B	= 15.1
high Vit B	= 6.7
low H_2O_2	= 3.5
high H_2O_2	= 4.3

**the ferric iron interfered with BTEX analysis so this rate lacks certainty*

Figure 11. Lab biodegradation results. The sterile control compared to the live control provides evidence that the reduction in BTEX was due to microorganisms. Biodegradation was moderately slow under anaerobic conditions found in the live control. Supplements enhanced the rate of BTEX biodegradation.

Main findings:

1. The wells were contaminated with mono-aromatic compounds (xylenes, ethylbenzene, benzene; no toluene detected when these samples were collected)
2. Stabile water levels and specific conductance indicate wells RW1 & MW2 are relatively stagnant, with poor hydraulic conductivity
3. Sulfide, ferrous iron, and ammonia are geochemical indicators of anaerobic conditions in the wells.
4. Microbial communities characterized by BART assays were dominated by anaerobic sulfur-reducing, anaerobic heterotrophs, facultative anaerobic Pseudomonads, and iron-reducing bacteria
5. Natural biodegradation of BTEX in the well water was anaerobic and relatively slow
6. Biodegradation was enhanced with hydrogen peroxide or high Vitamin-B complex supplements