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FATE AND TRANSPORT OF POTENTIAL PATHOGENS: THE CONTRIBUTION FROM SEDIMENTS

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ABSTRACT: In this study, *Escherichia coli* was used as a bacterial tracer for the development of a watershed scale fate and transport model for a suite of reference pathogens. In this study, we investigated the *E. coli* densities in water and sediments from the Blackstone River Watershed, Massachusetts as phase I of the modeling study. *E. coli* and total coliforms from water and sediment samples were collected in three seasons during wet and dry weather events. The confirmed *E. coli* strains were identified by ribotyping for tracking the sources of *E. coli* and for determining the association of downstream *E. coli* isolates with isolates from upstream sediments. A majority of downstream samples were associated with upstream sediment sources of *E. coli*. A hydrologic model, WATFLOOD, will be used to predict the temporal and spatial variation of *E. coli* in the Blackstone River. *E. coli* densities ranged from 71 to 5968 MPN/100 mL in water samples and from 2 to 335 MPN/g in sediments. Pearson correlation analysis revealed significant correlations between *E. coli* and total coliforms in water ($r= 0.777$, $p<0.01$) and sediments ($r=0.728$, $p<0.01$). In addition, *E. coli* concentrations in water were weakly correlated with sediment concentrations ($r=0.298$, $p<0.01$).
KEY TERMS: *Escherichia coli*; watershed modeling; pathogens; fate and transport; ribotyping; sediments

INTRODUCTION

Concerns of microbial contamination of waters have increased as a result of documented waterborne disease outbreaks (Leclerc et al., 2002). Impairment of water quality due to the presence of pathogens is typically assessed by monitoring for microbial indicators such as total coliforms, fecal coliforms and *Escherichia coli*. Indicators have been associated with enteric pathogens present in water from human or animal fecal contamination (e.g. Hörman et al., 2004). However, the reliability of the use of indicators to predict pathogens has been challenged by some studies (Hegarty et al., 1999; Schets et al., 2005). As such, researchers have been developing new methods for the direct detection of pathogens in water samples (e.g. Guy et al., 2003) and have been studying pathogen fate and transport characteristics (e.g. Jiménez et al., 1989).

Most investigations into pathogen occurrence in watersheds have focused only on the water column and have seldom considered interactions with sediments. Sediments can affect the transport and survival of microorganisms in natural waters in several ways: (1) sediments at the bottom of rivers, lakes and estuaries may serve as reservoirs of viable indicator bacteria and enteric pathogens; (2) sediments and sediment particles can affect concentrations and transport processes of pathogens in the water column – they can be adsorbed to sediment particles and settle out of the water column more rapidly. These are important processes for microbial transport and removal in waters.

The types of particles have a great impact on the settling velocity. Generally, denser inorganic particles will settle out more quickly than others. The microbial adsorption to settleable particles is different for different types of microorganisms and the adsorption behavior of each organism changes between dry and wet weather (Characklis et al., 2005). The release of bacteria during sediment resuspension caused by storms, flood, tides or strong winds will result in high concentrations of bacteria in the water column (Jamieson et al., 2005; Muirhead et al., 2004). Lee et al. (2006) reported that the peaks of *E. coli* and enterococci levels in water and sediment were consistent with storm activity in the beaches which were exposed to fecal contaminants. Studies have shown that indictor organisms have a tendency to survive longer in sediments than in natural water (Burton et al., 1987; Davies et al., 1995). The persistence of pathogens in sediments creates a longer period of risk to public health.

In this study, we investigated the microbial contamination in sediment and water samples from the Blackstone River Watershed. The objective was to illustrate the role of sediments in the fate and transport of potential pathogens during wet weather events.

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Study Area

The area of this study is the Upper Blackstone River Watershed (Figure 1). The Blackstone River originates in the Worcester hills in central Massachusetts, and flows southeasterly into Rhode Island, discharging eventually into Narragansett Bay. Sediment and surface water samples were collected from three sites - MR02, BS04, and BS01 (Figure 1) for three wet weather events (May, October and November) and one dry event (September) in 2006. A wastewater treatment plant is located immediately downstream of site BS04. At times, sediment samples were not collected due to excessive flooding. Sediment samples were collected using long glass pipets (May event) and subsequently using sterile syringes (September, October and November events) and placed in small sterile bottles. All samples were placed on ice in a cooler and immediately sent for analysis. Analysis was done within 4 hours of sample collection.

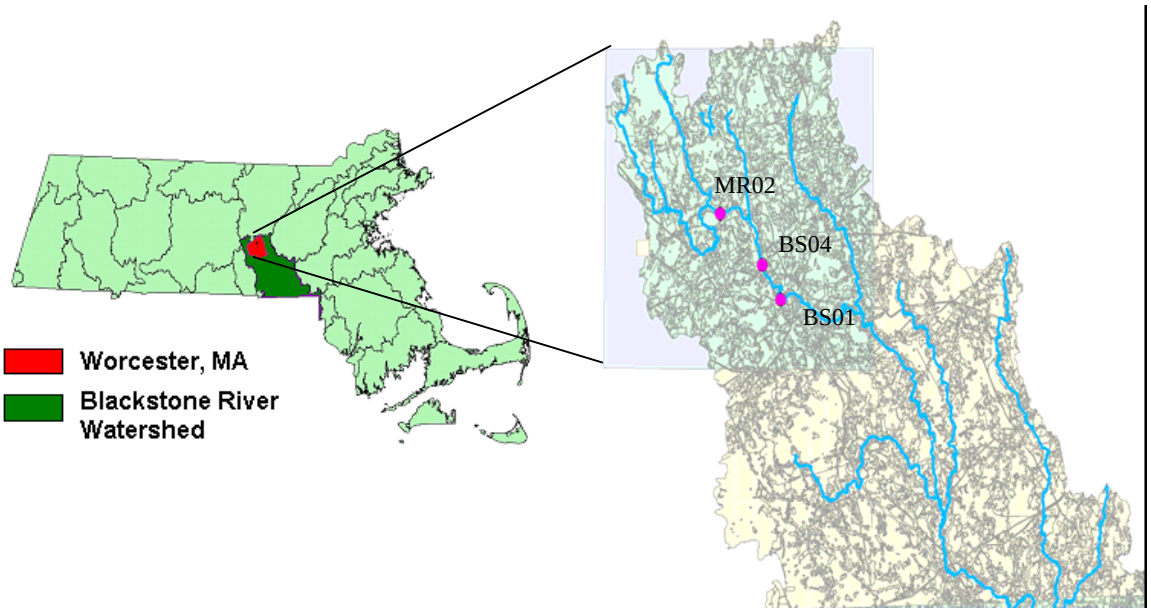

Figure 1. Study area in the Blackstone River Watershed. Sample Sites: (1) BS01, USGS streamflow gauging site at Millbury and downstream extent of Upper Blackstone River; (2) MR02, Middle River; (3) BS04, Upstream of wastewater discharge from the Upper Blackstone Water Pollution Abatement District.

Materials and methods

E. coli was enumerated using the Colilert method (IDEXX Company, USA). Sediment samples were analyzed according to the method presented by Craig et al. (2002). Twenty five g of sediments (wet weight) were placed into 75 mL of 0.1% peptone solution. Sediments were shaken and resuspended in solution for separating bacteria from the particles. Then 10 mL of liquid was placed into vessels and diluted 10 and 100 times. The mixtures were poured into Quanti-Tray®/2000**,** sealed in a Quanti-Tray® Sealer and placed in incubator for 24 hours at 35 ± 0.2 ºC. Total coliforms and *E. coli* were enumerated using an IDEXX Quanti-Tray®/2000 MPN table.

To grow isolates for ribotyping, a syringe was used to extract 0.5 mL of liquid from a random selection of fluorescent wells. The liquid was transferred into a tube with 10mL PBS buffer, mixed, and diluted 3 times as 10^{-1}, 10^{-2} and 10^{-3}, as described by Eckner (1998). A 0.1mL subsample was then spread onto the plate. A colony from the plate was selected and placed in a test tube containing inverted Durham tubes with EC broth (with MUG) medium as described by Feng and Hartman (1982). Then, the tubes were placed in an incubator at 35ºC for 24 hours. The tubes were observed under UV light, and the bacteria in the fluorescent tubes were confirmed as *E. coli*. The confirmed *E. coli* were made into slants for ribotyping.

Ribotyping was performed by the New York City Department of Environmental Protection using the RiboPrinter® Microbial Characterization System (DuPont Qualicon, Inc.). This system characterized the rRNA patterns of bacterial samples by matching the sample patterns against rRNA patterns that have previously been established and compiled into a

database. The bacteria were ultimately identified based on how similar, or different, the sample patterns were to the known patterns already existing in the Custom Identification library. Statistical analysis was carried out using SPSS 12.0 software. The Pearson correlation coefficients were calculated for *E. coli* and total coliforms in water and sediments.

Microbial fate and transport modeling is being performed using modeling code developed by Dorner et al. (2004, 2006). The model was developed for simulating and predicting peak pathogen concentrations in rivers and is currently being upgraded to improve simulations of the processes governing sediment resuspension during periods of increased streamflow. Unit values data were obtained from the U.S. Geological Survey database (USGS, 2006) for the Millbury station (#01109730). Streamflow data from 2006 are provisional and are subject to revision. Reference pathogen (e.g. *Cryptosporidium* spp., *Giardia* spp., *Campylobacter* spp. and *E. coli* O157:H7) fate and transport characteristics and model parameters are described by Dorner et al. (2006).

ArcGIS 9.2 (ESRI, 2006) software was used for GIS analysis for preprocessing of model input data files. The base maps (shapefiles), including the Blackstone River Watershed were acquired from MassGIS. The elevation data needed to simulate the water balance were acquired from Digital Terrain Model (DTM) downloaded from MassGIS website (http://www.mass.gov/mgis/). The studied area was divided into 226 grids, each with an area of 1 km^2. In each grid, the elevation of the center was assumed to represent the elevation of the grid. The slope, contour and aspect were calculated using the Spatial Analyst tools in ArcGIS. The flow direction and flow accumulation were calculated using the Hydrology Function in the Spatial Analyst tools. With the ArcHydro tool, the following steps were conducted: stream definition, stream segmentation, drainage line preprocessing, catchments grid delineation, catchment polygon processing, and hydro network generation. Land use data for the Blackstone River watershed was also obtained from MassGIS and was then input into the attribute table for the Blackstone River Watershed layer. In order to obtain the proportion of land use in each grid, each grid was clipped from the Blackstone River watershed layer. In the attribute table, land use was added as a new feature including both the type and extent in each grid. A total of 17 distinct land uses exist within the modeling area.

Results and Discussion

According to our monitoring data, *E. coli* densities at the three sites ranged from 71 to 5968 MPN/100 mL in water samples and from 2 to 335 MPN/g in sediments (dry weight). The spatial and temporal variation of *E. coli* in sediments is demonstrated in Figure 3. *E. coli* densities increased to high levels during storms and retuned to baseline levels during dry weather. In water samples, peak *E. coli* concentrations corresponded to the peaks in streamflow (Figure 2) Pearson correlation analysis indicated that *E. coli* concentrations in water were weakly correlated with sediment *E. coli* concentrations ($r=0.298$, $p<0.01$). There existed significant correlations between *E. coli* and total coliforms in water ($r=0.777$, $p<0.01$) and sediments ($r=0.728$, $p<0.01$). *E. coli* densities were higher at the two upstream locations (MR02 and BS04) as compared to the downstream location (BS01, below the discharge of the wastewater treatment plant).

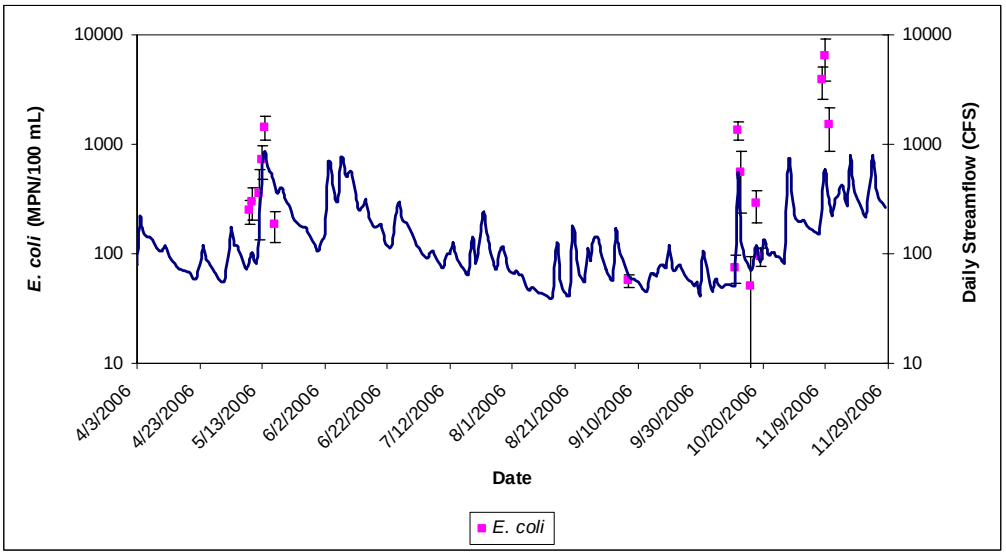

Figure 2. Daily streamflow and *E. coli* densities in the water column at site BS01 (Millbury Station).

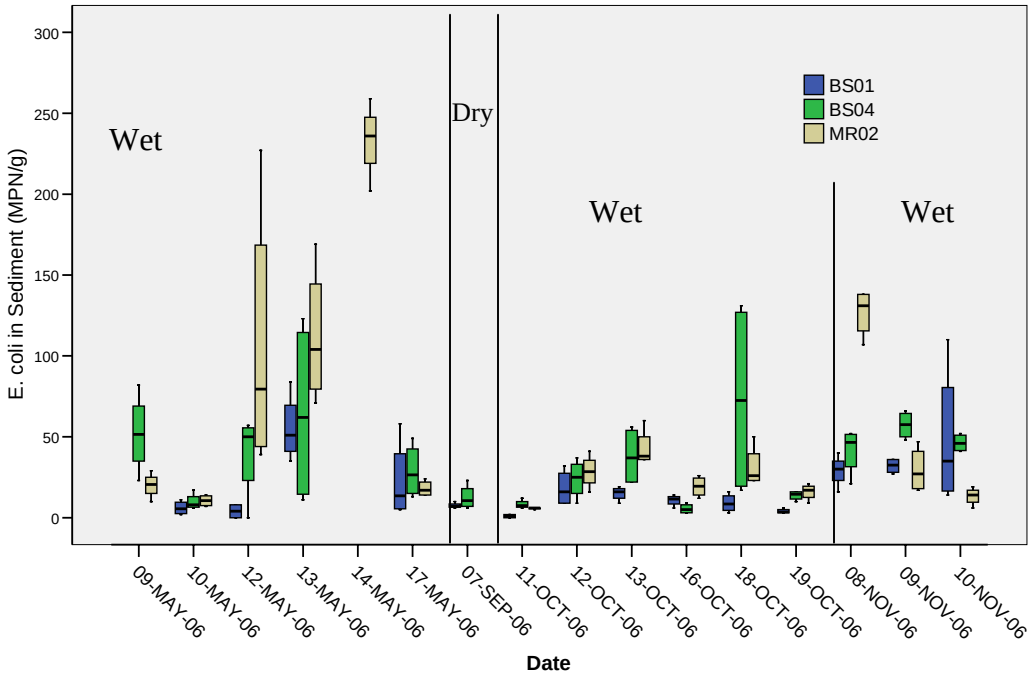

Figure 3. Temporal and spatial variation of *E. coli* in sediment samples.

In all, 49 isolates were processed for ribotyping. Ribotyping has been used successfully for the identification of the sources of food or water borne disease outbreaks (e.g. Schalch et al., 2003). The use of ribotyping for the general identification of host species within a watershed that are the sources of microbial contamination has met with moderate success (e.g. Stoeckel et al., 2004); however, this was not the objective of the current investigation. Five of the 49 were found to be organisms other than *E. coli*, and the remaining 44 were analyzed using restriction enzyme *Eco*R1. Of the 44 analyzed isolates, 21 (48%) successfully matched ribogroups of other *E. coli* collected during this study (Table 1). Isolates were only categorized into the same ribogroup if their genetic pattern (riboprint) matched other riboprints by 90% or greater similarity.

Examined *E. coli* from the upstream site, MR02, shared several of the same ribogroups as *E. coli* collected from the downstream sites, BS04 and BS01 (Table 1, shaded area). Both sediment and water isolated *E. coli* were found downstream; however, five of the seven matching MR02 *E. coli* were from the sediments. In some cases *E. coli* from MR02 on a given date were matched with downstream *E. coli* from an earlier date. This difference illustrates the complex transport dynamics in the natural environment and confirms the importance of sediments as a source of *E. coli*. Additionally, there were some *E. coli* recovered from site BS04 that were also found downstream at BS01 (Table 1 unshaded area). *E. coli* from a water sample collected at site BS04 on May 12 matched *E. coli* downstream at BS01 three days earlier, and also matched *E. coli* from BS01 collected one day later on May13, suggesting a long term upstream source of the ribotype that is transported downstream during events. Results also show that BS04 *E. coli* from the water column matched BS04 *E. coli* from the sediments collected on the same day.

Table 1. Matching RiboGroups of *E. coli* for sites sampled (Dorner et al., 2007)

RiboGroup	Date	Site*	Sample type	Site*(Sample type) and date matched
265-5	05/09/06	SMR02B	sediment	BS04B(water) 05/09/06
33-4	05/12/06	SMR02A	sediment	BS04B(water) 05/12/06
				SMR02A(sediment) 05/13/06
69-7	05/13/06	SMR02B	sediment	BS04B(water) 05/10/06
1000-4	05/13/06	MR02A	water	SBS04A(sediment) 05/13/06
48-8	05/14/06	SMR02B	sediment	BS01A(water) 05/17/06
				BS01B(water) 05/17/06

34-2	05/14/06	MR02A	water	SBS01B(sediment) 05/12/06
28-1	05/17/06	SMR02B	sediment	MR02B(water) 05/17/06
				SBS04B(sediment) 05/09/06
107-8	05/12/06	BS04A	water	BS01A(water) 05/09/06
				BS01A(water) 05/13/06
1000-3	05/13/06	BS04A	water	SBS04B(sediment) 05/13/06

*S in front of the site number (i.e. SMR02) indicates a sediment sample. A and B represent duplicates at a given site.

The Blackstone River was historically impaired by intense industrial development and urbanization. Fecal contamination has been documented at numerous locations throughout the Blackstone River watershed. During wet weather, resuspension of contaminated sediments in the river has been shown to be a source of water quality criteria violations (Wright et al. 2001). Our study showed that *E. coli* densities increased during wet weather events. This trend has been observed in other reports (Jamieson et al., 2005; Dorner et al., 2006). In water samples, peak concentrations of *E. coli* in wet weather were generally above an order of magnitude higher than that in dry weather. However, the temporal variation curves of *E. coli* were different between sediment and water samples. For example, the May 2006 event water samples at site BS01, had increasing *E. coli* concentrations from May 9 to May 14, which decreased to baseline levels by May 17. For the same event at site BS04, *E. coli* concentrations fluctuated with the peak concentration occurring on May 12. A similar trend was observed at site MR02. In sediment samples at site BS01, *E. coli* concentrations were highest on May 13 and decreased to baseline concentrations on May 17. At site BS04, the concentration of *E. coli* varied slightly over the course of the event. At MR02, *E. coli* concentration increased sharply from May 10 to May 14, and decreased to normal by May 17. The difference in temporal variation among sites suggests that the sediments are not the only source of *E. coli* in the water column. Stormwater runoff may also be transporting fecal bacteria that accumulate in the river during events.

Conclusions

The RiboGroups of *E. coli* isolates from water and sediment samples demonstrated that a greater number of downstream samples were associated with upstream sediment samples as compared to upstream water samples, which suggested that sediments have an effect on the sources and transport processes of *E. coli* in the water column. *E. coli* in sediments have a tendency to increase during wet weather, and sediments were associated with the sources and transport of *E. coli* in water column. However, the degree of the contribution of *E. coli* from sediments during wet weather is yet unclear since both storm water runoff and the resuspension of the sediment affected the *E. coli* concentrations in water. The relative importance of land-based versus sediment based sources of pathogens and indicators is important for the development of source water protection plans, as well as modeling the fate and transport of pathogens at a watershed-scale. The continued development of the hydrologic model for pathogen fate and transport will assist with the assessment of the relative and absolute contributions of the various sources of microbial contaminants over time. The model will also provide an estimate of the behaviour of other more environmentally persistent pathogens such as *Cryptosporidium* spp. and *Giardia* spp.

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