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Partitioning of *E. coli* and enterococci between planktonic and sorbed phases in runoff from pasturelands

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Abstract. *Pathogens are the leading cause of surface water impairments in Virginia. Currently, Nonpoint Source (NPS) pollution models are most frequently used to determine the maximum allowable loading rates of bacteria from identified sources and they typically simulate bacterial transport to surface waters as a planktonic or free pollutant. Very few models attempt to partition between the planktonic and attached phases primarily because data on bacteria partitioning during overland flow events are currently not available. A field study was conducted to evaluate the partitioning of E. coli and enterococci between the planktonic and attached phases in runoff from pasturelands and to identify the particle sizes to which the fecal indicators preferentially attached. Transport plots were constructed on pastureland with high vegetative cover to simulate well managed pastureland and bare box plots containing three different soil types were used to simulate bare or overgrazed pasturelands. Partitioning ratios were calculated for both studies and used to compare fecal indicator attachment in runoff from different pastureland conditions, from three soils types and between E. coli and enterococci. The average partitioning ratio in runoff from the plots with high vegetative cover was 0.06 for E. coli and 0.30 for enterococci. Partitioning ratios were much higher from bare soil box plots, ranging from 0.38 to 0.55 for E. coli and 0.42 to 1.79 for Enterococcus. In runoff from both the transport plots and the box plots, at least 50% of all attached cells were associated with particles retained by an 8µm screen. These partitioning ratios can be incorporated into NPS models and used to optimize selection of best management practices.*

Keywords. TMDL, *E. coli*, *Enterococcus*, nonpoint pollution, pastureland

Introduction

Pathogens are the leading cause of impairments in Virginia's surface waters and account for 51.8% of all impairments (USEPA, 2004). Pathogens originate from many different sources including agricultural operations such as allowing cattle to have direct access to streams; human sources such as leaking septic systems; or wildlife sources such as migratory birds. However, agricultural practices have been cited as the primary contributor to impairments of rivers and streams (USEPA, 2003). Soupir et al. (2006) compared concentrations of pathogen indicators in runoff from pastures receiving surface applications of turkey litter, liquid dairy manure and cowpies and reported significantly higher concentrations of fecal coliforms from the plots receiving cowpie treatments.

The three most common pathogen indicators in the United States include fecal coliforms, *E. coli* and enterococci (USEPA, 1986). Although fecal coliform have been traditionally used to detect the presence of pathogens in surface waters, *E. coli* and enterococci are thought to have a higher degree of association with outbreaks of gastrointestional illness (USEPA, 1986) and are also the pathogen indicators recommended by the Virginia Department of Environmental Quality (VDEQ, 2000). More than 150 pathogens found in livestock manure may be transmitted to humans, including *Campylobacter spp., Salmonella spp., Listeria monocytogenes, Escherichia coli* O157:H7*, Cryptosporidium parvum* and *Giardia lamblia*. Each of these organisms has a relatively low infectious dose in humans, which increases the potential for disease transmission (USEPA, 2003).

In an attempt to reduce pollutant loading to the nation's water bodies, TMDLs are being developed to assess water quality problems, identify pollution sources, and determine pollutant reductions needed to restore and protect rivers, streams and lakes. A TMDL is a calculation of the maximum amount of a pollutant that can be introduced to a water body, while still meeting the water quality standards, and an allocation of that amount to the pollutant's sources (USEPA, 2005). TMDL development is mandated under section 303d of the 1972 Clean Water Act. The most recent estimate of public and private costs associated with TMDL development and implementation over the next 15 years are approximately $1.0 billion for development of TMDL plans, $255 million for additional monitoring to support TMDL development, and $13.5 to $64.5 billion for implementation of TMDL plans (USEPA, 2002).

Because of the high costs associated with the development and implementation of TMDLs, it is essential that TMDLs be developed using sound scientific methods that are able to accurately reflect the pollutant loadings from the potential sources within a watershed. Currently, Nonpoint Source (NPS) pollution models are most frequently used to determine the maximum allowable loading rates of bacteria from the identified sources. However, most currently-used NPS models typically simulate bacterial transport to surface waters as a dissolved pollutant (Paul et al., 2004). Only the SWAT (Soil and Water Assessment Tool) model attempts to partition between the planktonic and sorbed phases, but data on bacteria partitioning are currently not available (Jamieson et al., 2004). Previous studies have determined that fecal bacteria preferentially attach to particulate matter (Auer and Niehaus, 1993; Henry, 2004; Ling et al., 2002) and as a result their survival time may increase (Burton et al., 1987; Gerba and McLeod, 1976). Other studies have found that *E. coli* is primarily transported in the dissolved state. Muirhead et al. (2005) found that on average only 8% of *E. coli* cells attached to sediment particles and most cells were not bioflocculated but instead transported as single cells in runoff from a portable rainfall simulator. Muirhead et al. (2006) identified *E. coli* attachment during overland flow to be primarily associated with particles <2 µm in diameter.

Many researchers have identified shortcomings in the existing methods used to model bacterial fate and transport (Jamieson et al., 2004; Paul et al., 2004). Representing bacteria as a dissolved pollutant might not accurately reflect the transport processes that occur in agricultural watersheds. However, before bacteria transport modeling can be improved, an in-field study of bacteria transport and the related associations with environmental factors, particulate matter and water quality indicators is needed.

Goal and Objectives

The goal of this research project was to investigate the transport of *E. coli* and enterococci from pasturelands to improve the predictive capabilities of current NPS models and design of best management practices. The first objectives of this study was to quantify partitioning between *E. coli* and enterococci in the planktonic phase and *E. coli* and enterococci sorbed to sediments and organic matter particles in overland flow from pasturelands. The second objective was to identify the particle sizes to which the bacteria preferentially attach.

Methods

To meet the stated research objectives, a combination of laboratory and field investigations was needed. A laboratory investigation was conducted to compare different physical and chemical dispersion techniques to improve enumeration of *E. coli* and *Enterococcus* attached to sediments. We also tested a multiple screen filtration technique to identify the particle sizes to which bacteria preferentially attach and optimized centrifugation speeds to separate attached from planktonic cells prior to the field study. Two sets of field plots were constructed and treated with cowpies and a rainfall simulator was used to create runoff. The first set of plots were constructed on pasturelands at the Virginia Tech Research Farm to investigate partitioning during overland flow events and are referred to as transport plots in this paper. Secondly, three different soil types were packed into boxes to investigate partitioning from overgrazed or bare pastures. This portion of the study is referred to as the box plot study.

Laboratory technique to partition planktonic and sorbed phases

Prior to validating separation techniques, a comparative laboratory study was conducted to identify the best method of dispersing wild strains of indicator organisms from sediment and organic matter particles present in runoff from pasturelands. Dispersion techniques are necessary prior to enumeration to release the bacteria that are attached to soil and organic matter. Total recovery from runoff samples collected from an area on which a standard cowpie was applied (Thelin and Gifford, 1983) was compared using three physical dispersion methods: hand shaker, ultrasonic bath, and vortex. Chemical dispersion techniques were tested by performing dilutions in different concentrations of Tween-80 (Yoon and Rosson, 1990), Tween-85 (Henry, 2004), and 0.1% sodium pyrophosphate (Trevors and Cook, 1992). Preliminary studies were conducted in triplicate and the most promising techniques were repeated using five replications. A 10-minute hand shaker treatment provided the most consistent results for both fecal indicators and was therefore selected as the optimal treatment (Figure 3). Previous research has found a great deal of variation in dispersion techniques used to recover viable bacteria. McDaniel and Capone (1985) suggested that response to dispersion techniques may be dependent upon the soil type and different techniques should be tested for each soil prior to their use.

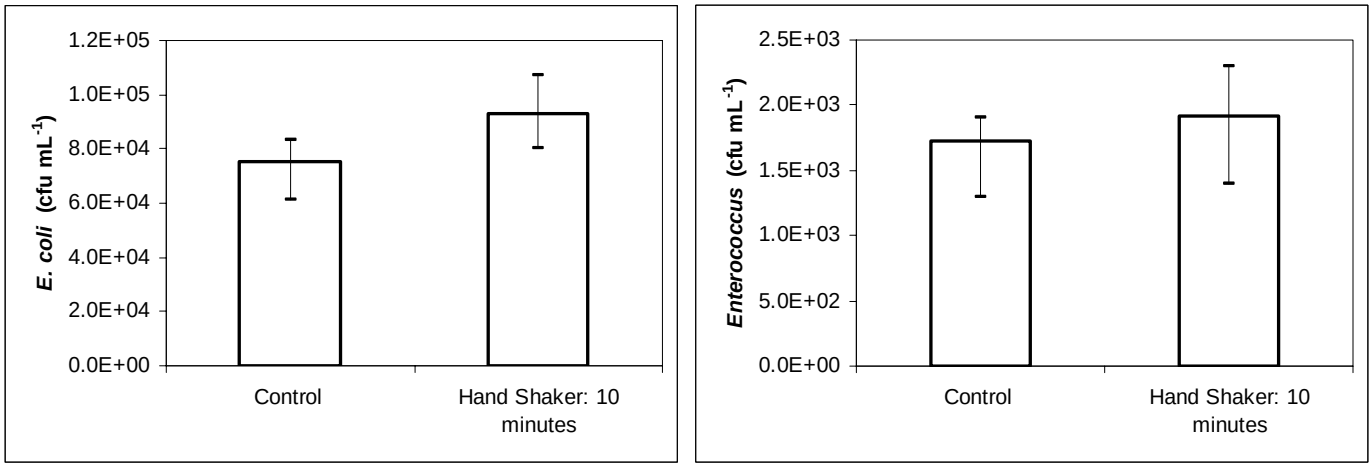

Figure 3. The hand shaker dispersion treatment increased bacteria concentrations when compared to the control treatment.

Screen filtration was used to identify the particle sizes to which *E. coli* and *Enterococcus* preferentially attach and the partitioning ratio (attached/planktonic) was also calculated. Multiple screen filtrations separated suspended solids into particle sizes while centrifugation was used to separate attached and bioflocculated from planktonic cells. A number 35 mesh screen was used to retain particles larger than coarse sand (>500 µm) and a 230 mesh screen retained medium, fine and very fine sand (63 - 500 µm). An 8 µm filter retained fine, medium, and coarse silt particles (Gordon et al., 2002; Henry, 2004; Mahler et al., 2000; Qualls et al., 1983) and a 3 µm filter retained some clays and very fine silt particles. Following filtration, the mesh screens and filters were placed in phosphate buffered water and gently rinsed to remove sediments from the filter. Preliminary studies found that >98% of particulates were removed during this process (Soupir, M., unpublished data). Samples were then centrifuged at 4,700 rpm for 15 seconds to separate planktonic cells from suspended particles (Huysman and Verstraete, 1993; Lago, 2005). The supernatant was enumerated for *E. coli* and enterococci concentrations on Modified mTEC and mE agar (USEPA, 2000) using membrane filtration to assess the planktonic bacterial concentrations. The sediment and organic particles were re-suspended in phosphate buffered water, treated using the dispersion technique (10-min hand shaker) and enumerated by membrane filtration to assess the total bacterial concentration.

The difference between the supernatant and total concentration was assumed to be the attached (sorbed) portion. The partitioning ratio was calculated using equation 1 and the percent attached was calculated using equation 2. A total suspended solids (TSS) analysis identified the particle sizes to which *E. coli* and enterococci preferentially attach.

$$Partitioning\ ratio = \frac{attached}{planktonic} \qquad (1)$$

$$Percent\ attached = \frac{attached}{attached + planktonic} \qquad (2)$$

Transport Plots

Transport plots were established to measure the partitioning of *E. coli* and *Enterococcus* between the attached and planktonic phases in overland flow from pastures with high vegetative cover. The transport plots were constructed at the Virginia Tech Prices Fork Research Farm at a location with no history of animal waste application on pasturelands dominated by a dense stand of Kentucky 31 Tall Fescue. All plots were located on Groseclose silt loam; a deep, well-drained soil (35% sand, 60% silt and 5% clay) with a slowly permeable subsoil.

Each transport plot was 3-m (9.8-ft) wide by 18.3-m (60-ft) long on an approximate 9-percent slope. A Topcon Total Station was used to verify the lengths and slopes of the plots. Plywood borders were placed to a depth of 15 cm along the plot boundaries to prevent water movement into or out of the plots. A "V" shaped outlet was placed at the down-slope end of each plot to direct runoff into a 0.15-m (6-inch) H-flume equipped with a stilling well and a stage recorder for flow measurement (Figure 1). Flumes were stabilized with wood stakes in order to assure that they remain level throughout the experiment period and to permit precise flow measurements by the stage recorders.

The transport of bacteria from plots applied with dairy cowpies was compared to that from the control plots on which no animal waste was applied. Cattle deposits were collected from six cows at the Virginia Tech dairy facility over a 24 hour period. Standard cowpies were formed using the collected manure and applied to the research plots. The size and shape of the standard cowpies were based on research results reported by Thelin and Gifford (1983), who developed standard cowpies to study fecal coliform release patterns. The fresh deposits were formed by taking fresh manure and mixing it in a cement mixer for fifteen minutes. The manure was next placed in molds with a diameter of 20.3 cm (8 in) and a depth of 2.54 cm (1 in). Fecal matter was added to the mold until a weight of 0.9 kg (2.0 lbs) was reached. Approximately 106 cowpies were applied to each plot. This application rate was based on the available volume of manure and represents a rotational grazing system or an area where pastured cattle tend to congregate, such as feeding, watering, or shaded areas.

Figure 1. A rainfall simulation on transport plots constructed on pasture (left) and the H-flume equipped with a stilling well and a stage recorder for flow measurement (right).

Due to the unreliability of natural precipitation for short-term field research, the Virginia Tech Department of Biological Systems Engineering's rainfall simulator (Dillaha et al., 1988) was

used to generate storm events to produce runoff from the field plots. Rainfall was applied at a uniform rate (2.8 cm/h) to all plots (Figure 1). During runoff events, discreet grab samples were taken at the outfall of the flumes at ten minute intervals.

Box Plots

Box plots were established to measure the partitioning of *E. coli* and enterococci between the attached and planktonic phases in overland flow from bare or overgrazed pastures from various soil types. A total of eighteen boxes were packed with soils collected from the Ap horizon. Six boxes were packed with Grosclose silt loam, containing approximately 60% silt; six boxes were packed with Levy silty clay loam, containing approximately 40 to 60% clay; and the remaining six boxes were packed with Eunola loamy fine sand, containing approximately 60% sand. Soils were compacted and leveled at least 24-hrs prior to each rainfall simulation. Each box plot was 100-cm × 20-cm × 7.5-cm (SERA-17, 2005) in size and was placed on an approximate 8-percent slope.

A single standard cowpie (Thelin and Gifford, 1983) was applied to five of the six box plots containing each soil type (Figure 2). The sixth box plot was left bare to act as a control. This application rate was twice the rate applied to the transport plots. As indicated previously, the bare soil box plots represent a poorly managed or overgrazed pasture where cattle are allowed direct access to streams.

A Tlaloc 3000 portable rainfall simulator, based on the design of Miller (1987), with a ½50WSQ Tee Jet nozzle (Spraying Systems Co., Wheaton, IL) was used to apply rain to the box plots (Figure 2). The nozzle was placed in the center of the simulator and a pressure regulator was used to establish a water flow rate of 210 mL/s at the nozzle. Rainfall simulations were conducted within 24 hours of the manure application to represent a condition where rainfall occurs immediately after direct deposition. Simulated rain was applied until 30 minutes after the initiation of runoff, as recommended by the National Phosphorus Research Project (SERA-17, 2005). Grab samples were collected after 10, 20, and 30 minutes of runoff. After the 30 minute sample was collected, the rainfall simulation ended.

Figure 2. A Tlaloc 3000 portable rainfall simulator (left) and a single cowpie was applied to each of the box plots (right).

Results and Discussion

Partitioning ratios were calculated for both the transport and box plot studies and were used to compare fecal indicator attachment in runoff from different pastureland conditions, from three soils types and between *E. coli* and enterococci indicators.

Partitioning during runoff events – Transport Plot Study

The average *E. coli* partitioning ratio for all samples collected from the transport plots was 0.06 which corresponded to 4.8% attachment. The partitioning ratios, averaged over each six minute increment following the beginning of runoff, are presented in Figure 4. The majority of partitioning ratios fell between 0.01 and 0.1 throughout most of the runoff event. Following the onset of runoff, samples were collected in ten minute increments from the outfall of the flume. Therefore, more samples were collected from the plots from which runoff began earlier. The rainfall simulation lasted a total of 3 hours 20 minutes and the longest runoff event lasted 90 minutes (plot 2). The simulation ended when steady state flow rates were reached from all plots.

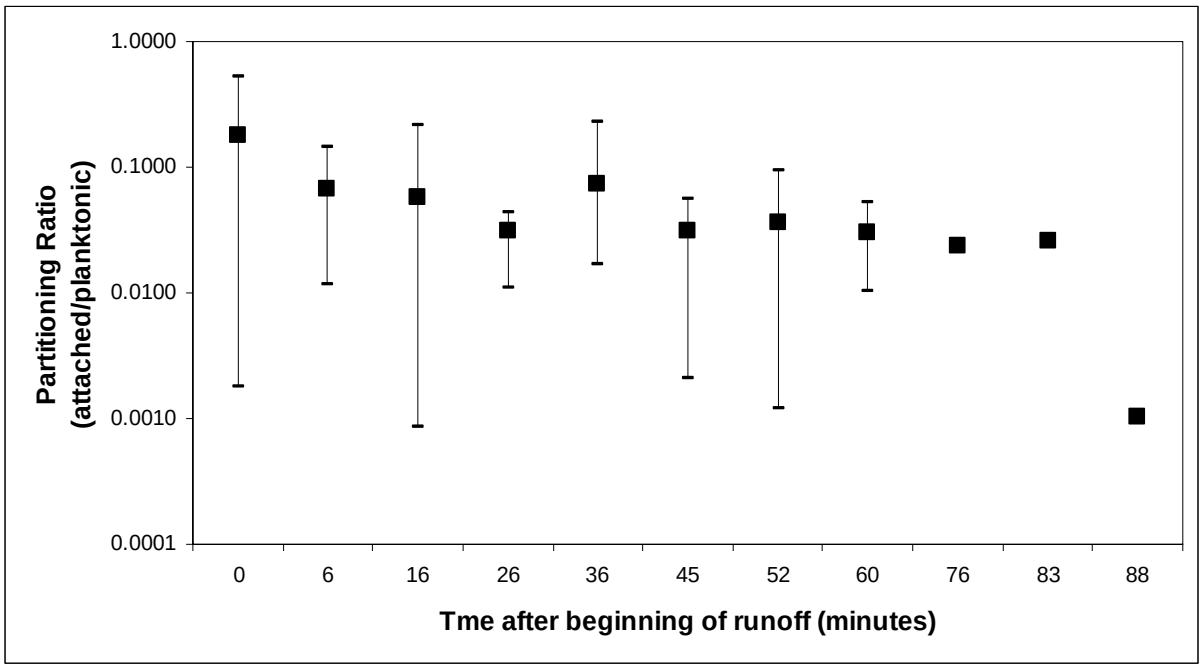

Figure 4. Average partitioning ratios (attached/planktonic) of *E. coli* in runoff from pasturelands. High and low bars indicate the range of partitioning ratios during each sample time.

The average percent attachment of 4.8% was lower than values previously published from stormwater runoff partitioning studies. Jeng et al. (2005) found *E. coli* attachment to range from 21.8% to 30.4% in stormwater samples while Characklis et al. (2005) found and average attachment ranging from 20% to 35% in grab samples collected during storm events. Differences between this study and previous findings could be due to several factors, including the laboratory methods used to partition between planktonic and attached phases, the time between introduction of the bacterium into the system and sample collection, and the sources from which the fecal indicators originated. Jeng et al. (2005) separated between the two phases using a static settling technique, allowing particulates to settle for five hours before assessing

the suspended portion and average TSS concentrations ranged from 170 to 203 mg L^{-1}. Characklis et al. (2005) separated between the attached and planktonic phases by centrifuging stormwater samples at 1164*g* for 10 minutes and TSS concentrations ranged from 51 to 157 mg L^{-1}. Both studies collected grab samples during storm conditions. The source of *E. coli* from the stormwater studies is unknown as is the time since introduction of the fecal indicators into the system. There is some indication that strains of *E. coli* introduced into a system from different sources (eg. waterfowl, cattle, domestic pets) may exhibit different attachment properties (Lago, 2005). In addition, cells exposed to an oligotrophic environment are more likely to attach to particulates in an effort to obtain nutrients and increase survival (Morita, 1997). In this study the average TSS concentration was 155 mg L^{-1}, similar to concentrations present in the stormwater samples. Because many different environmental strains of dairy cow *E. coli* were applied to the plots, it is difficult to attribute lower attachment ratios to differences in strains, even though the source species in the stormwater studies are unknown. Therefore, it is most likely that *E. coli* attachment is lower in runoff from lands treated with fresh manure samples since the cells are not stressed and nutrients and moisture are in abundant supply. Although the average TSS concentrations were similar between the stormwater studies and this study, the thick vegetative cover, present in our study, reduced total suspended solids concentrations when compared to levels that might be observed from poorly managed pasturelands. Therefore, it is possible that attachment would be higher from different pastureland management scenarios.

Figure 5 presents the enterococci partitioning ratios averaged over each six minute increment following the start of runoff. The average partitioning ratio for all samples was 0.30 which corresponded to 15.2% attachment. The majority of partitioning ratios fell between 0.1 and 1.0 throughout most of the runoff event (Figure 5).

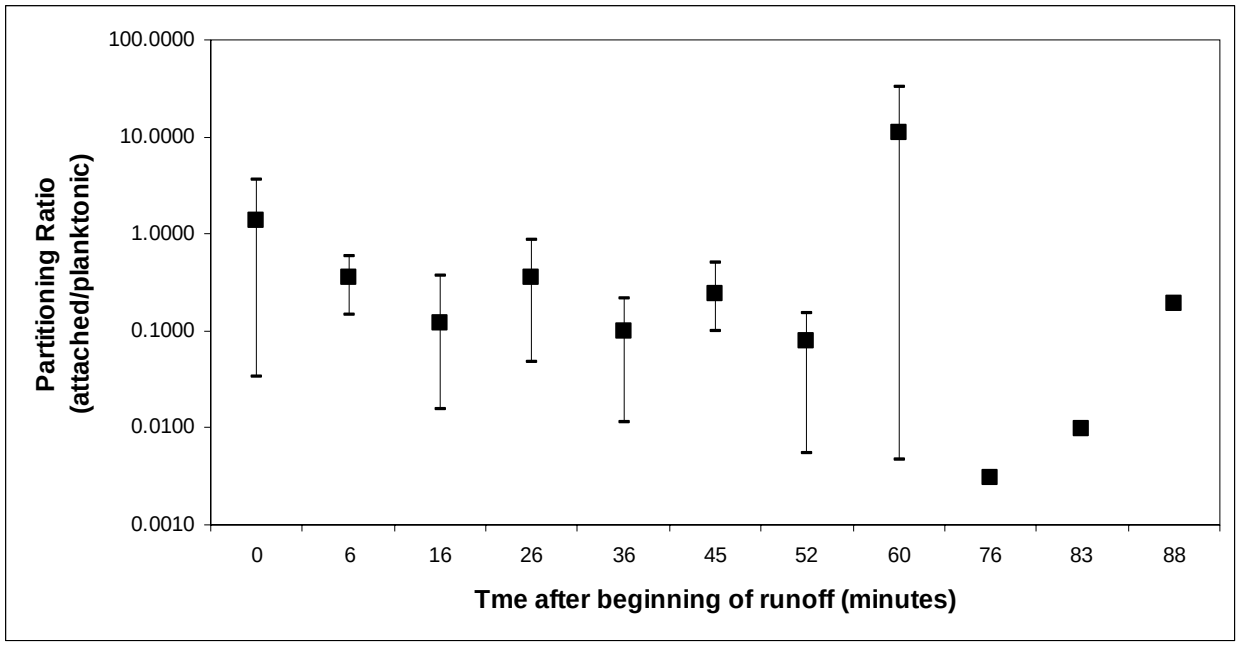

Figure 5. Average partitioning ratios (attached/planktonic) of enterococci in runoff from pasturelands. High and low bars indicate the range of partitioning ratios during each sample time.

Changes in runoff volume, lower flows at the onset of runoff and steady state flows which were reached prior to the end of the runoff, might have influenced partitioning ratios. *E. coli* and

enterococci partitioning ratios were slightly higher in the first sample collected from each plot. In addition, TSS concentrations averaged 522 mg L^{-1} in the first sample collected from each plot (representing low flows) and dropped to an average of 99 mg L^{-1} in the last sample collected from each plot (representing steady state flows). This indicates that the partitioning ratios are likely a function of flow, the availability of attachment sites, and the physical state of the cells.

The average attachment ratio was much higher for enterococci (15.2%) than *E. coli* (4.8%). Characklis et al. (2005) concluded that *E. coli*, fecal coliforms and enterococci all displayed relatively similar partitioning behavior from background samples, but during storm events the attached fractions of fecal coliforms and enterococci increased at all three sites while the attached fraction of *E. coli* decreased at two of the three sites. While it is difficult to attribute a single factor to the increased attachment exhibited by enterococci, it is possible that enterococci are more stressed and therefore more likely to exhibit higher affinity for attachment to soil particles than *E. coli* originating from a fresh manure sample.

Preferential attachment to particulates – Transport Plot Study

The TSS associated with each screen size were weighed and used to identify the particle sizes to which fecal bacteria preferentially attach. If the TSS concentration associated with a screen size weighted less than 1 mg, the solids were considered to be negligible and it was assumed that all cells retained by that screen size either remained in suspension or were bioflocculated but not attached to particulates. If a sample had a higher concentration of suspended cells than total cells due to sample variability, it was assumed that all cells were transported in the planktonic state.

The majority of sediments were retained on the 8 µm screen and the highest concentrations of *E. coli* and enterococci were both associated with particles retained by this screen size (Table 1). Very few solids passed through the 8 µm filter and only one sample had measurable solids retained by the 3 µm screen. This could be due to the particle size distribution of the Grosclose silt loam soils, since they contained only 5% clay. It is also likely that the clay sized particles present in runoff were transported as aggregates and were trapped by the 8 µm filter and unable to pass through it.

Table 1. Particle sizes to which *E. coli* and enterococci preferentially attach in runoff samples collected during an overland flow event.

		E. coli		**enterococci**	
Particle Size	Average TSS (mg/L)	cfu/mg solids	% attached	cfu/mg solids	% attached
>500 µm	43.9	851	27%	240	11%
63 - 499 µm	70.7	433	14%	549	25%
8 - 62 µm	171.9	1,766	55%	1,095	50%
3 - 7 µm	445.0	157	5%	299	14%

Previous studies have identified preferential attachment of fecal indicators to smaller particle sizes (>10 µm) through the use of fractional filtration (Auer and Niehaus, 1993; Schillinger and Gannon, 1985); however, these studies assumed that all cells retained on each screen were attached while our study accounted for planktonic cells trapped by the filter or sediments. The higher surface area associated with finer particles allows for more attachment sites, possibly explaining the higher association of *E. coli* and enterococci to the particles retained by the 8 µm filter. Jeng et al. (2005) found that *E. coli* tended to attach to a wide range of particle sizes while

enterococci preferentially attached to particles ranging from 10 µm to 30 µm. The authors hypothesized that the *E. coli* can attach to different particle sizes because of its motility and rod shape which allows for adsorption at several faces or angles.

Partitioning during runoff events – Box Plot Study

While the transport plot study was used to determine partitioning ratios and particle sizes to which fecal indicators preferentially attach during overland flow conditions from high quality pasturelands, the box plot study simulated poor quality pasturelands (bare soils) on three different soil types. The partitioning ratios from the box plot study varied by both fecal indicator and soil type (Figure 6). The percent attachments for *E. coli* and enterococci, respectively, were 31% and 49% in runoff from the silty loam soils, 43% and 28% from the loamy fine sand soils, and 41% and 42% from the silty clay loam soils. The partitioning ratio was highest in samples collected from the Grosclose silt loam soils, containing approximately 60% silt. The enterococci partitioning ratio was greater than the *E. coli* partitioning ratio in runoff samples collected from the silty loam and silty clay loam soils, while the partitioning ratios for *E. coli* and enterococci were similar in samples collected from the loamy fine sand. Enterococci had a higher percent attached to the silty loam while *E. coli* had a higher percent attached to the loamy fine sand. The general trend towards greater enterococci attachment in the transport plot study (silty loam soils) was again observed in the box plot study. It is possible that enterococci are more stressed and therefore more likely to exhibit higher attachment affinity than *E. coli* in a fresh manure sample. The rod shaped *E. coli* might have greater ability to attach to a variety of particle sizes and therefore a higher percent of *E. coli* are able to attach to loamy fine sand soils.

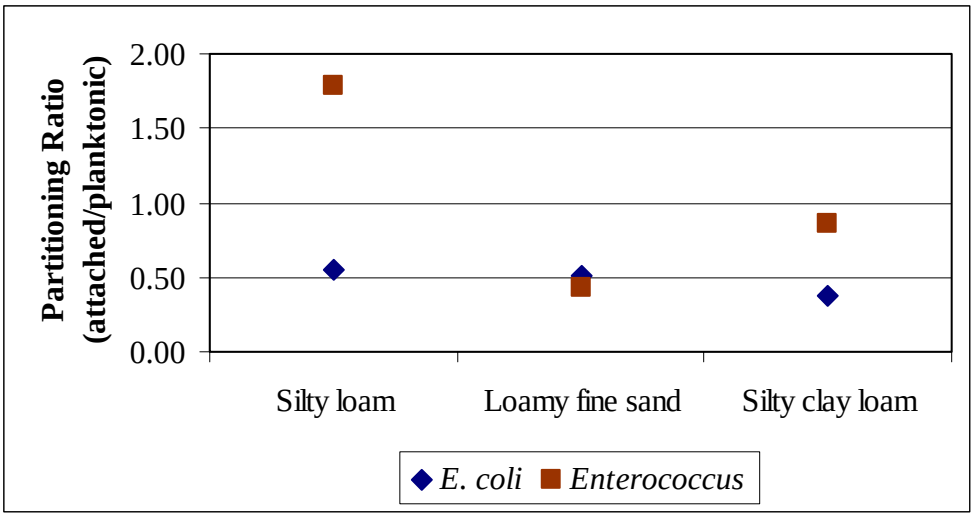

Figure 6. Partitioning ratio (attached/planktonic) of *E. coli* and enterococci in runoff from pasturelands

Partitionig ratios were much higher from the box plot study than the transport plots study. Again, enterococci exhibited greater attachment than *E. coli* in runoff from the silty loam soils and the percent attached increased by 26% and 34% for *E. coli* and enterococci, respectively, in the box plot study, compared with those from the transport plots. The bare soil conditions simulated by the silty loam soil box plot study resulted in an average TSS concentrations of 4,680 mg L^{-1}. In the transport plot study, higher TSS concentrations at the beginning of a runoff

event corresponded to slightly higher partitioning ratios. While a variety of factors might influence the differences in attachment to different soil types for these indicators, it appears that higher TSS concentrations, which correspond to a greater number of attachment sites, predominately influence partitioning ratios.

Preferential attachment to particulates – Box Plot Study

Similar to the results from the transport plot study, both fecal indicators preferentially attached to sediments retained by the 8µm filter. At least 50% of all *E. coli* and enterococci were associated with sediment and organic particles passing through the 63µm filter. No samples contained measurable solids retained by the 3 µm filter even though the Levy silty clay loam soils contained approximately 60% clay. We again assume that the clay sized particles present in runoff were most likely detached and transported as aggregates.

Table 2. Particle sizes to which *E. coli* and enterococci preferentially attach in runoff samples collected from bare soil box plots.

	Particle Size	**Average TSS (mg/L)**	***E. coli***		**enterococci**	
			cfu/mg solids	**% attached**	**cfu/mg solids**	**% attached**
Grosclose silt loam	>500 µm	4,363	9,038	2%	28	3%
	63 - 499 µm	9,256	51,007	10%	209	20%
	8 - 62 µm	4,930	435,668	**88%**	800	**77%**
Eunola loamy fine sand	>500 µm	3,209	8,596	1%	38	1%
	63 - 499 µm	5,306	168,640	12%	507	14%
	8 - 62 µm	1,621	1,254,993	**88%**	2,971	**84%**
Levy silty clay loam	>500 µm	536	67,321	25%	248	3%
	63 - 499 µm	517	1,151,284	24%	3,204	35%
	8 - 62 µm	1,096	2,134,764	**51%**	5,179	**63%**

The distribution of suspended solids did not appear to correspond to the distribution of *E. coli* or enterococci attachment. Both the silty loam and loamy fine sand soils had higher concentrations of sediments classified as sand (retained by the 63 µm filter), but cells preferentially attached to the particles retained by the 8 µm filter. The clay loam soils had the greatest concentration of particles retained by the 8 µm filter and *E. coli* and enterococci both preferentially attached to these particulates. This is most likely due to the larger surface area of the smaller particles which correlates to a higher number of sites available for attachment. Again, the motility and rod shape of *E. coli* might explain the greater attachment of *E. coli* to particles >500 µm in runoff from the silty clay loam soils.

Summary and Conclusions

A field study was conducted to evaluate the partitioning of *E. coli* and *Enterococcus* between the planktonic and attached phases in runoff from pasturelands and to identify the particle sizes to which the fecal indicators preferentially attach. Transport plots were constructed on well-managed pastureland with high vegetative cover to determine partitioning ratios of *E. coli* and enterococci in runoff samples collected at the edge-of-the-field. Soil box plots were packed with three different soil types to represent poorly managed pasturelands dominated by bare soils. Partitioning ratios were calculated for both studies and used to compare fecal indicator attachment in runoff from different pastureland conditions, from three soils types and between *E. coli* and enterococci indicators.

The average partitioning ratio in runoff from the transport plots was 0.06 for *E. coli* which corresponds to 4.8% attachment and 0.30 for enterococci which corresponds to 15.2% attachment. Greater attachment of enterococci than *E. coli* might indicate that enterococci were more stressed in the fresh cowpie samples and increased attachment as a means to enhance their survival. Partitioning ratios were much higher from bare soil box plots, ranging from 31% to 49% attachment for both indicators. The partitioning ratio was highest in samples collected from the soil boxes containing approximately 60% silt, which also had the highest TSS concentrations associated with all three particle sizes. Greater sediment losses from the box plot study provided additional attachment sites for both indicators. In runoff from both the transport plots and the box plots, at least 50% of all attached cells were associated with particles passing through a 63 µm screen. The larger surface area of the smaller particles corresponds to a higher number of sites available for bacterial attachment.

Partitioning ratios developed from this study can be incorporated into NPS models that allow for partitioning between the attached and planktonic phases. Different partition ratios appear to be necessary to simulate pasturelands that are well-managed and contain high percentages of vegetative cover versus poorly-managed pasturelands with a great degree of exposed soils that are prone to erosion. Because the majority of cells were transported in the planktonic state from pasturelands with high vegetative cover, best management practices should focus on reduction of planktonic bacterial transport. Pasturelands contributing high sediment loads may require management practices that reduce particulate-attached bacterial transport in addition to planktonic bacterial transport to meet water quality standards.

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