Upper Sugar Creek Watershed, Ohio: A Model Watershed for Study of Pathogen Origin, Fate and Transport

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Abstract

Transport of human and animal wastes into natural waters can result in contamination with pathogenic microorganisms, which has become an increasingly serious health concern. The inability to differentiate these non-point sources of microbial pollutants creates conflicts among agricultural, environmental, and public health interests. There is also a need to discover practical intervention strategies to interrupt transmission from the source to the receiving water. We are investigating the origin and transport of microbial contaminants from non-point sources on a watershed scale and identifying the social and cultural keys to good management practices within the watershed. This study is novel in its combination of both scientific research and community outreach.

This project will be conducted through three phases. First, we will associate land use patterns with bacterial contamination in the headwaters of the Sugar Creek watershed, which is one of the most polluted in the state of Ohio, using a targeted sampling approach. We will use qPCR (quantitative PCR) techniques to identify the magnitude of indicator bacteria concentrations at key points in the watershed, and host-specific traditional PCR to determine potential host sources. For the second phase we will conduct a highly focused follow-up sampling to associate specific land management practices with host source diversity of fecal indicators, detection of select pathogens, and microbial indicator concentrations. In the last phase we will identify social and cultural keys that impact land management decisions so that microbial contamination in the watershed will be reduced.

Impact statement

This project advances the research on the fate and transport of microbial contaminants from non-point sources that are diffuse and difficult to control, by using an innovative microbial source tracking method. It is innovative in its combination of targeted sampling, novel bacterial indicator methods, and pathogen detection to relate local land use and management practices to host sources of microbial contamination. As a further investigation, the association of management practices such as exclusion fencing, no-tillage, buffer strips and manure spreading with the source and density of fecal indicators in the watershed will be studied.

The First Phase --- Materials and Methods

► Sample collection: Stream water samples were taken from 21 sampling sites on September 2007 and November 2007 for this preliminary study. At the upper Sugar Creek watershed we have different land uses with potential contaminant sources such as residential areas, crop fields, livestock operations (dairy, sheep, horse, and swine), and natural forested areas. Water samples were kept in a cooler box, taken back to the laboratory, and frozen at -20 ºC until further processing.

►Sample filtration and DNA extraction: Water samples were thawed and duplicate 100ml samples were filtered (0.2µm filter) to collect the bacterial cells. DNA was extracted directory from whole filters using PowerSoil DNA Kit (Mo Bio, CA). DNA was quantified using a NanoDrop ND-1000 UV spectrophotometer (NanoDrop Technologies, DE). Most samples showed less than 2 ng/ul of DNA concentration. PCR was performed with DNA extraction with no dilution.

►Quantitative PCR: General 16S rRNA gene *Bacteroidales* makers were used to assess the magnitude of indicator bacteria concentrations in the samples. Within each qPCR run, all samples were analyzed in triplicate. Also, the potential impact of PCR inhibiters was determined with 10 fold and 100 fold of selected samples. There was no inhibition observed.

►Host specific PCR: Human specific 16S rRNA gene *Bacteroidales* markers were used to assess the sources of fecal pollution. Human fecal DNA extraction and sewer influent DNA extraction were used as positive controls of PCR assay.

Result- qPCR

All samples except sampling point 9* contained detectable amount of *Bacteroidales* (Figs. 1 and 2), which indicates any type of fecal contamination. November samples had higher concentrations than September samples except sampling point 4. Overall, results showed a wide range of concentration, anywhere from 10^3 to 10^7 of target DNA segments per 100 ml sample.

This preliminary study would suggest the possible association between qPCR results and land use pattern. The sub-watershed of sampling point 18 is a more developed area than the one of sampling point 16 (See Fig. 3). qPCR results showed relatively higher values for sampling point 18, and lower value were obtained from sampling point 16.

*(Sampling point 9 was a community maintained natural spring used as a drinking water supply.)

Result- Human specific *Bacteroidales* PCR

September samples did not show positive result for human specific *Bacteroidales* at any of the 21 sampling sites. This is probably due to the low impact of human activity contributing to this specific marker (data not shown). However November 2007 samples showed positive results with human specific marker (Fig. 4). This result suggests the possible association between host specific PCR results and land use pattern. Sampling point 18, which is mentioned above, also showed strong positive for the human specific marker as did sampling point 1 downstream of the Smithville city sewage treatment plant. However, the bands were quite weak compared to the positive controls.

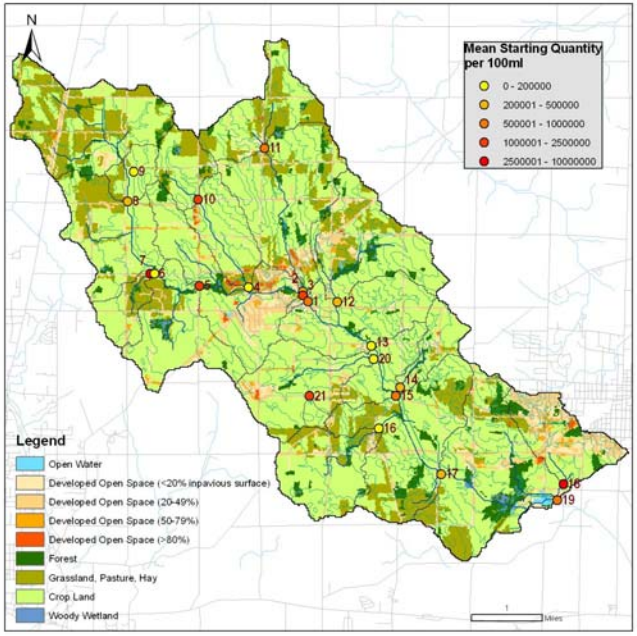

Fig. 1 Upper Sugar Creek Watershed mapped with land use data (NLCD 2001, USDA), A total of 21 sampling points in sub-watersheds are identified. The color of the sampling points indicates the magnitude of mean standard quantity per 100 ml water sample (November 2007).

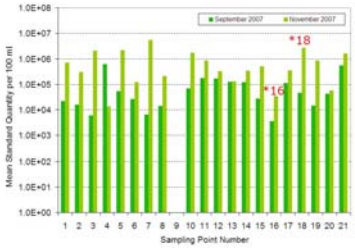

Fig.2. qPCR results with general *Bacteroidales* 16S rRNA gene markers. Values are geometric mean of PCR duplicates and filtration duplicates (total of four).

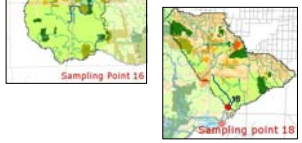

Fig.3. Examples of sub-watershed.
#16: agricultural area is dominant.
#18: developed area is dominant.

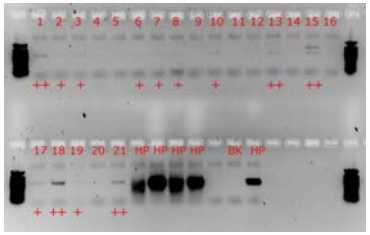

Fig.4. Host Specific PCR results with human specific *Bacteroidales* 16S rRNA gene markers for November 2007 samples.

Numbers for each lane indicate the sampling points. HP indicates human fecal as positive controls, BK indicates the PCR negative control.

Strong positives: Sampling points 1,13,15,18, and 21.
Weak positives: Sampling points 2,3,6,7,8,10,17, and 19.

Discussion

These preliminary results suggest the possibility of using qPCR to identify and quantify the *Bacteroidales* 16S rRNA gene as a microbial contamination indicator. However, the DNA recovery rate for the extraction process was not stable (data not shown) and needs to be improved.

Host specific PCR analysis shows the origin of the indicator bacteria *Bacteroidales*. However, the fate and transport of these indicator bacteria are not well studied yet. Also, the sensitivity of the method should be improved in order to detect lower concentrations of the host specific indicator bacteria.

Future Work

Phase 1

Complete sampling at all watershed sites and do analyses with qPCR/host specific PCR.

•Improvement of extraction method with stable recovery rate
•Improvement of host specific PCR sensitivity
•More watershed wide sampling

Study of association between **land use** and bacterial contamination

Phase 2

Conduct focused follow-up sampling for study of:
•Association between **land management practices** and bacterial contamination
•Detection of select **pathogens**

Phase 3

Identify **social and cultural keys to land management decisions to improve management of potential** microbial contaminants in the Sugar Creek watershed.

Acknowledgements

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