Effectiveness of traditional septic systems for fecal-indicator microorganism removal in Ohio soils with seasonally high water tables

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Abstract

The purpose of this investigation was to evaluate effectiveness of fecal-indicator organism removal at residential onsite septic systems in Ohio on soils with seasonally high water table. The working design parameter for septic systems in Ohio is that fecal-indicator microorganisms are removed within the first two feet of leach field. Although this assumption is based on bacterial travel through unsaturated soil, in practice the design parameter is applied to all permitted leach-field-type septic systems. Deep perimeter curtain drains are sometimes installed to lower the water table and extend the zone of unsaturated soil.

Sampling wells were drilled at horizontal spacing (5-23 feet) from the leach line and at various vertical increments (4-8, 8-12, 12-16 ft) from land surface. Water collected from sampling wells was analyzed for the presence of *Escherichia coli*, a fecal-indicator bacterium. When water was present, the curtain drain also was sampled. *E. coli* subtypes were discriminated by rep-PCR genotyping. Subtypes detected in sampling wells were compared with subtypes present in the septic tank and in animal feces at the land surface to provide evidence of origin. In one of the six soil types evaluated, *E. coli* were detected at a distance of 4 feet or more from the leach line (Miami-Kokomo-Eldean region; Greene county). At two intensively sampled sites, *E. coli* were detected at distances as great as 10 feet from the leach line and at soil depths reaching down to the 4- to 8-foot increment. Comparison of subtypes found in the soil groundwater to possible sources allowed evaluation of whether fecal contamination of subsurface groundwater was from the leach field or feces at the land surface.

Introduction

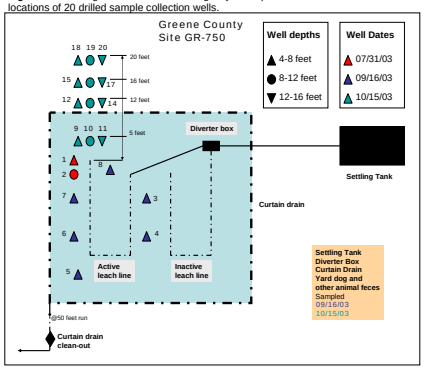
Ohio's state guidelines for citing household sewage treatment system (HSTS) leach lines are based on the assumption that treatment of bacterial pathogens occurs within the first 1.5 to 2 feet of soil (Duncan et al, 1994). It has been estimated that only 6.4% of Ohio soils are suitable for traditional leach line or mound septic systems (Mancl and Slater, 2002). The goal of this study is to assess the performance of standard leach-line septic systems in environments in which they are likely to fail but show no evidence of surfacing effluent. This information may be used by the Ohio Department of Health (ODH) to revise state minimum construction standards for on-site sewage systems. Additionally, relatively experimental microbial source tracking (MST) techniques may be indicated as useful tools with which to test whether nearby ground-water resources have been contaminated by septic systems.

In this poster we describe results of the evaluation of three sites in Greene and Licking Counties, Ohio. Fecal contamination of ground water was assessed by sampling for the fecal indicator *Escherichia coli* and characterizing this organism using rep-PCR DNA fingerprinting techniques. Known-source samples collected directly from septic tanks were characterized to represent the host population. Samples were collected from near-surface ground water at increasing distances from selected septic leach fields and from curtain drain effluent. Comparisons were made to determine whether *E. coli* cultivated from ground water and curtain drain locations were genetically identical to *E. coli* isolated directly from the septic system. The proximity of the test samples to the septic system makes association of the two likely, but *E. coli* could also originate with droppings on the land surface (pets) or from widespread contamination of off-site, near-surface soil water.

Materials and Methods

Sample Collection:
Only 3 sites of 20 tested were characterized within the project timeline because of low volumes of available ground water and insufficient *E. coli* concentrations at numerous sites. Two of the sites are in Greene County: GR-750 and GR-752, and the third site is in Licking County: LI-16. The site diagram in Figure 1 illustrates the typical orientation of sampling locations for one site, GR-750. Samples were collected at varying depths (4-8 feet, 8-12 feet, and 12-16 feet) and varying distances from the septic leach lines (4-20 feet). *E. coli* were found within the 4-8 feet depth range and up to 20 feet from the leach lines. Isolates were also cultivated from the originating sewage settling tanks, diverter boxes, and the curtain drain effluent, where possible. Where found, animal scat samples were collected from the land surface for cultivation and analysis.

Figure 1. Site diagram of GR-750 showing major components of HSDS and locations of 20 drilled sample collection wells.



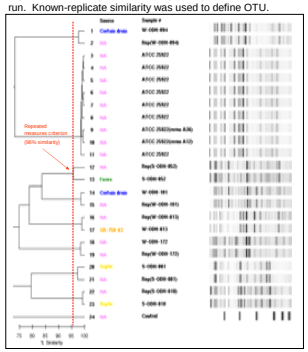
All samples were either plated by membrane filtration or streaked directly onto mTEC agar to cultivate presumptive *E. coli*; this was done either in the field or at the U.S. Geological Survey Ohio District Microbiology Laboratory (ODML), Columbus, Ohio. Individual colonies were picked to TSB and streaked onto EMB agar as a purity check. Pure cultures were stored in LB with 40% glycerol at -70°C until further use. Genomic DNA extraction was then performed using the MoBio UltraClean™ microbial DNA extraction kit. All DNA extracts were quantified with the PicoGreen® dsDNA quantitation kit and normalized to 50 ng/µL for use as rep-PCR DNA templates. Isolate extracts were stored at -20°C until analysis by rep-PCR.

Data Generation:
Rep-PCR using BOX A1R primer was done by use of the DiversiLab™ system from Bacterial Barcodes, Inc., a proprietary commercial system adapted from Versalovic et al. (1994). Complete protocol details can be found at http://www.bacbarcodes.com. A master PCR reaction cocktail was prepared for each run of 48 isolates and dispensed into individual PCR vessels. Normalized DNA extracts were added to the dispensed PCR cocktail as templates. Each PCR run was accompanied by a negative control in which sterile reagent grade water was substituted for template and a positive control utilizing a reference ATCC strain of *E. coli* as template. Each run included a within-run and between-run replicate isolate. All reactions were performed in 0.2 mL thin-walled PCR tubes, and the reagents and vessels were kept cold during preparation using thermal blocks. PCR conditions were similar to those described in Versalovic et al. (1994). All PCR products were held at 4°C pending gel electrophoresis.

DNA amplification products were separated by microcapillary gel electrophoresis by use of LabChip® DNA chips (Agilent 2100 Bioanalyzer®). Visualized DNA fragments ranged in size from 150bp to 7,000bp. The Bioanalyzer® uses microfluidic technology to integrate the individual steps of traditional electrophoresis. DNA samples are separated within micro-channels of the DNA chip. As the separated DNA fragments pass through a laser detector, a DNA fingerprint is generated in the form of an electropherogram. PCR products from 10 isolates, a negative and positive control, and a DNA molecular weight ladder were run on each DNA chip. Following genetic analysis, isolates were subjected to phenotypic tests recommended by USEPA in order to confirm each as *E. coli* (urease activity, indole production, growth on minimal citrate, fermentation of lactose at elevated temperature, oxidase activity).

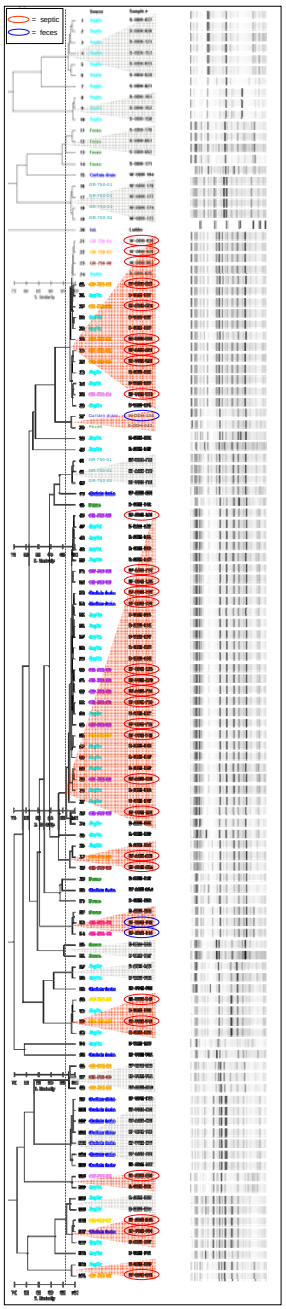
Data Analysis:
DiversiLab® software was used to calculate the Pearson similarity coefficient for each pair of rep-PCR fragment patterns (electropherograms) generated. Genetic similarity between isolates was observed in two ways: a hierarchical dendrogram was produced using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) and a scatterplot was created showing a two-dimensional representation of similarities among all isolates.

Figure 2. Dendrogram containing all repeated measures run. Known-replicate similarity was used to define OTU.



The dendrogram was used to define *E. coli* subtypes, or operational taxonomic units (OTU). OTU were established by first using replicate isolate analysis to determine the analytical variability inherent to the method (Figure 2). Multiple isolates were considered from the same presumptive OTU if the variability among them was less than or equal to the criterion set by the repeated measures. In all cases, professional judgment was used to check dendrogram-based assignment of OTU and, occasionally, override the presumptive OTU assignment.

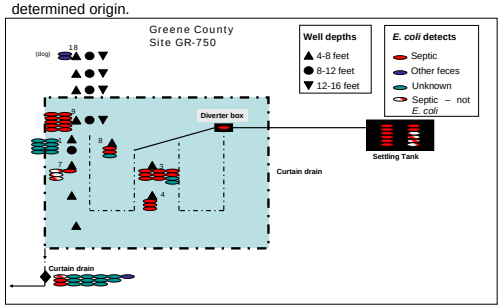
Figure 4. Dendrogram displaying results from site GR-750. OTU containing test isolates of known origin indicated in red ( ).



Results

At each of the three study sites, *E. coli* were detected at horizontal distances of at least four feet from the septic leach line at shallow depth (4-8 feet), but not in deeper soil water. At one site, GR-750, *E. coli* were detected up to 20 feet from the septic leach line, again at the shallow (4-8 feet) depth. Curtain drain effluent was sampled at two sites (GR-750 and GR-752); *E. coli* were detected at both sites. At site GR-750, *E. coli* were detected at 7 of the 20 wells sampled and in the curtain drain effluent. At site GR-752, *E. coli* were detected at 3 of the 8 wells sampled and in the curtain drain effluent. At site LI-16, *E. coli* were detected at 2 of the 3 wells sampled and no effluent could be sampled from the curtain drain.

Figure 3. Site diagram for GR-750 illustrating *E. coli* detections and their determined origin.



At the most rigorously tested site, GR-750, considerable evidence of fecal-bacteria breakthrough was found (Figures 3 and 4). *E. coli* isolates at five separate sampling wells and the curtain drain effluent were genetically indistinguishable from *E. coli* isolates in the septic system. In total, 55 *E. coli* isolates from these wells and curtain drain were analyzed, of which 30 were assigned to OTU shared with isolates of septic origin. Data used to reach this interpretation are provided in Figure 4. *E. coli* from deposited scat samples and 3 test isolates were also genetically indistinguishable: 2 from well 18 and one from the curtain drain effluent (Figures 3 and 4). In one sample well and the curtain drain, a total of four isolates did not confirm as *E. coli,* despite their apparent origin in the septic system. While this finding may not have the same regulatory implications as genetic similarity among confirmed *E. coli* isolates, it still indicates that bacterial cells of fecal origin, both *E. coli* and non-*E. coli*, were capable of surviving travel from the septic leach line to nearby ground water.
These results from site GR-750 were supported by findings at the two less thoroughly samples sites. At GR-752, *E. coli* isolates consistent with septic origin were identified in 2 out of the 3 sampling wells in which *E. coli* isolates were found. Limited results from site LI-16 indicate that one well out of two analyzed had *E. coli* originating from the septic leach line (2 out of 7 isolates tested). Despite their ability to grow on mTEC agar, nearly all isolates cultivated from site LI-16 failed to confirm as *E. coli*.

Conclusions

•*E. coli* originating from home sewage treatment systems (HSTS) was detected up to 5 feet from septic leach lines at soil depths of 4-8 feet.

•Microbial source tracking using rep-PCR can be a useful tool for evaluating whether off-site fecal bacteria originate with HSTS.

•Leachate from HSTS can carry *E. coli* and other fecal-origin bacteria through more than the design guideline of two feet of soil; leachate-carried bacteria can be intercepted and transported with curtain drain effluent, but septic-origin bacteria were not detected outside the curtain drain perimeter.

References

Dombek, P.E., L.K. Johnson, S.T. Zimmerley, & M.J. Sadowsky. 2000. Use of repetitive DNA sequences and the PCR to differentiate *Escherichia coli* isolates from human and animal sources. Appl. Environ. Microbiol. 66(6): 2572-2577.
Duncan, C.S., R.B. Reneau, Jr., and C. Hagedorn. 1994. Impact of effluent quality and soil depth on renovation of domestic wastewater. Proceedings of the Seventh International Syposium on Individual and Small Community Sewage Systems. 219-228.
Mancl, K. and B. Slater. 2002. Suitability of Ohio soils for treating wastewater. Ohio State University Extension Bulletin 896. Columbus, OH.
Versalovic, J., M. Schneider, F. J. de Bruijn, and J. R. Lupski. 1994. Genomic fingerprinting of bacteria using repetitive sequence-based polymerase chain reaction. Methods Mol. Cell. Biol. 5:25-40.