

The Role of Suspended Sediment in *E. coli* Transport

Pamela Struffolino*, Von Sigler¹, Daryl Dwyer¹

pstruff@utnet.utoledo.edu

¹Lake Erie Research Center, ¹University of Toledo, Department of Earth, Ecological and Environmental Science.

Introduction

Beach closings or warnings due to bacterial pollution are common during the recreational season. The source of the pollution is often unknown but is commonly a result of fecal pollution, which is indicated by the presence of *Escherichia coli*. Recent studies have shown that bacteria survive better in sediments than in the overlying water column. Therefore the resuspension of sediments might have an impact on *E. coli* population density and structure. This study was conducted to determine the role of the resuspension of bottom sediments on *E. coli* densities, population structure and their relationship to beach closings.

Methods

Experimental site and sampling

Sediment traps were placed at several locations in Maumee Bay of Lake Erie (Figure 1) to collect suspended sediment from the water column. The sediment traps consisted of 5 pairs of vertical cylinders attached to a stable base (Figure 2). The traps were placed in at least three feet of water to ensure that the cylinders remained submerged even during transient periods of low water levels and were located at the mouths of several local streams and ditches (Table 1).

At approximately two-week intervals, trapped sediment, bottom sediment, and water were collected and placed on ice to be enumerated in the laboratory (Figure 3).

Table 1. Site identifications and descriptions.

Site ID	Site Description
ST02	Otter Creek in Maumee River
ST04	Near the Edison Outfall (where water used in a cooling tower is discharged)
ST05	Heckman Ditch
ST06	Joliet Ditch
ST08	Tobias Ditch
ST09	McHenry Ditch
ST13	Between McHenry and Berger Ditches
ST14	East of Maumee Bay State Park, West of the mouth of Berger Ditch
ST15	East of the mouth of Berger Ditch
ST16	Berger Ditch

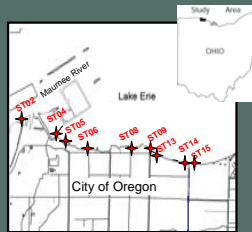


Figure 1. Location of sediment trap locations throughout Maumee Bay

E. coli enumeration

E. coli densities in trapped and bottom sediment were estimated using the Coli-18 method. *E. coli* densities in water were estimated using the modified m-TEC filtration method (USEPA, 2002).

E. coli community analysis

DNA was extracted from trapped and bottom sediments from sites ST02, ST04 and ST08 according to the method of Sigler and Zeyer (2002). The resulting DNA samples were pooled, assessed for concentration by measuring absorbance at 260 nm and purity was estimated from the ratio of A_{260}/A_{280} .

The structure of the *E. coli* population in each of the samples was assessed by polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE). Specifically, a 166 base pair sequence of the β -glucuronidase gene (*uidA*) was amplified using primers UAL-1939-F and UAR-2105-R, which targeted the carboxyl end of the *uidA* gene of *E. coli* and some *Shigella* spp. (Bej et al., 1991). To facilitate the separation of PCR products in DGGE, a GC-clamp (Muyzer et al., 1993) was attached to the 3' end of UAL-1939.

DGGE was performed in 8% polyacrylamide gels containing a denaturing gradient of 20-60%. The gel was run at 60° C for 1000 V-h (5 h at 200 V). Following electrophoresis, gels were stained with a 1:10,000 dilution of Gel Star nucleic acid stain (BioWhittacker) and visualized using a Gel-Logic image analysis system (Kodak).



Figure 2. Design of sediment trap. Two graduated cylinders with funnels attached comprised one set of collectors. There were five collectors attached to a circular base with pole, light, and sign for identification.



Figure 3. One set of collectors are recovered after two weeks. Bottom sediment and water were also collected at the same time. All samples were placed on ice until laboratory analysis.

Results and Discussion

E. coli enumeration

E. coli densities in trapped sediments ranged from 67 MPN/g_{DW} to 3433 MPN/g_{DW} (Figure 4). Densities in the bottom sediment ranged from <1MPN/g_{DW} to 479 MPN/g_{DW}. The means were statistically different (Student's-t test, $p < 0.01$). These results suggest two considerations;

1. *E. coli* attrition is associated with older, bottom sediments, while viable *E. coli* are associated with suspended sediments.
2. The difference in mean *E. coli* densities between trapped and bottom sediments indicates that the sediment traps effectively collected fresh inputs of sediment rather than resuspended bottom sediments.

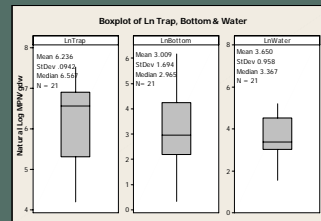


Figure 4. Boxplot of water, bottom and trapped sediment *E. coli* densities. Inner box represents the middle 50% of data. The vertical lines extending from the ends of the boxplot represent the general extent of the data. The horizontal line represents the median of the data.

- Among all sampling sites, *E. coli* densities in trapped sediment exhibited less variance than those in bottom sediments (Figure 5). One possible explanation for this result is that trapped sediments likely harbor a great proportion of fresh *E. coli* inputs, whereas bottom sediments exhibit a mixture of fresh, viable *E. coli* populations as well as older, less viable populations (Figure 6). Experiments are ongoing to test this hypothesis.

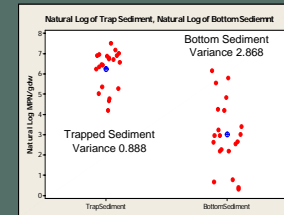


Figure 5. Plot of *E. coli* densities of trapped sediment vs bottom sediment. The variance of *E. coli* densities for the trapped sediment have less variance than the bottom sediments.

E. coli community analysis

- PCR-DGGE fingerprints of *E. coli* from the trapped sediment differed from those of bottom sediments suggesting that discrete *E. coli* populations differentiate trapped from bottom sediments throughout the Bay (Figure 7). This is likely a result of differing population structure associated with fresh inputs of *E. coli* versus older populations in bottom sediments.

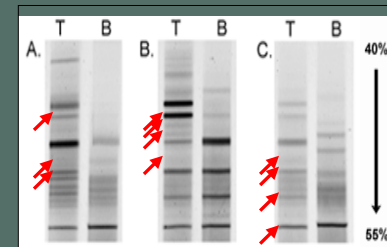


Figure 7. DGGE profiles of *E. coli* populations in trapped (T) and bottom (B) sediments from sites ST02 (panel A), ST04 (panel B), and ST08 (panel C). Percentages refer to approximate percent denaturant concentration in the selected region of the gel.

- Several bands in the DGGE profiles of trapped sediment do not appear in those of the bottom sediment (arrows, Figure 7). These bands appeared to represent novel populations indicative of the sediment-related transport of *E. coli* that originated from sources distant from the trap site. This provides further evidence that the sediments in the trap originated from fresh inputs and not resuspended bottom sediments.

Conclusion

Fresh inputs of suspended sediments harbored higher *E. coli* densities than bottom sediments. Additionally, *E. coli* population structural differences between trapped and bottom sediments suggest that suspended sediment inputs harbor unique *E. coli* populations. Therefore, while *E. coli* has been found to persist in bottom sediments, the majority of sediment collected in the traps appear to be fresh inputs rather than resuspension of bottom sediment as indicated by the higher densities of *E. coli* found in the traps. Finally, sediment suspension and transport of this sediment is an important factor to the bacterial pollution of recreational beaches. We are continuing to conduct research to establish the contribution of sediment inputs to bacterial pollution in recreational waters.

References

- Bej, A. K., Dicesare, J. L. Haff, L. Atlas, R. M., 1991. *Detection of Escherichia coli and Shigella ssp. in water by using polymerase chain reaction and gene probes for uid.* Appl. Environ. Microbiol. 57:1013-1017.
- Muyzer, G., de Waal, E. C., Uitterlinden, A. G., 1993. *Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA.* Appl. Environ. Microbiol. 59:695-700.
- Sigler, W.V. and J. Zeyer. 2002. *Microbial Diversity and Activity along the Forefields of Two Receding Glaciers.* Microb. Ecol. 43:397 - 407.
- United States. Environmental Protection Agency. 2002. Office of Water., *Method 1603: Escherichia coli (E. coli) in Water by Membrane Filtration Using Modified membrane-Thermotolerant Escherichia coli Agar (Modified mTEC.* [Washington, D.C.]: United States Environmental Protection Agency Office of Water.