Report as of FY2006 for 2006KY71B: "The Mobility of Fecal Indicator Microoganisms within a Karst Groundwater Basin in the Inner Bluegrass Region, Kentucky"

Publications

- Conference Proceedings:
  - Ward, J.W., A.E. Fryar, G. M. Brion, and M. S. Coyne, 2007, Solute and Particle Tracer Movement Under Various Flow Conditions in a Karst Groundwater Basin, Inner Bluegrass Region, Kentucky, in Proceedings of the Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, Lexington, Kentucky, p 13-14.

Report Follows

Problem and Research Objectives

Preferential flow paths, such as conduits and sinkholes, characteristic of karst aquifers make these ground-water systems highly vulnerable to point and non-point-source pollutants. Some of these non-point-source pollutants include microorganisms that can pose a considerable health concern. The primary focus of this research was to assess the mobility of fecal indicator microorganisms within the Blue Hole Spring karst basin located in Versailles, Woodford County, Kentucky. At this site, a series of groundwater traces using solute tracers (conservative ions and fluorescent dyes), latex microspheres and isotopically tagged bacteria occurred to evaluate the mobility of microorganisms under various-flow conditions within the karst system.

The primary area of interest is the karst conduit network between Blue Hole Spring and a swallet in Big Spring Park, ~ 500 m away, where tracer tests have been conducted via slug injection into the karst conduit through a piezometer. At this location, a solute trace was conducted under low-flow conditions on June 2, 2006 using rhodamine WT fluorescent dye and the conservative ion bromide (Br^-). A second trace on July 11, 2006, under storm-flow conditions used the same solutes in combination with 1-µm-diameter fluorescent latex microspheres (used to mimic microorganisms). A third trace was conducted on April 1, 2007, under storm-flow conditions using the same solutes and latex microspheres in combination with a mass growth of ^{15}N-enriched wild-type *E. coli*.

Methodology

Analyses from these traces were conducted at the Kentucky Geological Survey (KGS) and at Environmental Research and Training Laboratory (ERTL) located at the University of Kentucky. A method was developed using a Nikon E-600 series epifluorescent microscope, located at ERTL and used primarily for counting viruses, to detect the fluorescent labeled 1-µm diameter latex microspheres (Bangs Laboratories, Inc., Fishers, IN). Samples were stored in 1-L polypropylene bottles at 4ºC at ERTL until analysis. Prior to filtration, samples were mixed by inverting 25 times, sonicated for approximately 30 seconds and then inverted another 25 times to obtain a homogeneous suspension and dislodge any microspheres possibly attached to the sides of the sample bottles. Microspheres were removed from solution using a typical membrane filtration assembly used for microbial filtration, which was washed between samples to prevent cross-contamination. A maximum of 100 mL of sample can be filtered before sediments begin to interfere with viewing the microspheres. Microspheres were removed from solution through 47-mm diameter, black, gridded cellulose nitrate filters with a nominal filter size of 0.8 µm. Once the sample was filtered, the filter was placed on a pre-washed 50-mm microscope slide with the grid parallel to the edge of the slide. The filters were then viewed with the 40× objective and counted beginning at the top and moving downward in a side-to-side motion. Typically a filter can be scanned in 30 minutes; any longer will result in drying of the filter, causing it to detach from the slide.

Methods for isotopically enriching a wild strain of *E. coli* were also conducted over the past year. This strain was isolated from Blue Hole Spring water using a MUG

auger technique, stored in soy auger slants and placed in a 4ºC cooler, allowing for culture preservation over extended time periods. Mass cultures of the isolated *E. coli* were grown in 1-L volumes of M-9 media using substrates enriched in ^{15}N to obtain isotopically labeled microorganisms via metabolic processes. The 1-L volumes of *E. coli* were then centrifuged down and resuspended into 6 L of autoclaved Blue Hole water for storage until use. This will allow for analysis of samples by an isotope-ratio mass spectrometer (IRMS). Proof-of-concept laboratory experiments to assess isotopic labeling of cells have been performed and indicate that these labeled microorganisms should work successfully as an environmental tracer.

Rainfall was limited prior to the June 2, 2006 low-flow trace, totaling 0.25 cm within 2 weeks prior to the slug injection. For this trace, 125 mL of 20 % rhodamine WT and 2 kg of sodium bromide (NaBr) salt were combined with on-site water in a 20-L carboy. The 20-L solution was inverted and shaken for 1 minute to facilitate homogeneous mixing of the slug. The slug was then poured into the piezometer and chased with ~ 100 L of water. Base-flow discharge for the trace averaged 0.079 m^3/s. Rhodamine WT arrived at the spring ~ 6.16 hours post-injection with the maximum concentration occurring at 7.8 hours; concentrations fell below detection after 21.16 hours. Bromide arrived at the spring ~ 6.5 hours after injection with a maximum concentration occurring at 7.75 hours; concentrations fell below detection 12.66 hours after injection (Fig. 1). The analytical detection limit (DL) was 0.1 µg/L for rhodamine WT (determined by fluorescence spectrophotometer) and 0.1 mg/L for Br^- (determined by ion chromatograph). Calculated mass recoveries were 79.15 % for rhodamine WT and 84.19 % for Br^-. EC averaged 608 $\mu S/cm^3$ @ 25 ºC and water temperature averaged 14.65 ºC for the duration of the low-flow trace.

The July 11, 2006 summer storm-flow trace included 250 mL of 20 % rhodamine WT, 6 kg of NaBr and 20 mL of dragon green microspheres ($1.875x10^{10}$ microspheres/mL, Bangs Laboratories, Inc., Fishers, IN), which were combined with on-site water in two 20-L carboys. The solutions were mixed, injected, and chased as before. Rainfall totaled 2.4 cm over a 5-hour period prior to injection, with another 11.6 cm of rainfall occurring during the 2-week monitoring period. Spring discharge during the storm-flow trace averaged 0.165 m^3/s, with a maximum of 0.262 m^3/s. Breakthrough began ~ 2.33 hours post-injection for the solutes and ~ 2.5 hours post-injection for the microspheres. Rhodamine WT and Br^- concentrations at the spring peaked ~ 2.67 hours after injection. Rhodamine WT was < DL 14 hours after injection, while Br^- was < DL 5.5 hours after injection. Calculated mass recoveries were 56.67 % for rhodamine WT and 52.61 % for Br^-. Microspheres were detected at the spring until 164 hours after injection (Fig. 2). Breakthrough curves for the solutes are very smooth, but the microsphere breakthrough curve is very jagged, with a pronounced peak at ~ 2.67 hours post-injection (Fig. 3). EC averaged 506 $\mu S/cm^3$ @ 25 ºC (maximum 575 $\mu S/cm^3$ @ 25 ºC) and water temperature averaged 17.55 ºC (maximum 18.83 ºC) for the duration of the trace.

The April 1, 2007 spring storm-flow trace included 200 mL of 20 % rhodamine WT, 7 kg of NaBr and 20 mL of plum purple microspheres ($1.875x10^{10}$

microspheres/mL, Bangs Laboratories, Inc., Fishers, IN), which were combined with on-site water in two 20-L carboys. The solutions were mixed, injected, and chased as before. Rainfall totaled 2.06 cm over an 8-hour period prior to injection, with another 7.5 cm of rainfall occurring during the 2-week monitoring period. Spring discharge during the early spring storm-flow trace averaged 0.100 m^3/s, with a maximum of 0.357 m^3/s. Breakthrough began ~ 0.75 hours post-injection for the solutes and ~ 1.08 hours post injection for the microspheres. Rhodamine WT and Br^- concentrations at the spring peaked ~ 0.92 hours after injection. Rhodamine WT was < DL 5.75 hours after injection, while Br^- was < DL 2.75 hours after injection. Calculated mass recoveries were 79.32 % for rhodamine WT and 27 % for Br^- (Fig. 4). Microsphere analysis of all samples is currently being completed. Like the summer storm-flow trace, breakthrough curves for the solutes were relatively smooth with steep upward limbs, while the microsphere breakthrough curve remained very jagged. EC averaged 514 µS/cm^3 @ 25 ºC (maximum 645 µS/cm^3 @ 25 ºC) and water temperature averaged 14.0 ºC (maximum 16.5 ºC) for the duration of the trace. Analyses of isotopically enriched *E. coli* samples are pending.

Principal Findings and Significance

These data demonstrate differences in tracer behavior under low- and storm-flow conditions. Mass recoveries for rhodamine WT and Br^- were similar (within 5 %) for the first two traces, suggesting that both tracers are conservative under both flow regimes, yet the mass recoveries for rhodamine WT and Br^- were very different for the early spring storm-flow trace. This difference in mass recovery is possibly due to the quick breakthrough of this trace: the Br^- slug may not have had sufficient time for complete mixing to occur within the system and the primary plume was most likely missed during sampling. Rhodamine WT mass recoveries were lower for the summer storm-flow trace, which may indicate that part of the solute breakthrough curve was missed, that some discharge from the conduit network occurs downstream of Blue Hole Spring (such as via high-level overflow passages, which have not been delineated), or possibly, that loss of the tracers in portions of the epikarst occurred due to the higher stage within the system. Near-simultaneous breakthrough occurred for all tracers for each trace. Rhodamine WT tailing relative to Br^- may be an artifact of the lower DL for the dye. Continued detection of microspheres after rhodamine WT became undetectable may reflect sedimentation and/or resuspension of the particles (Fig. 3) (Marshall et al., 1998). Our results demonstrate that bacteria-sized particles can remain mobile for at least several days after introduction, which is consistent with studies in other karst terranes (Goldscheider et al., 2006; Davis et al., 2005).

Literature Cited

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Marshall, D., Brahana, J.V., and Davis, R.K., 1998, *Resuspension of viable sediment-bound enteric pathogens in shallow karst aquifers*: In J.V. Brahana, Y. Eckstein, L.K. Ongley, R. Schneider, and J.E. Moore (eds.), Proceedings of the Joint Meeting of the XXVIII Congress of the International Association of Hydrogeologists and the Annual Meeting of the American Institute of Hydrology. AIH, St. Paul, MN, p. 179-186.

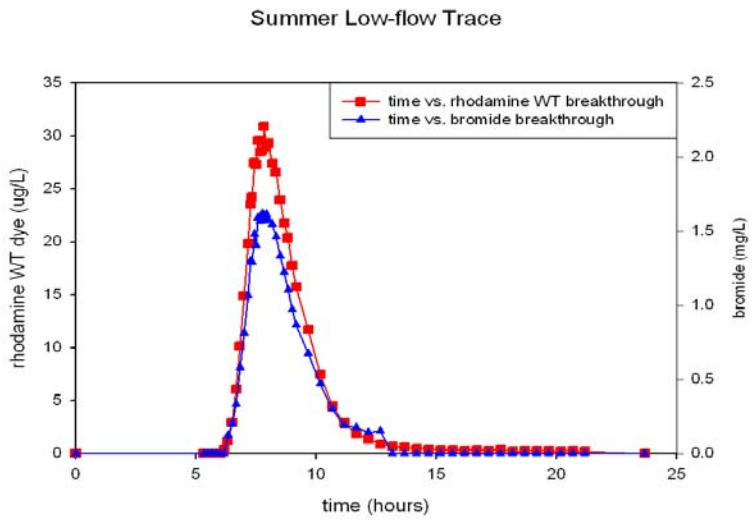

Figure 1, Rhodamine and bromide breakthrough vs. time during summer low-flow trace.

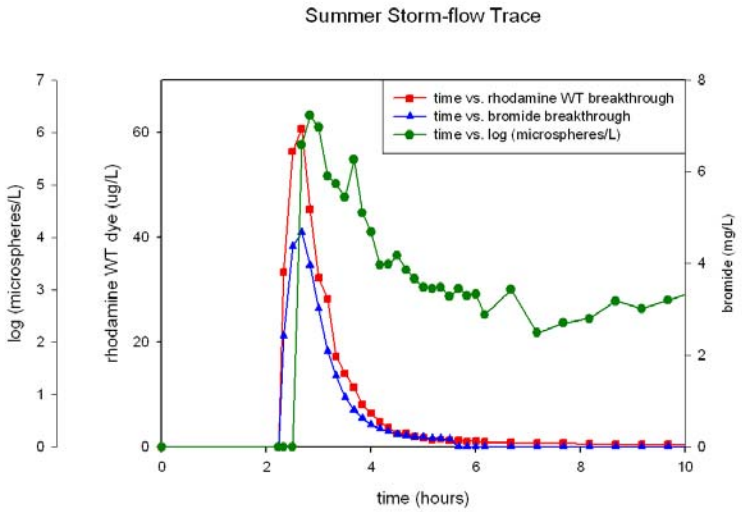

Figure 2, Rhodamine, bromide and microsphere breakthrough vs. time during summer storm-flow trace.

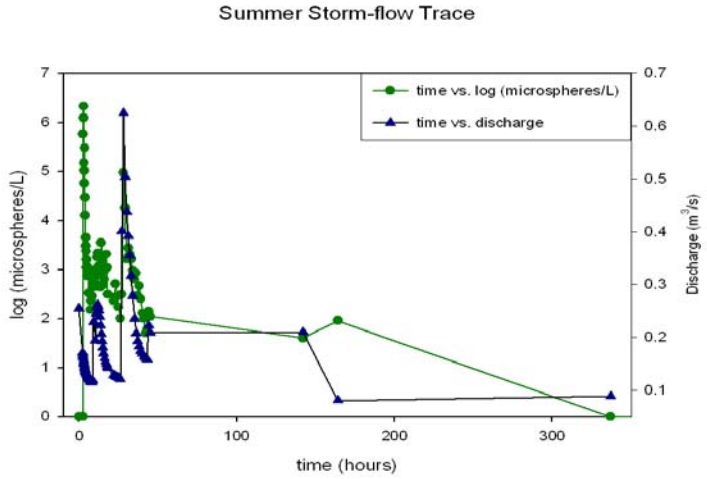

Figure 3, Latex microsphere breakthrough and remobilization over time compared to discharge during summer storm-flow trace.

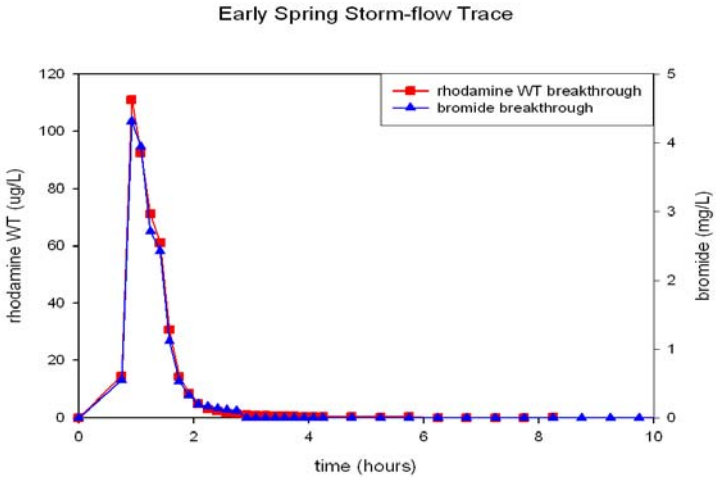

Figure 4, Rhodamine and bromide breakthrough vs. time during early spring storm-flow trace.