Fate and Transport of Pathogen Indicators from Pasturelands

Michelle L. Soupir

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Saied Mostaghimi
Theo A. Dillaha, III
Daniel L. Gallagher
Charles Hagedorn, III
Nancy G. Love

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Abstract

The U.S. EPA has identified pathogen indicators as a leading cause of impairments in rivers and streams in the U.S. Elevated levels of bacteria in streams draining the agricultural watersheds cause concern because they indicate the potential presence of pathogenic organisms. Limited understanding of how bacteria survive in the environment and are released from fecal matter and transported along overland flow pathways results in high uncertainty in the design and selection of appropriate best management practices (BMPs) and in the bacterial fate and transport models used to identify sources of pathogens.

The overall goal of this study was to improve understanding of the fate and transport mechanisms of two pathogen indicators, *E. coli* and enterococci, from grazed pasturelands. This goal was addressed by monitoring pathogen indicator concentrations in fresh fecal deposits for an extended period of time. Transport mechanisms of pathogen indicators were examined by developing a method to partition between the attached and unattached phases and then applying this method to analyze runoff samples collected from small box plots and large transport plots. The box plot experiments examined the partitioning of pathogen indicators in runoff from three different soil types while the transport plot experiments examined partitioning at the edge-of-the-field from well-managed and poorly-managed pasturelands.

A variety of techniques have been previously used to assess bacterial attachment to particulates including filtration, fractional filtration and centrifugation. In addition, a variety of chemical and physical dispersion techniques are employed to release attached and bioflocculated cells from particulates. This research developed and validated an easy-to-replicate laboratory procedure for separation of unattached from attached *E. coli* with the ability to identify particle sizes to which indicators preferentially attach. Testing of physical and chemical dispersion techniques identified a hand shaker treatment for 10 minutes followed by dilutions in 1,000 mg L^{-1} of

Tween-85 as increasing total *E. coli* concentrations by 31% (*P* value = 0.0028) and enterococci concentrations by 17% (*P* value = 0.3425) when compared to a control. Separation of the unattached and attached fractions was achieved by fractional filtration followed by centrifugation. Samples receiving the filtration and centrifugation treatments did not produce statistically different *E. coli* (*P* value = 0.97) or enterococci (*P* value = 0.83) concentrations when compared to a control, indicating that damage was not inflicted upon the cells during the separation procedure.

In-field monitoring of *E. coli* and enterococci re-growth and decay patterns in cowpats applied to pasturelands was conducted during the spring, summer, fall and winter seasons. First order approximations were used to determine die-off rate coefficients and decimal reduction times (*D*-values). Higher order approximations and weather parameters were evaluated by multiple regression analysis to identify environmental parameters impacting in-field *E. coli* and enterococci decay. First order kinetics approximated *E. coli* and enterococci decay rates with regression coefficients ranging from 0.70 to 0.90. Die-off rate constants were greatest in cowpats applied to pasture during late winter and monitored into summer months for *E. coli* ($k = 0.0995\ d^{-1}$) and applied to the field during the summer and monitored until December for enterococci ($k = 0.0978\ d^{-1}$). Decay rates were lowest in cowpats applied to the pasture during the fall and monitored over the winter ($k = 0.0581\ d^{-1}$ for *E. coli* and $k = 0.0557\ d^{-1}$ for enterococci). Higher order approximations and the addition of weather variables improved regression coefficients (R^2) to values ranging from 0.81 to 0.97. Statistically significant variables used in the models for predicting bacterial decay included temperature, solar radiation, rainfall and relative humidity.

Attachment of *E. coli* and enterococci to particulates present in runoff from highly erodible soils was evaluated through the application of rainfall to small box plots containing different soil types. Partitioning varied by indicator and by soil type. In general, enterococci had a higher percent attached to the silty loam (49%) and silty clay loam (43%) soils while *E. coli* had a higher percent attached to the loamy fine sand soils (43%). At least 50% of all attached *E. coli* and enterococci were associated with sediment and organic particles ranging from 8 – 62 μm in diameter.

Much lower attachment rates were observed from runoff samples collected at the edge-of-the-field, regardless of pastureland management strategy. On average, 4.8% of *E. coli* and 13% of enterococci were attached to particulates in runoff from well-managed pasturelands. A second transport plot study found that on average only 0.06% of *E. coli* PC and 0.98% of enterococci were attached to particulates in runoff from well-managed pasturelands, but percent attachment increased slightly in runoff from poorly-managed pasture with 2.8% of *E. coli* and 1.23% of enterococci attached to particulates. Equations to predict *E. coli* and enterococci loading rates in the attached and unattached forms as a function of total suspended solids (TSS), phosphorous and organic carbon loading rates appeared to be a promising tool for improving prediction of bacterial loading rates from grazed pasturelands (R^2 values ranged from 0.61 to 0.99).

This study provides field-based seasonal die-off rate coefficients and higher order approximations to improve predictions of indicator re-growth and decay patterns. The transport studies provide partitioning coefficients that can be implemented into NPS models to improve predictions of bacterial concentrations in surface waters and regression equations to predict bacterial partitioning and loading based on TSS and nutrient data. Best management practices to reduce bacterial loadings to the edge-of-the-field from pasturelands (regardless of management strategy) should focus on retention of pathogen indicators moving through overland flow pathways in the unattached state. Settling of particulates prior to release of runoff to surface waters might be an appropriate method of reducing bacterial loadings by as much as 50% from highly erodible soils.

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Dedication

I would like to dedicate this work to my husband, Steven Soupir, for his steady encouragement.

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List of Abbreviations and Symbols

θ ……..……temperature correction coefficient
μ_1 ……...…… first order die-off rate constants (day^{-1}) at the first stage of decay
μ_2 ……...……first order die-off rate constants (day^{-1}) at the second stage of decay
υ …….flow velocity ($cm\ h^{-1}$)
BMP……...……..Best Management Practice
C ……..……bacteria concentration in solution ($cfu\ mL^{-1}$ or $cfu\ cm^{-3}$)
CFU……..colony forming unit
DOC……...……dissolved organic carbon
DOP……..……..dissolved organic phosphorus
D-value……..Decimal Reduction Time
EPA……....Environmental Protection Agency
k ……..……..first order die-off rate constant (day^{-1})
k_{T2} ……die-off rate adjusted to T_2
k_{T1} ……..…….die-off rate measured at T_1
K_d ……..…… linear partitioning coefficient
MPN……...……most probable number
N……..……number of bacteria at time *t*
N_o ……...……initial number of indicator bacteria
NPS……...…..Nonpoint Source
NS……...……Not significant
PAF……..……..particulate associated fraction
PC……..……partitioning coefficient
PDA……. previous day average
PDH……previous day high
PDT…….previous daily total
PWA……..previous week average
PWH……...previous week high
PWT……previous weekly total
q ……...bacterial flux per unit cross-sectional area ($cfu\ cm^{-1}\ h^{-1}$)
R^2……..…coefficient of determination
RH……...relative humidity
S …….. attached bacteria density ($cfu\ g^{-1}$)
SOC…….……suspended organic carbon
SOP…….……..suspended organic phosphorus
SR……solar radiation
SWAT……..Soil Water Assessment Tool
t ……elapsed time (day)
t_1 ……..…….. time when the first stage of decay ends (day)
T_1 ……..temperatures (°C)
T_2 ……..temperatures (°C)
TC……..……total concentration
TDP……...……total dissolved phosphorous
TMDL……...Total Maximum Daily Load
TOC……..……..total organic carbon
TOP……..……..total organic phosphorus

TP……..……..total phosphorus
TSP…….total suspended phosphorous
TSS……total suspended solids
VBNC……..…Viable But Non-culturable

Chapter 1. Introduction

1.1. Introduction

The U.S. EPA (2005b) has identified bacteria as a leading cause of impairments in rivers and streams in the U.S. Bacteria pollution in agricultural watersheds originates from many different sources including agricultural practices such as allowing cattle to have direct access to streams; human sources such as leaking septic systems; or wildlife sources such as migratory birds. Although many different sources may exist within a watershed, agricultural practices have been cited as the primary contributor to bacteria impairments in rivers and streams (U.S. EPA, 2003). Elevated levels of bacteria in agricultural watersheds cause concern because they indicate the potential presence of pathogenic organisms. The three most common bacteria indicators in the United States include fecal coliforms, *E. coli* and enterococci. 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 (U.S. EPA, 1986). 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 (U.S. EPA, 2003).

In an attempt to reduce pollutant loading to the nation's water bodies, Total Maximum Daily Loads (TMDL) are being developed to assess water quality problems, identify pollution sources, and determine pollution 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 discharged to a water body, while still meeting the water quality standards, and an allocation of that amount to the pollutant's sources (U.S. EPA, 2007a). TMDL development is mandated under section 303(d) of the 1972 Clean Water. 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 TMDLs, and $13.5 to $64.5 billion for TMDL implementation (U.S. EPA, 2002a).

Because of the high costs associated with the development and implementation of TMDLs, it is essential that TMDLs be developed using sound scientific methods 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. Most current NPS models completely ignore bacterial subsurface transport (Jamieson et al., 2004) and typically simulate bacterial transport to surface waters as a dissolved or unattached pollutant (Paul et al., 2004). Only the Soil and Water Assesement Tool (SWAT) model attempts to partition between the dissolved and sorbed phases, but sufficient data on bacterial partitioning are currently not available (Jamieson et al., 2004). Previous studies have determined that fecal bacteria preferentially attached 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). In addition, first order decay equations that are most often used to express bacterial die-off do not account for external environmental influences on the fate of bacteria applied to the land as a result of different agricultural practices.

Many researchers have identified shortcomings in the existing methods used to model bacterial fate and transport. Representing bacteria as a dissolved pollutant does not accurately reflect the transport processes that occur in agricultural watersheds and first order decay equations do not account for external environmental influences on the fate of bacteria. However, before bacteria modeling can be improved, in-field studies of bacteria fate and transport and the related associations with environmental factors, particulate matter and water quality indicators are needed.

1.2 Goals and Objectives

The overall goal of this study was to improve understanding of the fate and transport mechanisms of two pathogen indicators, *E. coli* and enterococci, from grazed pasturelands. This in-field study of bacteria decay and transport mechanisms and the related associations with weather variables, flow, particulates, and water quality indicators will provide much needed information to improve TMDLs and Best Management Practice (BMPs) effectiveness. Many NPS models already partition between nutrient phases; thus, identifying possible correlations

between bacterial and nutrient partitioning might improve predictive capabilities of bacterial transport models by modification of existing nutrient overland transport process algorithms. In addition, if attachment is a significant edge-of-field transport factor, design and selection of management practices could be improved to encourage settling of particulates and the attached fecal indicators for reduction of pathogen transport to surface waters.

The specific objectives of this study were to:

1. Evaluate various laboratory procedures for dispersing and separating *E. coli* and enterococci into attached and unattached phases and to identify particle size ranges to which *E. coli* and enterococci preferentially attach

   Hypothesis: Sonification is the best method of recovering attached bacteria from sediments

2. Assess *E. coli* and enterococci re-growth and decay patterns in cowpats applied to pasturelands

   Hypothesis: First order decay does not adequately describe die-off rates for *E. coli* or enterococci.

3. Quantify partitioning of *E. coli* and enterococci between the attached and unattached phase in runoff from three bare Virginia soils.

   Hypothesis: *E. coli* and enterococci partitioning during runoff events is related to the dissolved phosphorus/suspended phosphorus ratio and the majority of cells are associated with particles retained by the 8 μm filter.

4. Quantify partitioning of *E. coli* and enterococci between the attached and unattached phase from different pastureland management scenarios during overland flow.

   Hypothesis: *E. coli* and enterococci partitioning during runoff events is related to the dissolved phosphorus/suspended phosphorus ratio and the majority of cells are associated with particles retained by the 8 μm filter.

1.3 Study Design

This study was designed to first identify an easy-to-replicate method to disperse and separate particulate-attached and bioflocculated cells and identify particle sizes to which indicators preferentially associate. Three field studies were conducted to assess in-field bacterial survival and transport mechanisms. The influence of environmental parameters on bacterial decay patterns were evaluated by long-term monitoring of cowpats applied to pastureland during four seasons. Overland transport mechanisms from different pastureland management scenarios were examined through the use of small box plots containing three Virginia soils and large transport plots on a single soil type representing transport at the edge-of-the-field. Partitioning coefficients from the different management scenarios were then compared and the ability to predict bacterial partitioning and total bacterial concentrations based on water quality indicators were examined. The methodology, results, and discussion for each of the objectives are presented in separate chapters.

Chapter 2. Review of Pathogen Indicator Fate and Transport

Many factors must be considered as we strive to improve understanding of bacterial fate and transport mechanisms. Bacterial die-off is typically assumed to follow first-order decay patterns, neglecting the potential for re-growth and the impact of the external environment. The attachment of fecal bacteria to sediment and organic particulates during overland flow processes is often not considered because very little is known about the partitioning between the attached and unattached phases. Varied laboratory techniques have been utilized to separate fecal indicators between phases, possibly explaining the mixed results between previous laboratory and field-based studies. In addition, previous studies have yet to examine indicator partitioning at the edge-of-the-field or to identify the particulate sizes to which indicators preferentially attach. By improving understanding of bacterial fate and transport mechanisms, predictive capabilities of NPS models used in the development of TMDLs can be improved along with the design and placement of management practices to mitigate bacterial transport to surface waters.

Terminology to describe the attached and unattached fractions varies among studies (Characklis et al., 2005; Jeng et al., 2005; Krometis et al., 2007) and the selected terminology is often a function of the method used to separate cells into the attached and unattached fractions. The terminology adapted here is attached and unattached as used in various publications by Muirhead et al. (2005; 2006a; 2006c; 2006b). Attached cells are identified as those associated with particulates (sediments or organic particles) or bioflocculated to the point that they are pulled from suspension during a centrifuge treatment; however, Muirhead et al. (2005; 2006a; 2006c; 2006b) previously identified *E. coli* present in runoff from a fresh fecal source as being primarily transported as individual cells and not bioflocculated. Unattached cells are referred to as remaining in suspension, suspended, free, planktonic, dissolved or buoyant in other studies discussed in this chapter. Attached cells are also referred to by other authors as being sorbed, associated with particles or the settable fraction.

2.1. An Overview of Bacterial Fate and Transport Modeling Processes

A process-based approach to modeling microbial fate and transport in a watershed includes release from manure, overland transport, infiltration into the soil, in-stream transport, vadose

zone and groundwater transport, and die-off and re-growth throughout the storage and transport processes (Benham et al., 2006) as illustrated in Figure 2.1. This study specifically focuses on two components: the in-field bacterial die-off and potential re-growth patterns over time, following excretion, and the phase in which fecal indicators are transported to surface waters during overland flow. The following chapter discusses each of these processes starting with the current methods used to model pathogen indicator fate and transport. Previously utilized dispersion, separation and enumeration techniques are reviewed along with factors impacting indicator decay and transport processes.

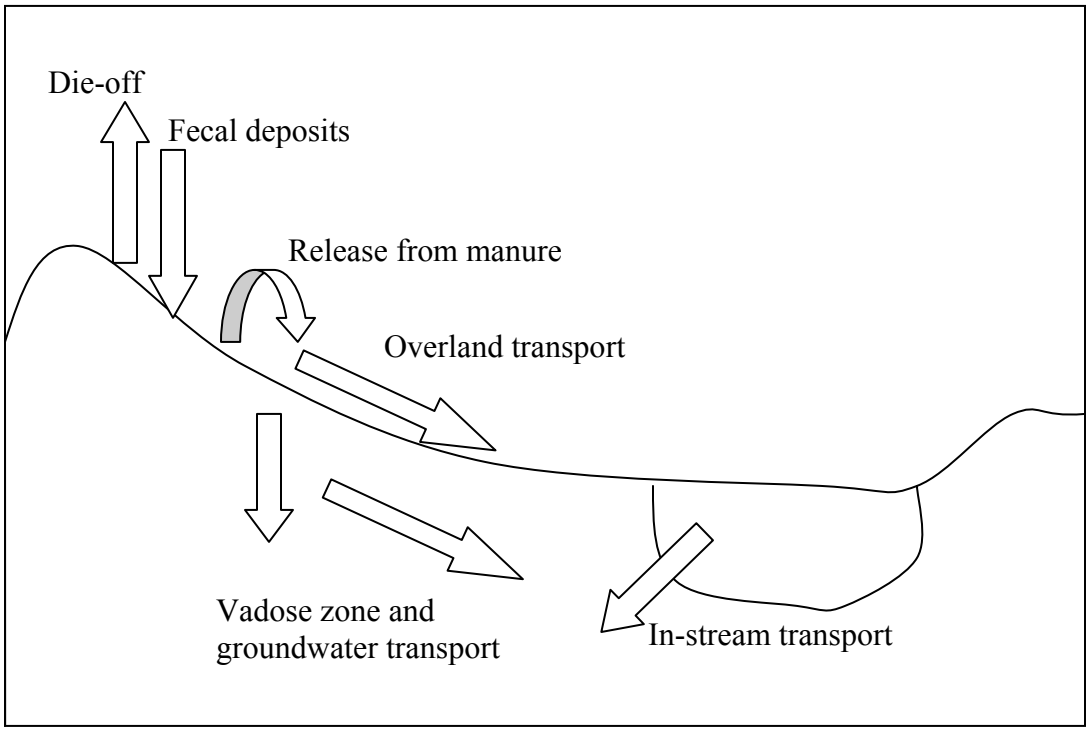

Figure 2.1. Fate and transport of pathogens. Adapted from Haydon and Deletic (2006).

2.1.1. Die-off

The three commonly observed patterns of indicator bacteria die-off are first order decay, bacteria growth followed by first-order decay; and first order decay with variable die-off rates (Crane and Moore, 1986; Mancini, 1978). Since very little is actually known about the individual influences and interactions between the many parameters affecting die-off, first order decay is most often used to express bacterial die-off. Chick's Law (Crane and Moore, 1986; Moore et al., 1988), presented as Equation 2.1, is the first order decay equation most often used to express bacterial

die-off in stored manure, soil, land applied manure, streams and groundwater (DeGuise et al., 1999; Pachepsky et al., 2006; Wang et al., 2004):

$$N = N_o \exp^{-kt} \quad [2.1]$$

where N= number of bacteria at time t; N_o = initial number of indicator bacteria; k = first order die-off rate constant (day^{-1}); and t = elapsed time (day). The first order die-off rate constant k can be modified is a function of temperature and can be described using the Arrhenius equation as follows:

$$k_{T_2} = k_{T_1}\, \theta \exp(T_2 - T_1) \quad [2.2]$$

where k_{T2} = die-off rate adjusted to T_2, T_1 and T_2 are temperatures (°C), k_{T1} = die-off rate measured at T_1, and θ = temperature correction coefficient.

Mubiru et al. (2000) found that a first-order decay model with a two-stage function better represented the longevity of *E. coli* inoculated in soils and Zhai et al. (1995) found that a two-staged exponential decay model better described mortality rates of fecal coliforms in poultry waste. The two-staged die-off model was presented in Crane and Moore (1986):

$$N = \begin{cases} N_0 \exp(-\mu_1 t), & t < t_1 \\ N_0 \exp(-\mu_1 t_1) \exp[-\mu_2 (t - t_1)], & t \geq t_1 \end{cases} \quad [2.3]$$

where N= number of bacteria at time t; N_o = initial number of indicator bacteria; μ_1 and μ_2 are first order die-off rate constants (day^{-1}) at the first and second stage of decay; and t_1 is the time when the first stage of decay ends (day).

Modifications have been made to Chick's Law to adjust for some of the environmental factors including temperature, soil moisture content, solar radiation, and pH (Mancini, 1978; Polprasert et al., 1983; Reddy et al., 1981). It is unclear whether the equations developed under laboratory

conditions are representative of field conditions. In addition, existing equations have yet to represent after-growth that might occur following excretion or land application of waste (Conner and Kotrola, 1995; Crane et al., 1980; VanDonsel et al., 1967; Wang et al., 1996; Wang et al., 2004). New or improved equations are needed to better capture the bacterial growth and die-off dynamics for extended periods of time (Wang et al., 2004).

2.1.2. Transport to Surface Waters

Many researchers and practitioners recognize the shortcomings in the existing methods used to model bacterial transport (Jamieson et al., 2004; Paul et al., 2004). Interactions between overland indicator transport and sediments vary among different models. The widely used HSPF (Hydrological Simulation Program – FORTRAN) model (Bicknell et al., 1997), as typically implemented for microbial transport, ignores microbial transport associated with sediment and simulates the convective transport of planktonic bacteria (Pachepsky et al., 2006). A model described by Haydon and Deletic (2006) assumed that pathogens are transported by overland flow because most adsorb to particulates <16 μm in size. Other models have described pathogen transport as being sorbed to sediment and particulates during overland flow (Fraser et al., 1998) while the SWAT (Soil and Water Assessment Tool) model allows for partitioning between microbes associated with sediment and transported along with overland flow (Pachepsky et al., 2006).

Partitioning of fecal bacteria into the attached and unattached phases occurs during initial release from manure, overland and subsurface transport, and stream and bed transport (Benham et al., 2006). A linear partitioning relationship is typically assumed:

$$S = K_d C \qquad [2.4]$$

where S is the attached bacteria density (cfu g^{-1}), C is the unattached bacteria concentration in solution (cfu mL^{-1}) and K_d is the linear partitioning coefficient.

An advective algorithm is used to simulate bacterial mass flux in the unattached state along overland transport pathways:

$$q = \nu C \quad [2.5]$$

where q is bacterial flux per unit cross-sectional area (cfu cm^{-2} h^{-1}), υ is flow velocity (cm h^{-1}) and C is bacteria concentration (cfu cm^{-3}) (Benham et al., 2006).

Representing bacterial transport during overland flow as either a dissolved or sorbed pollutant might not accurately reflect the transport processes that occur in agricultural watersheds. Use of a partitioning coefficient to separate between attached and unattached phases should improve predictions of in-stream microbial concentrations, but data on bacteria partitioning are currently limited or not available (Jamieson et al., 2004).

2.2. Bacterial Dispersion, Separation and Enumeration Techniques

There is currently no standardized technique for separating attached and unattached bacteria in soil or water samples. The separation techniques most commonly presented in the literature are filtration, fractional filtration and centrifugation. Frequently chemical or physical dispersion techniques are employed to separate attached and bioflocculated bacteria following filtration or to increase enumeration in a control. The advantages and disadvantages of each method have been previously identified and many researchers have obtained their best results by using a combination of these dispersion and separation techniques. The recovery of stressed cells from environmental samples is discussed; however, membrane filtration followed by plating is the standard technique to enumerate *E. coli* and enterococci.

2.2.1. Separation Techniques

Filtration: Filtration has been identified as one method of separating unattached from bioflocculated and attached bacteria. Typically, cells passing through the filter or screens are assumed to be in the unattached or free state. However, during the filtration process it is possible that bacteria retained by the filter are bioflocculated (and not actually associated with sediment or particulates) or that free bacteria could attach to the filter. Both of these scenarios result in free bacteria being incorrectly classified as sorbed (Henry, 2004). Despite these potential drawbacks, filtration has been used in multiple studies to separate bacterial fractions. Previous

researchers have defined unattached *E. coli* as those able to pass through an 8 μm screen (Henry, 2004; Mahler et al., 2000; Qualls et al., 1983). Since a typical *E. coli* cell is 1 × 3 μm (Madigan et al., 2000), the filtration method assumes that only unattached bacteria or those attached to very small particles are able to pass through the 8 μm filter. Smaller, stressed bacteria that are clumped together could also pass through. Mahler et al. (2000) validated the use of an 8 μm filter to partition between phases by passing a known suspension of *E. coli* through the 8 μm filter. The authors successfully recovered 99% of the unattached *E. coli*, demonstrating that only a small proportion of the free cells were retained would have been incorrectly classified as attached. Henry (2004) partitioned water samples into two sub-samples, passing one sample through an 8 μm filter where bacteria passing through the filter were classified as planktonic (unattached). The total count from the second sample was obtained following dispersion treatment. The attached bacteria fractions were determined by calculating the difference between the total *E. coli* concentrations and the *E. coli* passing through the 8 μm filter.

Multiple screen or fractional filtration has been used to identify the bacteria attached to different particles sizes. Fractional filtration of urban stormwater was performed by Schillinger and Gannon (1985) to separate sediment particle sizes. Screen sizes of 52 μm, 30 μm, 10 μm, and 5 μm mesh openings were selected. The smallest size of 5 μm was selected because particles smaller than 5 μm have a negligible settling velocity in natural systems. The filtration system was disinfected with boiling distilled water and then prefiltered stormwater was used to preload the columns with liquid. Between 2 and 8 mg of stormwater solids were filtered to prevent clogging the screens. Downward air pressure was applied to remove any remaining liquids. Screens were then removed aseptically and inserted into a tube with 10 mL of sterile 0.02% polysorbate 80. The tubes with screens were shaken vigorously 100 times to disperse the particle-attached bacteria. Microscopic observations determined that the screens were able to retain particles in the correct size category while allowing unattached cells to pass through the screen system. Auer and Niehaus (1993) used a similar method on sediment particles collected from Onondaga Creek during storm events. The authors selected Nitex nylon mesh screens of 102, 53, 20, 10, 6, and 1 μm followed by a 0.45 μm membrane filter. The screens and filter were removed and placed in a bottle with phosphate buffered water and 5 drops of Tween 85. The

bottles were shaken for 5 minutes to separate cells from the screen, sediments and to suspend the cells prior to enumeration.

Centrifugation: Centrifugation is another method frequently used by researchers to determine partitioning between unattached and particulate attached bacteria. The percent attached is typically determined by subtracting the total count from the concentrations remaining in the supernatant following centrifugation. The attached portion could be enumerated by resuspending the sediment/bacteria pellet and applying dispersion techniques; however, Henry (2004) noted that during centrifugation, the super concentrating or increased cell-particle or particle-particle "collisions" of the soil and bacteria aggregates may alter the attachment properties and strengthen the existing bonds. Characklis et al. (2005) stated that using centrifugation to separate microbial fractions assumes that microbial sorption is not affected during the process. Henry (2004) also noted that free bacteria can be similar in size to clay particles, so determining proper centrifuge settings to separate sediments from nonattached bacteria can be difficult.

Characklis et al. (2005) evaluated partitioning between suspended and settleable particles using a procedure calibrated with standard particle suspensions. Surrogate particles were selected based on their settling properties. Latex particles (10 μm mean diameter, 1.05 g/cm^3) represented free phase microbes and organic particles while glass beads (5-50 μm diameter, 2.5 g/cm^3) represented inorganic particles. Centrifugation of the surrogate particles at 1164 g (2000 rpm) for 10 min with a break of 4 at 4°C removed 97% of the glass beads larger than 5 μm while 80% of the latex particles remained in suspension. The authors felt this centrifugation procedure reasonably separated particles and microbes into settleable and suspended fractions and that the procedure's affect on microbial sorption would be minimal because of the dilute concentration of the samples.

Several other studies have also applied centrifugation procedures to separate microbial fractions, but few details are provided on how the centrifugation settings were determined. Huysman and Verstraete (1993) used centrifugation to determine attachment of *E. coli* to montmorillonite and kaolinite. A 0.1 g clay mixture was added to a known concentration of *E. coli* cells that had been cultivated to the exponential phase. The mixture was first vortexed vigorously for 30 s and then

mixed slowly on a rotary shaker for one hour to promote sorption. Next, the tubes were centrifuged for 30 s at 120 g. Following centrifugation, the bacteria in the supernatant were enumerated by plate counting. The difference between the original concentration and the concentration in the supernatant was assumed to be the attached concentration. Adhesion to the clay particles was nearly complete after 15 – 20 minutes of mixing. Lago (2005) also used centrifugation to evaluate the effects of strain and water quality on attachment of *E. coli* to sediments. *E. coli* cells were grown to log phase, washed twice in the incubation medium, and resuspended in 10 mL at a concentration of about 10^7 mL^{-1}. Sediment was added to the solution and mixed for 20 minutes at room temperature. The free and attached bacteria were separated by centrifugation at 4700 rpm for 15 seconds. The bacteria in the supernatant were enumerated by plate counting and the difference between the original concentration and the concentration in the supernatant was assumed to be the attached portion. Schillinger and Gannon (1985) used the same procedure but centrifuged samples for 3 minutes in a bench-top centrifuge at the Number 2 setting to separate attached from unattached bacteria while Sayler et al. (1975) centrifuged samples collected from the Chesapeake Bay at a relative centrifugal force of 2,100 g for 15 minutes. Muirhead et al. (2005) separated between phases by injecting a Nycodenz solution below the suspension and then centrifuging the samples.

2.2.2. Dispersion Techniques

Dispersion techniques, such as sonification or chemical surfactants, disrupt the attachment of bacteria to surfaces. Davies et al. (1995) used sonification to disperse bacteria present in bottom sediments. Samples were collected and 10 g of sediments were mixed thoroughly with 90 mL of sterile deionized water. These samples were sonicated at 100W for 30 seconds using a Braun Labsonic U ultrasonic probe (19 mm diameter). Samples were allowed to settle for 10 minutes before the bacteria in the supernatant were enumerated by membrane filtration.

Craig et al. (2002) evaluated different separation techniques and found that the binding properties between the bacteria and sediment depend upon the size and composition of the particle. The authors reported that treatment by sonication bath for 10 minutes was the most effective separation technique. For better recovery from silt/clay sediments, the authors recommend treating the sample twice. McDaniel and Capone (1985) compared homogenization,

sonication and two chemical treatments for separating sorbed bacteria from particles collected from intertidal mud flat of a north temperate salt marsh. The study found homogenization and sonication to be the best methods, cautioning that each sediment type responded differently to each enumeration procedure. Sonication at 20 kHz and 900 W was the best method of extracting sediments and also reduced variance when compared to the other methods. The authors were unable to define trends between the effectiveness of any of the techniques and sediment size, organic content, porosity or salinity.

Epstein et al. (1997) combined *in situ* radioisotope labeling of sediment bacteria, bacteria dislodging by ultrasonic treatments, and enumeration using fluorescent staining to determine bacteria enumeration in sandy sediments. This protocol accounted for between 88 to 98% of all bacteria present in sediments. The optimum time for sonication was between 80 to 160 s. Previously Epstein and Rossel (1995) determined that the ultrasonic cell disruptor performed better than a commercial blender or an ultrasonic cleaner. Samples were diluted and incubated for 15 minutes in a surfactant such as sodium pyrophosphate or Tween 80 to aid in dislodging the bacteria from sediments. The solution then received a sonic treatment for 180 s at 109 μm amplitude. The solution was washed with autoclaved sea water, the sediment was allowed to settled, and the supernatant was collected. Samples were stained and enumerated by epifluorescent illumination.

Yoon and Rosson (1990) treated turbid seawater samples with Tween 80 surfactant prior to sonification to improve enumeration of bacteria attached to sediments. Tween 80 concentrations were varied (0, 2.5, 5, 10, 25, 50, and 100 mg/L) and it was determined that the optimum concentration of Tween 80 was 10 mg/L. Tween concentrations greater than 25 mg L^{-1} increased bacteria die-off while sonification (10 W for 30 s using a half-wave step 1.3 cm diameter titanium probe) alone was only able to disperse between 42 to 72% of the attached bacteria.

Each method of separating microorganisms from particles has advantages and disadvantages. Filtration techniques separate unattached from particulate-attached organisms, but higher percentages of organisms could be incorrectly classified as attached due to clogging of the filter or bioflocculation of microbes. Centrifuging can separate suspended from settleable particles

(and thus microorganisms), possibly providing a more realistic picture of microbe mobility within the water column. However, the centrifugation procedure increases interactions between cells and particles, possibly increasing or strengthening attachment during centrifugation (and therefore increasing percent attached). Previous dispersion studies have found optimal removal of bacteria from particles using different methods and the performance of each technique appear to be dependent upon sediment type. Thus a comparative study is recommended to compare previously employed chemical and physical dispersion techniques prior to applying these techniques to manure, sediment or water samples.

2.2.3. Enumeration Techniques

There are many conflicting ideas about the state in which bacteria survive in natural waters, as well as many problems with the methods used to enumerate the various forms in which these bacteria exist. Microscopic observations of cells in natural environments frequently exceed those which can be recovered and cultured, often by orders of magnitude (Mukamolova et al., 2003). Bacterial growth is usually presented as a sequence of events including i) initial stationary, ii) lag or growth acceleration, iii) logarithmic growth, iv) negative growth acceleration, v) stationary, vi) accelerated death, and vii) logarithmic death phases (Roszak and Colwell, 1987a). Over the past 20 years, studies have identified an extended steady state phase, but the definition and method of identifying this state are still unclear, as are the potential virulent properties that cells in this state might maintain. Roszak and Colwell (1987b) identified this extended steady state phase as viable but non-culturable (VBNC). They described the VBNC stage as one where bacterial cells are intact and alive when tested for criteria such as enzyme activity, photosynthesis, respiration, and energy charge, but do not undergo cell division on routinely employed bacteriological media.

The bacteriological water quality criteria were developed by the U.S. EPA using estimates of bacterial indicator counts and the resulting gastrointestional illness rates (USEPA, 1986). These criteria were established based on results from epidemiological surveys conducted following marine and freshwater bathing. Surveys were conducted following weekend swimming events and participants showing illness symptoms were interviewed. Illness symptoms were classified as gastrointestional, respiratory, eye, ear and nose, and other (including fever or backaches);

however, gastrointestional illnesses were always observed at the most polluted beaches, while other illnesses did not seem to be associated. The EPA tested multiple indicators of water quality so they could develop a statistical relationship between the water quality indicators and the swimming associated illnesses. Bacterial indicators *E. coli* and enterococci were enumerated using two membrane filtration methods developed for the EPA study, mE agar for enterococci and mTEC agar for *E. coli*. Modification to the original agars have been recommended to reduce analysis time and improve analytical quality (USEPA, 2000). The revised method for enterococci (mE Agar) uses a single medium, reduces analysis time from 48 to 24 hours, and improves analytical quality. The modified mTEC method for analysis of *E. coli* concentrations is now a single step method that only requires the use of one medium. After water samples are filtered through a membrane, the membrane is placed on the modified mTEC agar and incubated at 35±0.5°C for 2 hours to resuscitate the stressed or injured organisms and then incubated at 44.5±0.2°C for 22 hours (USEPA, 2000). Francy and Darner (2000) compared methods for enumerating *E. coli* in recreational waters. The study compared the Colilert method (a most probable number method) to the modified mTEC, and MI membrane filtration methods. These were compared to the mTEC method, which requires a two step procedure. No statistically significant differences were found between the mTEC method and MI methods, but they were found between the modified mTEC or Colilert methods and the mTEC method. The modified mTEC method recovered statistically fewer *E. coli* than the mTEC method.

Although there is much concern about the existence of the VBNC state that fecal bacterial indicators may take on when exposed to environmental stresses, the presence of these cells was accounted for during the development of the EPA criteria. Water quality criteria are based on epidemiological studies, which use statistics based on traditional culture enumeration of bacteria, implicitly including VBNC bacteria. By using culture methods, it is assumed that cells in the VBNC state will be implicitly accounted for by comparison to water quality criteria.

2.3. Bacterial Die-off from Agricultural Sources

Studies examining the decay patterns of pathogens and pathogen indicators have been conducted under controlled laboratory conditions and in monitored field plots, exposing microorganisms to a wide variety of environmental conditions. The fit of these die-off patterns to a first-order

model is typically examined along with the potential fit to two-staged, first-order models. Environmental factors, most frequently moisture content and temperature, are often examined for their impact on decay rates. This section presents a summary of previous laboratory and field-based die-off studies; the environmental factors impacting decay; and first order decay coefficients for conditions comparable to those that will be obtained in this study.

2.3.1. Environmental Factors Influencing Bacterial Survival

Prior to the development of improved equations to model bacterial survival in the environment, data are needed to clearly link environmental factors with bacterial survival in animal waste. Pathogens and organisms with the capabilities to form spores can survive free-living in the soil for years, but most pathogens encounter conditions that prevent normal cell functions once they leave the host. Crane et al. (1983) summarized the variables that affect the survival of enteric organisms in the environment: physical and chemical properties of the soil including pH, porosity, organic matter content, texture and particle size distribution, elemental composition, temperature, moisture content, absorption and filtration properties, and availability of nutrients; atmospheric conditions including sunlight, humidity, precipitation, and temperature; biological interaction of organisms including competition from indigenous microflora, antibiotics, and toxic substances; and the waste application method including the technique, frequency, and density of the organisms in the waste material. It is also known that some potential pathogens are free-living in the soil and may be nourished by animal wastes (Ellis and McCalla, 1978).

While many variables are thought to influence bacteria survival, temperature and moisture content of the soil or manure are thought to be key factors affecting die-off rates. Wang et al. (2004) found that temperature (but not moisture content) had a significant effect on indicator bacteria die-off in dairy cow manure. Wang et al. (1996) found that *E. coli* O157:H7 inoculated in fresh dairy cow manure survived for 63 to 70 days when incubated at 5°C with a high (74%) moisture content compared to survival times ranging from 42 to 59 days at incubation temperatures of 22°C and 37°C with lower (10% at the end of the study) moisture content. Howell et al. (1995) found that fecal coiform mortality rates decreased as the sediment particle size became finer and as temperature decreased. The study did not find evidence of interaction between temperature and particle size in determining fecal bacteria persistence. Mubiru et al.

(2000) attributed differences in *E. coli* survival inoculated in two soils to differences in available water in the soil matrix. Most rapid die-off of *E. coli* O157:H7 and *Salmonella typhimurium* were observed at 37°C when compared with 4 and 20°C (Himathongkham et al., 1999). *E. coli* O157:H7 inoculated in feces in the laboratory survived best when incubated at temperatures below 23°C but also survived for shorter periods of time than manure exposed to the external environment, emphasizing the difficulty in applying laboratory results to field conditions (Kudva et al., 1998).

Van Donsel et al. (1967) studied the effects of seasonal variation on the survival of FC and FS in soil. The survival of fecal coliform and fecal streptococcus was studied for several years at shaded and exposed outdoor soil plots. The 90% reduction for fecal coliform occurred after 3.3 days in the summer and 13.4 days in the autumn, while the FS 90% reduction times ranged from 2.7 days in summer to 20.1 days in the winter. Taylor and Burrows (1971) found that *E. coli* survived 7 to 8 days and *Salmonella dublin* persisted up to 18 days on growing pastures. Cutting the pastures reduced the bacterial survival time on the grasses, most likely through its effect on drying rates and increased exposure to solar radiation. Manure storage may reduce bacterial survival by allowing die-off to occur before it is applied to the soil and also allows manure to be spread under optimum climatic conditions. Waste spread on frozen soils may not infiltrate into the soil and bacterial survival may be prolonged due to the low temperatures (Moore et al., 1988). A study by Crane et al. (1983) showed a trend toward minimal bacterial losses from applied liquid waste systems and greatest losses for solid spread methods, but the differences were not significant (Crane et al., 1983, unpublished data).

Several studies have detected bacterial re-growth following land application of waste. Crane et al. (1980) applied poultry manure to bare soil plots in a controlled environment. The manure was applied at approximately 36.5 and 164 t/ha on Norfolk loamy fine sand from the coastal plains and Davidson clay loam from the Piedmont region. Die-off of fecal coliform was rapid immediately following the manure application until day seven. The first seven days were followed by a period of re-growth lasting five days and then the organism concentrations remained constant. Although the re-growth could not be attributed to a single factor, the high soil moisture content and the mild unfluctuating temperature most likely contributed to the re-

growth. VanDonsel et al. (1967) noticed after-growth of both tracer fecal coliforms and nonfecal coliforms. The re-growth was thought to be stimulated by nutrients remaining from the broth inoculum used to apply a cultured fecal coliform to the field plots. During nonfreezing conditions, an increase in the nonfecal coliforms appeared most often after a rainfall. The increase seemed to be related to the temperature conditions following the rain rather than the amount of rain. The study concluded that very warm weather following a rain could cause up to 100-fold increase in the soil coliforms. Laboratory studies have also found increased concentrations of fecal coliforms and *E. coli* in manures up to a week following excretion (Conner and Kotrola, 1995; Wang et al., 1996; Wang et al., 2004).

2.3.2. First-order Decay Rates

Several researchers have concluded that first-order decay adequately describes die-off kinetics of fecal indicators from agricultural sources (Crane and Moore, 1986; Himathongkham et al., 1999; Oliver et al., 2006), however, most die-off studies have been conducted under laboratory conditions making it difficult to compare these laboratory-based findings to the field-based findings presented in this study. Table 2.1 presents a summary of *E. coli* first order decay rates in freshly excreted dairy cow manure developed from both lab and field-based studies.

Table 2.1. *E. coli* die-off and first order decay rates in freshly excreted dairy cow manure.

Description of study		organism	Environmental variables	Length of study	Die-off rate, k ($days^{-1}$)	Reference
Die-off in freshly excreted dairy cow manure	lab-based	*E. coli*	three moisture contents (30%, 55%, and 83%) and three temperatures (4ºC, 27ºC, and 41ºC)	ranged from 35 to 103 days	0.11 d^{-1} at 4ºC 0.20 d^{-1} at 27ºC 0.32 d^{-1} at 41ºC	Wang et al., 2004
Die-off in the top layer of fresh dairy manure assessed separately from the middle and bottom layers, inoculated with E. coli O157:H7	lab-based	*E. coli* O157:H7	variable moisture and temperatures	ranged from 27 to 60 days	*Top layer* 0.111 d^{-1} at 4ºC (75% RH), 0.046 d^{-1} at 20ºC (50% RH) and 0.112 d^{-1} at 37ºC (30% RH) *Middle and bottom layer* 0.054 d^{-1} at 4ºC, 0.074 d^{-1} at 20ºC, and 0.279 d^{-1} at 37ºC	Himathongkham et al., 1999
Die-off in freshly deposited cattle feces (steers)	lab-based	*E. coli*	incubated at 15ºC, 25% and 50% moisture	111 days	0.054 d^{-1} (25% moisture) and 0.058 d^{-1} (50% moisture)	Oliver et al., 2006
Monitored bacterial die-off in milker, heifer and beef cowpats on grazed pastureland	field-based	*E. coli* and fecal coliform	Two seasonal studies, deposition in late April and mid-July		*April deposition*: 0.01593 d^{-1} *July deposition*: 0.02332 d^{-1}	Mostaghimi et al., 1999

Two-staged first order functions have been found to improve indicator decay patterns. Mubiru et al. (2000) inoculated *E. coli* O157:H7 and nonpathogenic *E. coli* strains in two soil types and enumerated concentrations weekly for an 8-week period. The decay rate constants for a first order approximation ranged from 0.09 d^{-1} to 0.17 d^{-1}, varying slightly by strain and soil type, with high regression coefficients ranging from 0.89 to 0.93. A two-staged first order function improved the fit and the initial mortality rates were much higher 0.15 d^{-1} to 0.25 d^{-1} than the second stage 0.05 d^{-1} to 0.08 d^{-1}. Mortality rates of fecal coliforms and fecal streptococci have also been adequately described by a two-stage exponential decay model (Zhai et al., 1995).

E. coli die-off rate coefficients developed under laboratory conditions at constant temperatures are generally higher than the field-based seasonal die-off rate coefficients (Table 2.1). If water quality models continue to use first-order decay to predict in-field bacterial concentrations, die-off rate coefficients should be developed in the field for utilization in these models. In addition, higher order approximations and inclusion of weather variables might more accurately represent in-field bacterial decay.

2.4. Bacterial Attachment to Particulates

Previous research has identified cellular properties (such as hydrophobicity of the cell and the electrostatic nature of the cell envelope) and external factors (beyond availability of attachment sites) that could increase fecal bacteria attachment to sediment and organic matter particles. Laboratory-based partitioning studies attempt to isolate the influences of individual parameters and identify the cellular properties or external factors responsible for bacterial attachment.

2.4.1. Cellular Properties Influencing Attachment

Bacteria adsorption to particles has been identified as either weak adsorption, due to van der Waals forces, which counteract repulsive forces (Jamieson et al., 2004), or strong adsorption due to cellular appendages such as fimbriae or pili (Henry, 2004) or adhesive polysaccharides and glycoprotein excreted by the cell (Madigan et al., 2000). Different strains of the same species may exhibit distinctive physiological properties depending upon the growth curve stage and environment surrounding the cell. A study by Dawson et al. (1981) found that cells in the early log, late log, and stationary phases exhibited few attachment properties. However, under starvation conditions, cells decreased in size and exhibited adhesive properties. Nearly all

ecosystems are oligotrophic, limiting in either the bioavailability of organic matter or nutrients (Morita, 1997). In oligotrophic environments, bacteria adhere to surfaces in an effort to obtain nutrients and increase survival (Morita, 1997). The exposed cell surface of the attached cells is decreased and the attached portion of the cell does not participate in substrate uptake. In addition to surface attachment, bacteria also bioflocculate or attach to one another and form aggregates. Bioflocculation of cells is genetically controlled and is thought to only be expressed in specific environments, usually when substrates are depleted and bacteria are stressed for nutrients (Morita, 1997).

To understand the growth of a particular organism, cells are placed in a flask containing nutrient broth and environmental conditions are controlled. The distinct phases that are observed during a growth curve include the lag phase, the exponential or log phase, the stationary phase, and the death phase (Maier et al., 2000). The lag phase occurs during the physiological adaptation of the cells to the culture conditions and is observed until the cells reach a population of approximately 10^6 cells mL^{-1}. During the exponential phase, the rate of increase of cells in the culture is proportional to the number of cells present at any time. As cells deplete the carbon and nutrient sources they enter into the stationary phase where growth is balanced by an equal number of dying cells. While no net growth occurs during this stage, a slight increase in the growth curve might occur due to the use of lysed cells as a carbon and nutrient source. During this period the cells detect the lack of substrate and begin to undergo metabolic and physiologic changes such as activation of dormant genes or suppression of active genes. Cells adapt individually to the low nutrient environment and use highly variable methods of survival; therefore, the population as a whole is very heterogeneous during stationary phase (Jones, 1997). As components necessary for growth become more and more limited, cells enter into the death phase where a net loss of culturable cells occurs, often at an exponential rate (Maier et al., 2000).

The outer surfaces of bacterial cells are normally negatively charged and are attracted to positively charged particles in the soil. However, electrical forces also do not appear to be fully responsible for attachment. Stenstrom (1989) found that adhesion of *Salmonella typhimurium* to mineral particles correlated with the hydrophobicity of the cell surface. A positive charge on the surface of bacteria contributed to the adhesion potential while, a negative charge on the bacterial

surface and changes in the pH (4 to 9) did not significantly change the adhesion process. Benthic cyanobacteria, which adhere to solid surfaces and bottom sediments were found to be hydrophobic while planktonic cyanobacteria were all found to be hydrophilic (Fattom and Shilo, 1984). These cellular properties indicate the potential for preferential attachment of cells to particulates (particularly those in the stressed state) during transport to surface waters.

2.4.2. External Factors Influencing Attachment

Factors external to the cell that have been found to influence cell sorption to particulate matter include ionic strength and pH of the carrying solution and size of the particulate matter. Jewett et al. (1995) studied the influence of ionic strength and pH on the retention of *Pseudomonas fluorescens* P17 in silica media. Changes in the pH (5.5 to 7.0) of the carrying solution and surface charge did not influence sorption. However, sorption was increased by increasing the ionic strength (2×10^{-2}, 5×10^{-4}, and 2×10^{-5} M) of the carrying solution. Fontes et al. (1991) evaluated the mineral grain size, ionic strength of an artificial groundwater solution (AGW), and cell size on the movement of microorganisms through porous media. Retention was highest for AGW of lower ionic strength (0.00089 m), larger cells (0.75 by 1.8 μm rods) and fine grained sand (0.33 mm). Grain size was determined to be more important than cell size and ionic strength in controlling the transport of bacteria. Mitchell and Chamberlin (1978) found that clays tend to adsorb coliforms more than silts or sands.

Equilibrium batch experiments examining the effect of dairy manure on *E. coli* attachment to soils found increasing manure content resulted in decreased *E. coli* attachment (Guber et al., 2005a). Other studies found that removal of dissolved organic carbon from a bacterial suspension increased attachment (Scholl and Harvey, 1992), and bacteria adsorption on quartz and iron-quartz particles was decreased by the addition of organic matter (Johnson and Logan, 1996). Guber et al. (2005b) hypothesized that the decreased bacterial attachment to particulates in the presence of manures could be caused by a variety of factors including competition between bacteria and dissolved organic matter for attachment sites on soil, modification of soil mineral surfaces by soluble manure constituents, or modification of bacterial surfaces by dissolved organic matter.

2.4.3. Laboratory-Based Partitioning Studies

Rather than collect environmental samples, some researchers have conducted experiments on a single strain of bacteria and grown cells to a certain stage on the growth curve or concentration prior to determining partitioning between unattached and attached phases. This technique might produce more consistent results since attachment can depend upon both the stage of the growth curve (Dawson et al., 1981) and different strains might exhibit different attachment properties when exposed to a low-nutrient environment. Although results obtained using these methods might be more reproducible, it is questionable whether findings from these studies will be be applicable to the natural environment. The sorption of a single strain of bovine *E. coli* to a sterile, homogeneous soil comprised of greater than 80% sand was evaluated by Henry (2004). Approximately 78% of the *E. coli* was found to be associated or attached to the sediment particles when attachment was defined as *E. coli* unable to pass through an 8 μm filter. Ling et al. (2002) evaluated strong and weak adsorption of *E. coli* onto two different soil types (14% and 35% clay content). Wild strains of *E. coli* were isolated from pasture runoff and grown to a desired concentration. Adsorption was determined by separating the *E. coli* from the soil particles using differential centrifugation. Weak adsorption was determined by centrifuging the mixture and separating the supernatant from the soil. Soils with 35% clay sorbed 99.2% of *E. coli* while soils with 14% clay content sorbed 24.5% of *E. coli*. Strong adsorption was determined by adding 0.85% NaCl in distilled water and shaking for 5 minutes before centrifugation at a higher g-force for a longer period of time. Soils with 35% clay sorbed 96.1% of *E. coli* while soils with 14% clay content sorbed 38.1% of *E. coli*. Results indicated that percent adsorption of *E. coli* was significantly higher in soils with a higher clay content. Soils with higher clay contents were recommended to receive higher application rates of waste as a method to reduce bacteria concentrations in runoff.

Oliver et al. (2007a) conducted batch sorption experiments with a clay loam soil to determine the particle size fractions to which *E. coli* preferentially attach (2 – 3 μm, 4 - 15 μm, and 16 - 30 μm). Thirty five percent of introduced *E. coli* cells were associated with soil particulates >2 μm diameter and 14% were associated with the 4-15 μm size fraction while the 16-30 μm fraction of soil particles contained the highest concentration of *E. coli* per unit area. *E. coli* association with different soil particle size fractions will impact delivery to surface waters. The

fraction that remains unattached or associated with ≤2 μm diameter particles are likely to remain suspended in the water column and avoid removal mechanisms of many management practices such as vegetated filter strips while the attached fraction are more likely to settle (Oliver et al., 2007a).

2.5. Bacterial Transport and Partitioning during Runoff Events

Runoff from pastureland and cropland receiving applications of animal manure has been well documented to be a source of fecal contamination of surface waters (Crowther et al., 2002; Edwards et al., 1994; Edwards et al., 2000; Khaleel et al., 1979b; Schepers et al., 1980; Schepers and Doran, 1980; Soupir, 2003; Tian et al., 2002). There is a long history of rainfall/runoff studies conducted to determine the concentrations of fecal indicators in runoff from agricultural lands. Indicator organisms present in soils along with pathogens may contaminate surface waters, through movement with surface runoff (either as sorbed to sediment and organic particles or in the unattached state) and groundwater, through downward leaching with infiltrating water (Reddy et al., 1981). However, pathogenic organisms are largely retained at or near the soil surface (Faust, 1982; Gerba et al., 1975), thus increasing the potential for pollution of surface runoff waters. This large body of literature has not produced sufficient understanding of the transport properties of fecal bacteria. Often environmental factors have not been linked to die-off, infiltration into the soil has not been monitored, and partitioning between unattached and attached forms has not been considered. It is unclear if cells sorbed to soil particles offer resistance to transport by overland flow or if they are transported along with eroding soil particles. In addition, many of the studies have only evaluated concentrations of fecal coliforms while indicators such as *E. coli* and enterococci are now preferred by the EPA as those more likely to detect the presence of pathogenic organisms (U.S. EPA, 1986).

2.5.1. Bacterial Transport into Surface Waters from Agricultural Sources

Previous research has linked the transport of bacteria in surface runoff from agricultural lands to rainfall duration, intensity, and frequency (Patni et al., 1985); method of manure application (Janzen et al., 1974; Moore et al., 1982; Soupir, 2003); fecal deposit age (Kress and Gifford, 1984; Thelin and Gifford, 1983); and sorption of cells to soil particles (Walker et al., 1990). Low amounts of manure can enhance the quality of the soil by improving the soil aggregate size and its water holding capacity. However, when applied at higher rates, the large contribution of

monovalent ions from incorporated manure increased soil erodibility (Mazurak et al., 1975) and thus the amount of bacteria detached by overland flow (Khaleel et al., 1979a). Mazurak et al. (1975) disked plots to a depth of 10 cm. Soil detachment from manured plots (application rate of 415 t ha^{-1} yr^{-1}) was 89 mg/cm^3 while soil detachment from non-manured plots was 55 mg/cm^3. Increased soil erosion due to land application of waste may increase transport potential of fecal bacteria sorbed to sediment particles.

Doran and Linn (1979) compared fecal coliform (FC) concentrations in runoff from a grazed cow-calf pasture and an ungrazed pasture in eastern Nebraska. The FC counts were 5 to 10 times higher in the runoff from grazed pasture. The FC counts in runoff from both the grazed and ungrazed pastures exceeded the water quality standard of 200 CFU/100 mL more than 90% of the time. Similar results were found in a study by Doran et al. (1981) on a 106 ac (43-ha) fenced pasture located in south central Nebraska that compared a grazed area to a control area with restricted cattle access. The grazing increased FC counts between 5 and 10-fold from the control area. The FC counts in both the grazed pasture and ungrazed control areas exceeded both primary and partial body contact more than 90 percent of the time. Greater wildlife activity was noted on the smaller, better-protected control area, possibly accounting for high fecal coliform levels in runoff. Schepers and Doran (1980) continued the research for an additional year, removing all cattle from the grazed pasture. After removing the cattle, the fecal coliform levels in the runoff from both the grazed and control pastures were similar. However, the average FC counts from both the previously grazed and ungrazed areas continued to exceed the recommended water quality standards of 200 CFU 100 mL^{-1}. This study concluded that a large background contribution existed from wildlife. The high bacterial concentrations in runoff from grazed pastures where the cattle had been removed may also be due to the build up of stable populations in the soil (Faust, 1982). In a review of the influence of dairy waste management systems and their influence on FC concentrations in runoff, Moore et al. (1982) concluded that background indicator bacterial concentrations in runoff most likely range from 10^3 to 10^5 organisms/100 mL, even with the implementation of best management practices.

Thelin and Gifford (1983) developed standard cowpies to study FC release patterns. Release refers to the availability of fecal bacteria from field manure sources prior to reductions from

overland transport processes. The standard cowpies were tested against naturally occurring fecal deposits for peak release concentrations. The peak release regression from the naturally occurring fecal deposits was not significantly different than the regression for the standard cowpies, so the authors concluded that the standard cowpies did not change the release patterns. Fecal deposits 5 days old or less released FC concentrations into the water on the order of millions per 100 mL. Fecal deposits that had not been rained on for up to 30 days released FC concentrations on the order of 40,000 per 100 mL (Thelin and Gifford, 1983). Kress and Gifford (1984) found that even 100-day old cowpies are still potential sources of FC contamination. The peak concentration from a 100-day-old deposit was 4,200 FC per 100 mL in runoff, using the most probably number (MPN) method. Approximately 1,000 100-day old fecal deposits were found to release FC concentrations equal to a single 2-day old fecal deposit. Hafez et al. (1969) found that fecal deposits from cattle were not uniformly distributed throughout a pasture. In certain areas, such as water troughs, gates, fence lines, and bedding areas, cowpie concentrations may be much higher. Cattle allowed to roam freely on pastureland will defecate an average of 12 times per day (Kress and Gifford, 1984).

Despite the large number of field scale studies attempting to explain the concentrations of fecal bacteria in runoff from agricultural lands, little information about the factors influencing microbial transport have been provided (Jamieson et al., 2004). This is demonstrated by the lack of progress made in reducing bacteria loadings from agricultural lands. High levels of fecal bacteria indicators or pathogens are currently the leading cause of impairments of rivers and streams in the United States (U.S. EPA, 2007b) with agricultural practices being the primary source of these impairments (U.S. EPA, 2003). These field studies have simply emphasized the fact that fecal bacteria applied to the land are present in surface runoff, and they have identified the need to fully understand hydrologic characteristics and other factors that affect the fate and transport of fecal bacteria in a watershed (Jamieson et al., 2004).

2.5.2. Partitioning of Fecal Bacteria in Agricultural Runoff

Muirhead et al. (2005) studied the transport state of *E. coli* cells by placing cowpats and fecal-material–soil mixtures on a metal tray 250 mm long and 200 mm wide, and runoff was created by placing a rainfall simulator nozzle 250 cm above the soil. On average only 8% of *E. coli* cells

attached to sediment particles and most cells were not bioflocculated (attached to one another to form aggregates), but instead transported in runoff as single cells. Experimental field plots that were 1m wide and 5m long were used to investigate the removal of *E. coli* from overland flow under saturation-excess runoff conditions (Muirhead et al., 2006a). Retention of bacteria on grassed and cultivated plots receiving dairy cow slurry applications at different flow rates were compared. The majority of *E. coli* were able to pass through a 20 μm filter (80%) and only 9% of *E. coli* were attached to large (dense) soil particles. The authors concluded that *E. coli* were primarily transported as particles of neutral buoyancy that remained suspended during overland flow thus explaining poor removal of bacteria from the plot studies (<50%).

2.5.3. Partitioning of Fecal Bacteria in Urban Runoff

While little information is available on the partitioning of fecal bacteria in runoff from agricultural lands, researchers have begun to assess the percent attached during urban runoff events. Jeng et al. (2005) studied the sorption of *E. coli*, fecal coliform and enterococci with estuarine sediment and stormwater particles in urban stormwater runoff using screen filtration. Researchers found that *E. coli* and fecal coliform attached to suspended particles over a broad range of diameters while enterococci attached primarily to particles between 10 and 30 μm in diameter. The authors attributed *E. coli* association with a broader range of particle sizes because of the motility and rod shape of the *E. coli* makes them more able to attach to different angles or faces of the particles (edge-to-edge or face-to-edge associations). Schillinger and Gannon (1985) and Auer and Niehaus (1993) also used a screen filtration method to determine the sediment sizes to which fecal coliforms were sorbed in urban stormwater. Schillinger and Gannon (1985) found that between 10 and 15% of the sorbed bacteria were retained by each screen size, but most of the bacteria were associated with particles retained on 52 μm and 30 μm screens (Schillinger and Gannon, 1985). Sheer stress on the bacteria due to the smaller screen sizes may have reduced the attachment to the smaller particles sizes. Auer and Niehaus (1993) found that fecal coliforms were primarily sorbed to particle classes 0.45 – 1 μm and 6 – 10 μm during storm overflow events. On average 90.5% of the fecal coliform bacteria were found to be sorbed to particle sizes ranging from 0.45 – 10 μm and 9.5% were sorbed to particles sizes greater than 10 μm.

Characklis et al. (2005) used centrifugation to separate settleable particles and associated microbes from particles that would remain suspended in the water column, primarily organic material and free phase organisms. Samples were collected from three urban locations near Chapel Hill, North Carolina. The study found that 20 – 35% of fecal coliforms, *E. coli*, and enterococci were associated with settleable particles during normal flow conditions and 30 – 55% during storm events. Krometis et al. (2007) examined attachment of fecal coliforms, *E. coli* and enterococci to settable particles throughout three separate storm events. Higher concentrations of settable particles and microbes were observed during the earliest stages of storm hydrograph and on average 40% of fecal coliforms, *E. coli* and enterococci were associated with settable particles during storm events. In samples collected from the Neuse River Estuary in eastern North Carolina an average 38% of *E. coli* and enterococci were associated with settable particulates (Fries et al., 2006).

2.6. Summary

Previous studies have produced mixed results as to whether enteric bacteria in soil and aquatic systems are present in the unattached state or sediment and particulate-attached. Interactions between bacteria and particulates influence survival on the soil surface and transport characteristics during overland flow events; thus, it is necessary to incorporate these relationships into NPS models. To advance current NPS modeling efforts, improved information is needed on the fate of *E. coli* and enterococci on agricultural lands and transport of *E. coli* and enterococci to surface waters. Best management practices (BMPs) are implemented to reduce the transport of pollutants to surface waters. Identification of the particulates to which bacteria preferentially attach would further aid in the design and selection of BMPs in watersheds impaired by fecal bacteria. Improved relationships to describe in-field die-off patterns and development of coefficients to describe partitioning between unattached and attached phases during overland flow can be used to improve in-stream predictions of indicator bacteria concentrations by NPS models.

Chapter 3. A Method to Partition between Attached and Unattached *E. coli* and Enterococci in Runoff from Agricultural Lands

3.1. Introduction

The three most common pathogen indicators in the United States include fecal coliform, *E. coli* and enterococci. 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 (U.S. EPA, 1986) and *E. coli* is typically the indicator preferred in fresh water systems. In an attempt to reduce pollutant loading to the nation's water bodies, total maximum daily loads (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 discharged to a water body, while still meeting the water quality standards, and an allocation of that amount to the pollutant's sources (U.S. EPA, 2007a). Nonpoint source (NPS) pollution models are most frequently used to assess bacterial transport to surface waters and most models typically simulate bacterial transport as a dissolved pollutant (Paul et al., 2004) primarily because sufficient data on bacterial partitioning are 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 be increased (Burton et al., 1987; Gerba and McLeod, 1976). *E. coli* attachment to particulates ranges from 20% to 35% during stream base flow conditions and 30% to 55% during storm events; while enterococci attachment to particulates ranges from 20% to 35% during stream base flow conditions and 8% to 55% during storm events (Characklis et al., 2005; Jeng et al., 2005). *E. coli* attachment to particulates averaged 8% when cowpats were placed on trays beneath a rainfall simulator (Muirhead et al., 2005). The variety of techniques used to assess partitioning between the unattached and attached phases could lead to differences in results and thus partitioning coefficients implemented into NPS models.

Most frequently, a separation technique such as filtration, fractional filtration or centrifugation is used to separate the unattached from the attached cells (Auer and Niehaus, 1993; Characklis et al., 2005; Fries et al., 2006; Henry, 2004; Jeng et al., 2005; Muirhead et al., 2005; Schillinger and Gannon, 1985) The unattached and total fractions are enumerated and the attached fraction is assumed to be the difference between the two. Chemical or physical dispersion techniques are often employed to separate sorbed bacteria from sediments to assess the total concentration. Dispersing the indicator organisms from sediments and organic particles allows each bacterium to form a separate colony; therefore achieving a better approximation of the total number of both unattached cells and those associated with particulates. Many studies have examined impacts of dispersion techniques on total bacteria or fecal coliforms (Craig et al., 2002; Epstein and Rossel, 1995; McDaniel and Capone, 1985; Trevors and Cook, 1992; Yoon and Rosson, 1990) and some researchers have obtained optimal results by using a combination of dispersion techniques (Yoon and Rosson, 1990). These previous studies have not examined the effects of different dispersion techniques on mixed environmental strains of *E. coli* and enterococci present in runoff samples.

Filtration is one technique used to separate unattached from attached bacteria. Typically, bacteria passing through the filter are assumed to be unattached, previously defined as cells able to pass through an 8 μm screen (Henry, 2004; Mahler et al., 2000; Qualls et al., 1983). Since a typical *E. coli* cell is 1 × 3 μm (Madigan et al., 2000) in size, the filtration method assumes that free bacteria, those sorbed to very small particles, or even bioflocculated clumps are able to pass through the 8 μm filter. Mahler et al. (2000) validated this method by passing a known suspension of *E. coli* through the 8 μm filter. The authors successfully recovered 99% of the unattached *E. coli*, demonstrating that only a small proportion of the free cells that were retained would have been incorrectly classified as attached. Fractional filtration has been used to identify the bacteria attached to different particles sizes (Auer and Niehaus, 1993; Jeng et al., 2005; Schillinger and Gannon, 1985). Fractional filtration of urban stormwater was performed by Schillinger and Gannon (1985) and Auer and Niehaus (1993) to separate sediment particle sizes; however both studies also assumed that all cells retained by screens were associated with particulates of that size. It is possible that bioflocculated or unattached bacteria could attach to the filters (Henry, 2004) or particulates could clog filters, retaining free cells and resulting in incorrect classification.

Centrifugation is another method frequently used by researchers to determine partitioning between unattached and sediment sorbed bacteria (Characklis et al., 2005; Dumontet et al., 1996; Huysman and Verstraete, 1993; Muirhead et al., 2005; Sayler et al., 1975; Schillinger and Gannon, 1985). The percent attached is typically determined by subtracting the total count from the concentrations remaining in the supernatant following centrifugation. Use of centrifugation to separate microbial fractions assumes that microbial sorption to particulates is not affected even though interactions between cells and particles are increased during the process (Characklis et al., 2005). In addition, unattached bacteria can be similar in size to clay particles, so determining proper centrifuge settings to separate sediments from nonattached bacteria can be difficult (Henry, 2004). Previous research has not identified a separation technique that will also identify particle sizes to which *E. coli* preferentially attach without the assumption that all unattached cells are able to pass through filtration devices (Henry, 2004; Jeng et al., 2005; Mahler et al., 2000).

A method to disperse and separate unattached from attached forms of environmental *E. coli* and enterococci present in runoff is needed to improve consistency between research results which will ultimately improve bacterial transport modeling. The goal of this study was to develop an easy-to-replicate laboratory procedure for separation of unattached from attached *E. coli* and enterococci which will also identify particle sizes to which *E. coli* and enterococci preferentially attach. The first objective was to compare previously employed methods for dispersing attached *E. coli* and enterococci from sediments and suspended particles and we hypothesized that sonification would be the best method of recovering attached bacteria from sediments. The second objective was to validate a sequence of previously employed methods for separation of unattached and attached *E. coli* and enterococci.

3.2. Materials and Methods

3.2.1. Sample Collection

A single, fresh cowpat was collected from the confined stalls at the Virginia Tech Dairy Farm. A cowpat fecal source was selected because previous research results indicated higher concentrations of indicator organisms and total suspended solids (TSS) in runoff from

pastureland plots treated with cowpats when compared to poultry litter and liquid dairy manure (Soupir et al., 2006b). The fresh cowpat was transported immediately to the Virginia Tech Prices Fork Research Farm and was placed on sloped pastureland of Grosclose silt loam soils (Creggar et al., 1985) with particle size distribution of 38% sand, 54% silt and 8% clay. Bare soils were selected to ensure sufficient transport of both sediment and organic particulates. High concentrations of particulates increased availability of bacterial attachment sites and likelihood of *E. coli* and enterococci attachment. Water from a local well was applied to the cowpat and the surrounding soils using a hand application watering can. Water was briefly applied over the cowpat until sufficient runoff could be collected approximately 0.30 m (1-ft) from the base of the cowpat to fill a 140 mL sterile bottle. High concentrations of organic matter from the cowpat and sediments were observed in the runoff samples. Cowpat runoff samples were used to compare the effectiveness of physical and chemical dispersion techniques and to develop the separation technique.

3.2.2. Comparison of Dispersion Treatments

A comparative study was conducted to identify the best method of dispersing environmental strains of *E. coli* and enterococci from sediment and organic matter particles present in runoff samples. Chemical and physical dispersion techniques were tested separately in preliminary studies before the optimal techniques were combined to evaluate if a combination of methods would further increase enumeration.

Based on findings from previous studies, selected chemical dispersion treatments included Tween-80 (Yoon and Rosson, 1990) and Tween-85 (Auer and Niehaus, 1993; Henry, 2004) at concentrations of 10, 100, 1,000, and 10,000 mg L^{-1} and 0.1% (w/v) sodium pyrophosphate (NaPP) combined with 1% (v/v) glycerol, 1% (v/v) peptone, and deionized water (Trevors and Cook, 1992). Runoff samples were diluted in the chemical solutions and enumerated on Modified mTEC agar (U.S. EPA, 2000) using membrane filtration procedures (Clesceri et al., 1998). The control samples were diluted in phosphate buffer solution (HACH Company, Loveland, CO) and enumerated by membrane filtration. All samples were vortexed for approximately two seconds to ensure mixing prior to membrane filtration.

Three physical dispersion treatments: hand shaker (Wrist Action Shaker, Burrell Corporation, Pittsburg, PA) treatment for 10 min, ultrasonic bath (Fisher Scientific, 50/60 Hz, 55W) treatment for 30 sec, 2 min, 6 min and 10 min, and a one minute vortex (Touch Mixer Model 231, Fisher Scientific, Pittsburg, PA) were also compared. After the runoff samples were collected, a 1 mL control subsample was extracted and diluted in phosphate buffer solution (HACH Company, Loveland, CO) and enumerated by membrane filtration (Clesceri et al., 1998). The remaining sample was treated with a dispersion technique prior to dilution and membrane filtration.

3.2.3. Development of Separation Technique

Multiple screen filtration separated suspended solids into particle sizes while centrifugation was used to separate attached from unattached cells. A Mini-Sieve Microsieve Set (Bel-Art Products, Pequannock, NJ, Figure 3.1) containing a number 35 mesh screen was used to retain particles larger than coarse sand (>500 μm) and a number 230 mesh screen was used to retain medium, fine and very fine sand (63 - 500 μm). An 8 μm filter (Poretics, Polycarbonate, GE Water and Processes Technologies) was used to retain 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 (Nuclepore Track – Etch Membrane Filtration Products, Whatman) was used to retain clay and very fine silt particles. Gravity flow was augmented with vacuum application. Following filtration, the mesh screens and filters were aseptically removed, placed in phosphate buffered solution, and gently rinsed to remove sediments from the filters. Preliminary studies found that >98% of particulates are removed from the filter during this process (Soupir, M., unpublished data). Samples were then centrifuged at 4,700 rpm for 15 seconds to separate unattached cells from suspended particles (Lago, 2005). The supernatant and filtrate passing through the 3 μm filter were enumerated for *E. coli* concentrations on modified mTEC agar using membrane filtration (Clesceri et al., 1998) to assess the unattached bacterial concentrations. The sediment and organic particles associated with each screen size were re-suspended in phosphate buffered water, treated with the optimal dispersion technique and enumerated by membrane filtration to assess the total bacterial concentration retained by each screen size. Total and unattached concentrations were converted to a mass basis based on filtration volumes and the difference between the total and unattached masses was assumed to be the attached portion. The attached portion associated with each screen size was divided by the total suspended solids associated

with each screen size to obtain the cfu (colony forming units) per gram of particulates and identify the particle sizes to which *E. coli* and enterococci preferentially attach.

Figure 3.1. Mini-Sieve Microsieve Set used to separate particles in runoff into 63 and 500 µm particle size categories.

Two separate studies were conducted to validate the separation technique. Suspended *E. coli* DH2 1030 collected from the stationary phase of the growth curve was centrifuged to ensure that unattached cells remained in suspension. Secondly, samples collected in duplicate at the base of the large cowpat-treated field plots described in Chapter 6 were enumerated for *E. coli* and enterococci concentrations by treatment with the dispersion technique followed by membrane filtration (control samples) and compared to *E. coli* and enterococci concentrations that were pre-treated by screen filtration and centrifugation followed by dispersion technique treatment and membrane filtration.

3.2.4. Data Analysis

The experimental design for the comparison of dispersion techniques was a randomized block design with preliminary comparisons of either chemical or physical treatments conducted in triplicate to identify the treatments most likely to disperse attached cells and increase *E. coli* and enterococci counts. Five replicates were used when the best chemical and physical dispersion

techniques were combined. Percent increase or decrease from the control was calculated by subtracting the control average concentration from the treatment average concentration and then dividing by the control average concentration. Experimental data were log-transformed and analyzed using the Mixed procedure of SAS (SAS Institute, 2004) and least square means were compared using Tukey's pairwise comparison (Ott and Longnecker, 2001). Statistical differences between total *E. coli* and enterococci pre-treated with screen filtration and centrifugation and total *E. coli* and enterococci analyzed by the dispersion technique and membrane filtration were determined by Analysis of variance (ANOVA) and a probability level of ≤ 0.05 was considered significant.

3.3. Results and Discussion

3.3.1. Comparison of Dispersion Techniques

A comparative study was conducted to identify the best method of dispersing wild strains of *E. coli* and enterococci from sediment and organic matter particles present in runoff from pasturelands. Previous researchers found a great deal of variation in the methods that result in greatest recovery of viable bacteria. While some methods increase enumeration, care must be taken when exposing the bacteria to either chemical or physical dispersion treatments so that the cells are not damaged, resulting in reduced enumeration.

A preliminary comparison of Tween-80, Tween-85 and sodium pyrophosphate solutions (Table A.1) indicated that the 1,000 mg L^{-1} Tween 85 solution increased *E. coli* concentrations by 36% and enterococci concentrations by 21% when compared to the control; however, this increase was not statistically significant. All other Tween treatments resulted in a decrease in *E. coli* concentrations that ranged from -3 to -34%. All other Tween 85 treatments decreased enterococci concentrations by -4 to -64% when compared to the control but Tween 80 concentrations had a varied response on enterococci concentrations, ranging from -20 to 60%, when compared to the control. Yoon and Rosson (1990) found that a specific concentration of Tween 80 resulted in greatest recovery of bacteria; however, the study did not specifically enumerate recovery of *E. coli*. They treated turbid seawater samples with Tween 80 concentrations ranging from 0 to 100 mg L^{-1} to improve enumeration of bacteria attached to sediments. Recovery was optimal in samples treated with the 10 mg L^{-1} concentration and

concentrations greater than 25 mg L^{-1} increased bacterial die-off. While Trevors and Cook (1992) found that sodium pyrophosphate increased enumeration of total aerobic colony forming units, in this study each treatment significantly (*P* value < 0.001) reduced *E. coli* concentrations by an average of 73% and enterococci concentrations by 1% when compared to the control. Sodium pyrophosphate might be effective in increasing counts of total aerobic bacteria; however, we found that it was not an effective dispersant when specifically enumerating wild strains of *E. coli*.

Others have found that chemical treatments have little or no effect on the dispersion of bacteria (Craig et al., 2002; Epstein and Rossel, 1995); therefore, physical dispersion techniques were also investigated. A preliminary comparison of physical dispersion techniques (Table A.2) revealed that the ultrasonic bath treatment resulted in up to 320% increase in *E. coli* and enterococci concentrations (*P* values < 0.0001) when compared to the control. Ten minutes of hand shaking also increased *E. coli* concentrations by 150% (*P* value = 0.0018) and enterococci concentrations by 33% (NS) while a one minute vortex treatment decreased *E. coli* counts by 33% and enterococci counts by 6%, but this decrease was not statistically significant.

The ultrasonic bath and hand shaker techniques were then combined with 1,000 mg L^{-1} dilutions of Tween 85 solution to evaluate if a combination of techniques would further increase cell recovery (Table 3.1). Contrary to findings from the preliminary studies, the physical treatments did not always increase enumeration of *E. coli*. The ultrasonic treatments differed in *E. coli* concentrations from the control by -19% to 2%; although none of the differences were statistically significant. It is possible that the cells were stressed prior to treatment with the ultrasonic bath and thus damaged by the treatments. The 10-minute hand shaker treatment increased *E. coli* concentrations by 24% and enterococci concentrations by 12% when compared to the control. Diluting samples treated with the hand shaker in the 1,000 mg L^{-1} concentration of Tween 85 resulted in a 45% (*P* value = 0.0028) increase in *E. coli* concentrations and a 21% increase in enterococci concentrations (NS). The 10-minute hand shaker treatment followed by dilutions in 1,000 mg L^{-1} Tween 85 was identified as the optimal treatment because this treatment provided the most consistent results for both indicators during both the preliminary study and when physical and chemical techniques were combined. The lower percentage of

increase in recovery of all samples may indicate that fewer *E. coli* and enterococci were bioflocculated or attached to sediment particles during the second sample collection. A previous study found that *E. coli* cells were released from cowpats and transported as single cells (Muirhead et al., 2005), possibly explaining the reduced effectiveness of all dispersion techniques. McDaniel and Capone (1985) suggested that response to dispersion techniques may be dependent upon the soil type and it might be necessary to test different techniques for each soil prior to use.

Table 3.1. Comparison of combined physical and chemical dispersion treatments on runoff samples collected below a dairy fecal deposit on pastureland (n=5).

	E. coli		enterococci	
Dispersion treatments (n=5)	**Concentration cfu mL^{-1} (std dev)**	**Average % change from control[1]**	**Concentration cfu mL^{-1} (std dev)**	**Average % change from control**
Control	2.44×10^4 (5639)		1.76×10^3 (764)	
Ultrasonic bath treatment 30 sec	2.5×10^4 (8746)	2%	1.38×10^3 (356)	-22%
Ultrasonic bath treatment 30 sec Dilutions in 1,000 mg L^{-1} Tween 85	2.56×10^4 (2302)	5%	1.53×10^3 (378)	-13%
Control	5.9×10^4 (7517)		Same as previous control	
Ultrasonic bath treatment 2 min	4.78×10^4 (7085)	-19%	1.88×10^3 (286)	7%
Ultrasonic bath treatment 2 min Dilutions in 1,000 mg L^{-1} Tween 85	6.2×10^4 (11662)	5%	1.72×10^3 (249)	-2%
Ultrasonic bath treatment 6 min	5.72×10^4 (10109)	-3%	1.78×10^3 (249)	1%
Ultrasonic bath treatment 6 min Dilutions in 1,000 mg L^{-1} Tween 85	6.56×10^4 (6269)	11%	1.66×10^3 (241)	-6%
Ultrasonic bath treatment 10 min	5.04×10^4 (5367)	-15%	1.98×10^3 (438)	13%
Ultrasonic bath treatment 10 min Dilutions in 1,000 mg L^{-1} Tween 85	6.12×10^4 (7085)	4%	1.88×10^3 (259)	7%
Control	7.54×10^4 (9100)		1.72×10^3 (239)	
Hand Shaker 10 minutes	9.32×10^4 (9628)	24%	1.92×10^3 (356)	12%
Hand Shaker 10 minutes Dilutions in 1,000 mg L^{-1} Tween 85	$10.9\times10^{4*}$ (10756)	45%	2.08×10^3 (482)	21%

[1]A negative value indicates a decrease in cells when compared to the control
[*]Indicates statistical significance when compared to the control treatment according to Tukey's pairwise comparison

3.3.2. Dispersion Technique Validation

A runoff sample was microscopically inspected to validate the selected dispersion technique. Samples were diluted in phosphate buffered water, stained with fluorochrome (acridine orange),

and viewed using the epifluorescence microscopic method as described by Clesceri et al. (1998). Samples were stained prior to any dispersion treatment (Figure 3.2a), after receiving the hand shaker treatment (Figure 3.2b), and after receiving the Tween-85 treatment (Figure 3.2c). Removal of bacteria from sediment and organic particles by the dispersion technique was confirmed by examining the samples prior to and after each treatment. The presence of dispersed cells, reduced clumping, and fewer cells attached to sediment or organic matter particles following the dispersion treatment validated this technique.

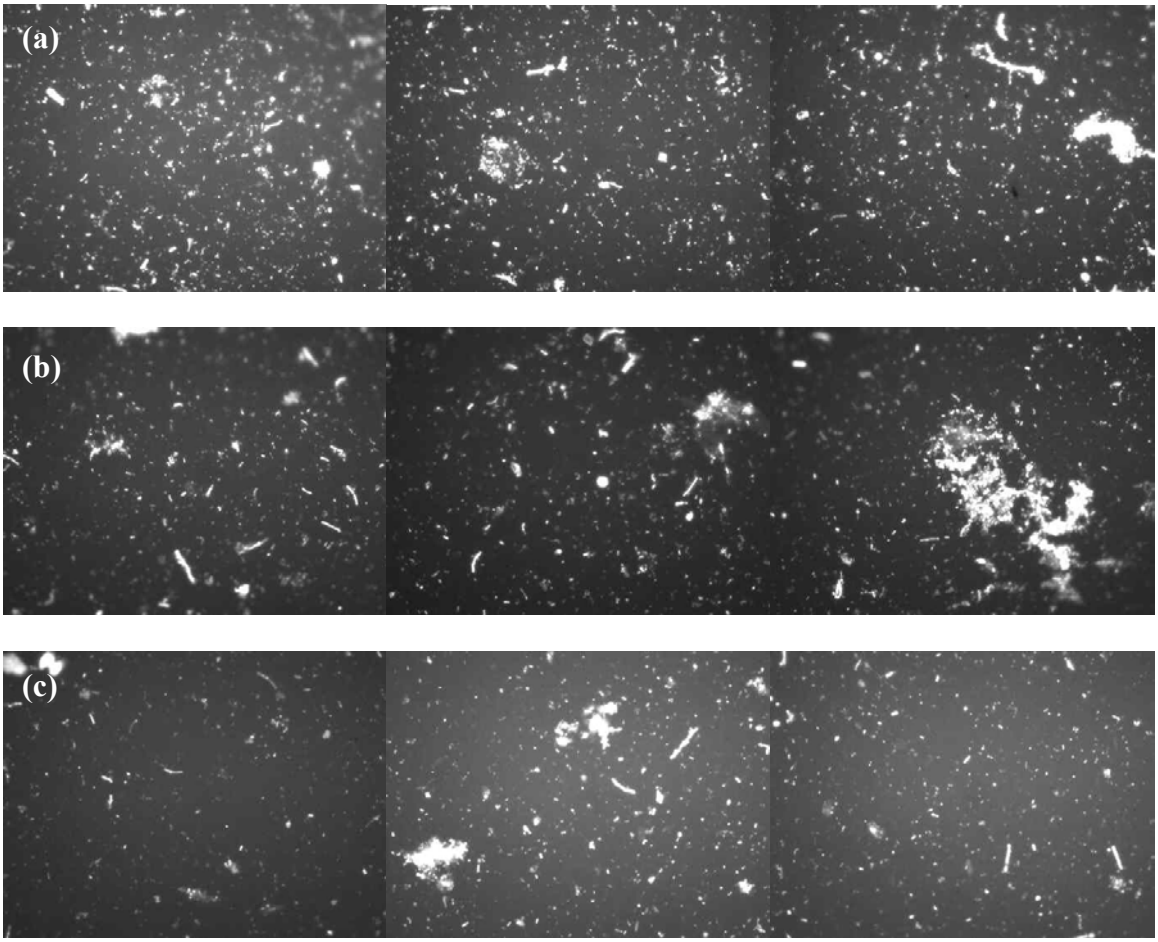

Figure 3.2. Cells receiving no dispersion treatment (a); cells treated with a hand shaker for 10 min (b); cells treated with a hand shaker for 10 min and diluted in 1,000 ppm Tween 85 (c).

3.3.3. Separation Technique Validation

Multiple screen filtration has been used previously to identify particle sizes to which bacteria are associated; however, centrifugation is more frequently used to separate unattached from attached

cells. We propose combining these two techniques to eliminate concerns associated with filtration techniques. While filtration using a single screen size of 8 μm has been identified previously as a viable method to separate attached and unattached bacteria (Gordon et al., 2002; Henry, 2004; Mahler et al., 2000; Qualls et al., 1983), the presence of sediments and organic particles in runoff from agricultural lands make it very likely that sediments could clog the filters, retaining unattached and bioflocculated cells.

To determine recovery of *E. coli* in filtrate passing through 8 and 3 μm filters, *E. coli* DH2 1030 was collected from the stationary phase of the growth curve (Figure A.1). *E. coli* cells were diluted in phosphate buffered water to approximately 10^5 cfu mL^{-1} and 10 mL of suspended cells were filtered through the 8 and 3 μm filters. The initial concentration and the concentration in the filtrate were enumerated by membrane filtration. Eighty-eight percent of cells passed through the 8 μm filter while only 3% of the cells present in the control passed through the 3 μm filter (Figures A.2 and A.3). This significant reduction indicated that an alternative method would be necessary to account for unattached cells retained on the filter surface or trapped by sediments and organic particles. Therefore, rinsing the screens and filters with phosphate buffered water and then centrifuging the solution to separate attached from unattached cells combined the benefits of fractional filtration, by identifying particle sizes to which cells attach, with the more commonly accepted practice of centrifugation. To validate the selected centrifugation technique, approximately 10^5 cfu mL^{-1} *E. coli* DH2 1030 was centrifuged at 4,700 rpm for 15 seconds to separate unattached cells from suspended particles (Lago, 2005). No reduction of unattached concentrations was observed (n=12, Figure A.4). While centrifugation could increase interactions between cells and particles, possibly strengthening existing bonds, we assumed that the application of the dispersion technique prior to enumeration of the total concentration should disperse any increased attachment that results from centrifugation.

Application of the separation technique to runoff samples collected at the base of 18.3-m long field plots (described in Chapter 6) provided an opportunity to conduct a mass balance and examine the technique for potential loss of cells during the filtration and centrifugation process. Field plots were constructed at the Virginia Tech Prices Fork research farm on the Grosclose silt loam soils examined in the dispersion portion of this study. Additional details about plot

construction, rainfall simulation and sample collection are presented in Chapter 6. A total of 68 samples were collected at the base of the plots, two samples collected at each sampling interval were used for the two different sets of analysis. The first was analyzed for total *E. coli* concentrations by treatment with the hand shaker for ten minutes followed by membrane filtration and is referred to as the control in Table 3.2. The second was analyzed for unattached and attached concentrations by the screen filtration and centrifugation procedure followed by treatment with the hand shaker for ten minutes and enumeration by membrane filtration. The number of attached and unattached *E. coli* associated with each screen size and the number of *E. coli* present in the filtrate were summed to determine the total for the separation technique. Results from both analyses were converted to concentrations and are presented in Table 3.2.

Table 3.2. Comparison of *E. coli* and enterococci concentrations enumerated by multiple screen filtration and centrifugation pre-treatment followed by dispersion and membrane filtration and total *E. coli* and enterococci concentrations enumerated by dispersion and membrane filtration (control).

	E. coli Mean Concentration cfu 100 mL^{-1} (STD)	Percent of total *E. coli* associated with screen sizes and filtrate	Enterococci Mean Concentration cfu 100 mL^{-1} (STD)	Percent of total enterococci associated with screen sizes and filtrate
Screen size 500 µm	2.91×10^3 (5.16×10^3)	0.26%	1.158×10^3 (1.44×10^3)	0.42%
Screen size 63 µm	2.49×10^3 (1.87×10^3)	0.23%	1.846×10^3 (2.24×10^3)	0.67%
Screen size 8 µm	3.54×10^4 (7.28×10^4)	3.20%	1.4833×10^4 (2.18×10^4)	5.35%
Screen size 3 µm	6.37×10^4 (1.17×10^5)	5.78%	4.0647×10^4 (4.77×10^4)	14.67%
Filtrate	9.99×10^5 (3.46×10^6)	90.53%	2.18521×10^5 (7.04×10^5)	78.89%
Total separation technique	1.10×10^6 (3.48×10^6) a[1]		2.76493×10^5 (7.24×10^5)	
Control	8.47×10^5 (9.12×10^5) a		3.79207×10^5 (8.47×10^5)	

[1]Means followed by the same letter do not differ at the 5% level of significance according to Tukey's pairwise comparison.

Interestingly, the combination of screen filtration and centrifugation, followed by treatment with the hand shaker and membrane filtration, resulted in a 22% increase in average *E. coli* sample concentration and a 37% decrease in average enterococci sample concentration per 100 mL when compared to the average concentration in the control. It should be noted that Tween 85 was not added to samples following treatment with the hand shaker for this portion of the study because the large number of samples and unknown *E. coli* and enterococci concentrations (and thus required dilution levels) make it likely that re-plating would be necessary and the impact of longer term exposure of Tween 85 on *E. coli* and enterococci viability is unknown. Serial dilutions were conducted using a phosphate buffer solution to preserve cells and minimize the

impacts of die-off between sample collection and membrane filtration. All samples were enumerated within five days of sample collection. Analysis by ANOVA determined that no statistically significant differences existed between the control and summed (total) separation technique values (P value = 0.97 for *E. coli* and P value = 0.83 for enterococci). We had hypothesized that screen filtration and centrifugation procedures could possibly damage the cells and decrease total concentrations when compared to the samples only treated with the hand shaker as observed by the enterococci populations. One possible reason for the differences in *E. coli* and enterococci concentrations when compared to the controls could be compounded sample variability as a result of summing the bacteria associated with each screen size. The exclusion of Tween 85 from the dispersion technique would not have impacted these results because Tween was not used in analysis of either the samples treated with the separation technique or the control. Regardless, the combination of screen filtration and centrifugation did not decrease concentrations of culturable *E. coli* and the means between the technique and the control did not differ statistically for either indicator. Ninety one percent of all *E. coli* cells and 79% of all enterococci cells passed through all screens in the fractional filtration device while the 3 μm screen retained the highest percentage of cells, 5.8% and 14.7% for *E. coli* and enterococci, respectively. These results indicate the *E. coli* and enterococci in runoff from the simulated pasture plots were primarily transported in the unattached state or associated with particulates less than 3 μm in size.

3.4. Summary and Conclusions

It has been widely reported that *E. coli* and enterococci preferentially attach to sediment and organic particles; however, most NPS models used to assess microbial transport assume that pathogen indicators are transported to surface water bodies in the unattached state. An improved understanding of bacterial transport mechanisms is necessary to correctly identify sources of fecal bacteria within a watershed and implement best management practices for reduction of pathogenic organisms. While many factors can be attributed to cellular attachment, there is currently no consistency among techniques used to separate unattached and attached *E. coli* and enterococci in runoff or surface water samples.

The goal of this study was to develop an easy-to-replicate laboratory procedure for separation of unattached from attached *E. coli* and enterococci and to identify particle sizes to which *E. coli* and enterococci preferentially attach. This was accomplished by comparing previously employed methods for dispersing attached *E. coli* and enterococci from sediments and suspended particles and by validating a sequence of previously employed methods for separation of unattached and attached *E. coli* and enterococci. The hypothesis that sonification would be the best method of recovering attached bacteria from sediments was not true. Physical and chemical dispersion techniques were evaluated and a combined treatment with a hand shaker for 10 minutes followed by dilutions in 1,000 mg L^{-1} of Tween-85 significantly increased total *E. coli* concentrations by 45% (*P* value = 0.0028) and also increased enterococci concentrations by 21% (NS) when compared to a control. To separate unattached from attached fractions two commonly used techniques, fractional filtration and centrifugation, were combined and validated. Centrifugation of suspended, unattached *E. coli* DH2 1030 did not reduce suspended concentrations and the filtration and centrifugation treatments also did not reduce *E. coli* concentrations when compared to a control (*P* value = 0.97).

This method is useful to determine partitioning coefficients for NPS models and identify the particle sizes to which *E. coli* and enterococci preferentially attach. The combination of these procedures resulted in an easy-to-replicate technique that could be applied to runoff and stormwater samples. The dispersion method was applied to cowpat samples monitored seasonally to determine die-off (Chapter 4) and both the dispersion and separation methods were applied to runoff samples collected at the base of small box plots (Chapter 5) and large transport plots (Chapters 6 and 7). This method was developed using samples with high sediment and organic matter concentrations and thus is applicable to runoff samples collected from a variety of agricultural landuses including feedlots, poor pastureland or well-managed pastureland as is demonstrated in other chapters of this dissertation. While the focus of this study is on runoff from agricultural landuses, it is possible that this method could also be applied to non-agricultural runoff samples such as urban stormwater samples.

While this method appears promising, future research is recommended to ensure that the identified dispersion technique is optimal for samples dominated by different particle sizes.

Runoff or stormwater samples with lower bacterial concentrations than used in this study might be difficult to assess using this method due to the level of dilution necessary to rinse particulates from screens. This method could be improved upon by including additional screen sizes to the screen filtration technique which would identify a finer distribution of *E. coli* and enterococci preferential attachment from different landuses.

Chapter 4. Die-off of *E. coli* and Enterococci in Dairy Cowpats

4.1. Introduction

Runoff from grazed pasturelands has been well documented as a source of fecal contamination of surface waters (Doran and Linn, 1979; Doran et al., 1981; Moore et al., 1982; Soupir et al., 2006b). Animal manure applied to agricultural lands is a potential source of pathogenic organisms as over 150 human pathogens have been found in livestock manure (USEPA, 2003). During runoff events pathogenic organisms can be transported to surface waters, leading to potential waterborne disease outbreaks. *E. coli* and enterococci are the two bacterial indicator organisms thought to have a higher degree of association with outbreaks of gastrointestional illness (U.S. EPA, 1986) and are, therefore, currently the two recommended bacterial indicator organisms (U.S. EPA, 1998; U.S. EPA, 2002b). Vinten et al. (2002) and Mubiru et al. (2000) determined that the *E. coli* O157:H7 die-off rate was the same as or faster than total *E. coli*, indicating that evaluation of total *E. coli* die-off should be representative of this particular pathogenic strain.

Total Maximum Daily Load (TMDL) plans, which are heavily dependent on modeling the fate and transport of bacterial indicators, are implemented to remediate waters impaired by fecal bacteria. Accurate predictions of bacterial concentrations in sources within a watershed, such as fecal deposits or land applied manure, are necessary for simulation of in-stream concentrations. Muirhead et al. (2005) found that a statistically significant linear relationship existed between the mean number of *E. coli* cells in cowpats and the mean number of *E. coli* cells in runoff, emphasizing the need to better model indicator concentrations in fecal sources to improve predictions of pathogen indicators transported to surface waters.

The three commonly observed patterns of indicator bacteria die-off are first order decay, bacteria growth followed by first-order decay; and first order decay with variable die-off rates (Crane and Moore, 1986; Mancini, 1978). Since little is actually known about the individual influences and interactions between the many parameters affecting die-off, first order decay is most often used to express bacterial die-off (Crane and Moore, 1986; DeGuise et al., 1999; Moore et al., 1988; Wang et al., 2004):

$$N_t = N_o \exp(-kt) \quad [4.1]$$

where N_t = number of bacteria at time t; N_o = number of bacteria at time 0; k = first order die-off rate constant (day^{-1}); and t = elapsed time (day).

Pathogens and organisms with the capabilities to form spores can survive free-living in the soil for years, but most pathogens encounter conditions that prevent normal cell functions once they leave the host. Crane et al. (1983) summarized the variables that affect the survival of enteric organisms: physical and chemical properties of the soil including pH, porosity, organic matter content, texture and particle size distribution, elemental composition, temperature, moisture content, absorption and filtration properties, and availability of nutrients; atmospheric conditions including sunlight, humidity, precipitation, and temperature; biological interaction of organisms including competition from indigenous microflora, antibiotics, and toxic substances; the application method including the technique and frequency; and density of the organisms in the waste material. It is also known that some potential pathogens are free-living in the soil and may be nourished by animal wastes (Ellis and McCalla, 1978).

While many variables are thought to influence bacteria survival, temperature and moisture content of the soil or manure are considered to be key factors related to die-off. Wang et al. (2004) found that temperature (but not moisture content) had a significant effect on indicator bacteria die-off in dairy cow manure. *E. coli* O157:H7 inoculated in dairy manure survived for 63 to 70 days when incubated at 5°C with a high (74%) moisture content, compared to 49 to 56 days when incubated at 22°C and 42 to 49 days when incubated at 37°C with lower (10% at the end of the study) moisture content (Wang et al., 1996). Mubiru et al. (2000) attributed variations in *E. coli* survival inoculated in two soils to differences in available water in the soil matrix. Most rapid die-off of *E. coli* O157:H7 and *Salmonella typhimurium* were observed at 37°C when compared with 4 and 20°C in manure (Himathongkham et al., 1999). *E. coli* O157:H7 inoculated in feces in the laboratory survived best when incubated at temperatures below 23°C but also survived for shorter periods of time than manure exposed to the external environment, emphasizing the difficulty in applying laboratory results to field conditions (Kudva et al., 1998).

Several studies have detected bacterial re-growth following land application of waste. Crane et al. (1980) applied poultry manure to bare soil plots in a controlled environment. The manure was applied at approximately 36.5 and 164 t/ha on Norfolk loamy fine sand from the coastal plains and Davidson clay loam from the Piedmont region. Die-off of fecal coliforms was rapid immediately following the manure application until day seven. The first seven days were followed by a period of re-growth lasting five days and then the organism concentrations remained constant. Although the re-growth could not be attributed to a single factor, the high soil moisture content and the mild unfluctuating temperature most likely contributed to the re-growth. Laboratory studies have also found increased concentrations of fecal coliforms and *E. coli* in manures for up to a week following excretion (Conner and Kotrola, 1995; Wang et al., 1996; Wang et al., 2004).

Shortcomings have been identified with the first order decay equations frequently used to model bacterial die-off, and the need is expressed for development of new equations to better predict the bacterial growth and die-off dynamics for extended periods of time (Wang et al., 2004). We hypothesized that the first order decay equations most frequently used to predict in-field bacterial decay do not adequately describe *E. coli* or enterococci die-off in seasonally monitored cowpats. To test this hypothesis, we monitored *E. coli* and enterococci re-growth and decay patterns in cowpats applied to pasturelands. The first objective was to compare seasonal variations in decay patterns using the decimal reduction times (*D*-values) and first order decay coefficients. The second objective was to evaluate higher order approximations and weather parameters by multiple regression analysis to identify parameters impacting in-field decay and to identify an alternative technique to improve modeling of *E. coli* and enterococci fate.

4.2. Materials and Methods

Freshly excreted dairy cow feces were collected from four to seven animals at the Virginia Tech dairy facility over a 24 hour period. Freshly excreted feces were transported in barrels to the Virginia Tech Prices Fork Farm. Standard cowpats (Thelin and Gifford, 1983) were formed by mixing the feces in a cement mixer for fifteen minutes. The homogenized manure was placed in molds with a diameter of 20.3 cm (8 in) and a depth of 2.54 cm (1 in) until a weight of 0.9 kg

(2.0 lbs) was reached. Approximately 100 cowpats were formed and applied to a mowed hay field which has not previously been grazed. The number of cowpats applied to the field plot varied slightly with each seasonal application based on the availability of freshly excreted feces. Cowpats were applied to the field in a randomly distributed pattern but the distance between pats was less than two to three feet with total plot areas of less than $93m^2$ (1,000 ft^2). During the growing season, grass was mowed when it began to shelter the cowpats to prevent accelerated degradation by the presence of vegetation. Vegetation appeared to accelerate degredation of the cowpats by breaking apart the fecal material.

Cowpats were applied to four separate field plots with no history of previous manure application during the spring, summer, fall and winter seasons (Table 4.1). While many of the sampling periods extended past a single season, each sampling period is referenced by the season in which sample collection began throughout the remainder of the chapter. The Biological Systems Engineering's weather station (Belfort Instrument) and the local weather station in Blacksburg, VA (NOAA, 2006) were used to collect environmental parameters including rainfall, temperature, solar radiation and relative humidity. Table 4.1 summarizes sampling dates and weather parameters recorded during the sampling periods. Raw data including indicator concentrations, manure moisture content, temperature, solar radiation, relative humidity, and rainfall are available in Appendix B.

Table 4.1. Sampling dates and average and high weather variables recorded during the four sampling periods.

Season	Sample collection dates	Sampling period (days)	Number of sampling events	temperature (ºC)		solar radiation (MJ)		relative humidity (%)	rainfall (cm)
				high	average	high	average	average	total
Spring	April 20 – August 30	133	22	32.8	19.1	3.24	1.16	77.3	21.7
Summer	June 28 – December 13	175	25	32.8	13.6	3.07	1.04	78.8	19.3
Fall	September 21 – April 5	196	24	27.2	6.22	3.24	0.84	67.5	23.0
Winter	February 13 – July 2	135	20	31.1	12.7	3.36	1.14	65.3	18.1

Samples from cowpats were collected three to five times within the first ten days following application to the field and then weekly thereafter. More frequent sample collection during the first ten days following application was conducted to ensure observation of any re-growth patterns. Five cowpats were randomly selected for sampling during each sampling event and manure was collected from both the outer crust and moist interior of the cowpat to obtain a

representative sample of the whole cowpat. Cowpats were not re-sampled unless a portion of the cowpat remained intact and appeared undisturbed. Sampling continued until *E. coli* and enterococci concentrations were near or below the detection limit of 10^2 cfu g^{-1} wet manure based on the minimum dilutions necessary to achieve enumeration or until cowpats could no longer be located in the field. Preservation of the cowpats in the field was greatly dependent upon the season and corresponding weather conditions. Cowpats applied to the field during the spring were monitored for 133 days, summer cowpats were monitored for 175 days, and fall and winter cowpats were monitored for 196 days and 135 days, respectively (Table 4.1).

Cowpat samples were analyzed for *E. coli* and enterococci concentrations. Fecal material was diluted in phosphate buffer solution (Hach Company, Loveland, CO) at a 1:10 mass ratio. Prior to enumeration, all samples were dispersed by treatment with a hand shaker for 10 minutes (Wrist Action Shaker, Burrell Corporation, Pittsburg, PA) and serial dilutions were performed in 1,000 mg L^{-1} dilutions of Tween 85 solution. The dispersion treatment improved enumeration by separating particulate-attached and bioflocculated cells prior to enumeration. This method was previously developed and validated and more detail is available in Chapter 3. *E. coli* and enterococci concentrations were enumerated on modified mTEC and mE agar (U.S. EPA, 2000) by membrane filtration (Clesceri et al., 1998). Manure moisture content was determined gravimetrically. At least 5 g of manure were weighted (PG 5002-5 Delta Range, Mettler Toledo, Columbus, OH) and then dried (1350 F Forced Air Oven, VWR Scientific, West Chester, PA) at 103 - 105 ºC until equilibrium was reached. Samples were cooled to room temperature in a dessicator and re-weighted. Initial source manure bacterial concentrations and moisture contents are presented in Table 4.2.

Table 4.2. Original source manure properties (n=5).

Season	Moisture content (%)	*E. coli* (cfu g^{-1} dry wt.)	enterococci (cfu g^{-1} dry wt.)
Spring	82.4	4.19×10^6	2.54×10^8
Summer	81.6	5.01×10^6	7.54×10^8
Fall	84.8	5.06×10^7	4.84×10^6
Winter	85.0	9.85×10^6	5.88×10^7

Statistical analysis of data was performed using the Statistical Analysis System (SAS Institute, 2004). *E. coli* and enterococci concentrations in the cowpats over time were normalized by

natural log transformation and linear regression was performed to determine if seasonal bacterial decay would fit a first order approximation. Dummy variables were used to develop a full model representing bacterial decay during all seasons and an F-test was used to determine differences between first order decay rates. Differences between initial experimentally determined bacterial concentrations and the statistically determined intercept were also determined by an F-test. Decimal reduction times (*D*-values), the time required for a 10-fold reduction in population density (Madigan et al., 2000), were calculated from the linear slope of the seasonal die-off curves. *D*-values could be directly implemented into field scale models to help identify bacterial transport mitigation strategies (Oliver et al., 2006). Higher order decay models were evaluated and environmental factors were incorporated into the decay models by multiple regression analysis to further improve the coefficient of determination and distribution of residual plots. Attempts were made to model *E. coli* and enterococci die-off with two sets of independent variables. Set 1 included average and maximum weather parameters during the time period since the previous sample collection date: time (d), maximum temperature (ºC), average temperature (ºC), maximum solar radiation (MJ), average solar radiation (MJ), average relative humidity (%), and total rainfall (cm). Set 2 included average and maximum weather variables during the day previous to the sample collection date: time (d), maximum temperature (ºC), average temperature (ºC), maximum solar radiation (MJ), average solar radiation (MJ), average humidity (%), and total rainfall (cm). Multiple regression analysis was conducted using the REG procedure in SAS and the final criteria to be included in the final model were selected based on the C_p statistic (Ott and Longnecker, 2001). A t-test was used to determine statistically significant slopes and intercepts.

4.3. Results and Discussion

Initial source bacterial concentrations for *E. coli* and enterococci averaged 1.78×10^7 cfu g^{-1} dry manure (SD = 2.67×10^7 cfu g^{-1} dry manure) and 2.97×10^8 cfu g^{-1} dry manure (SD = 3.35×10^8 cfu g^{-1} dry manure), respectively. Average *E. coli* concentrations were similar among the four seasonal studies but enterococci concentrations in fresh fecal deposits were slightly higher during spring and summer studies (Figure 4.1) with summer concentrations being a magnitude of about 2logs greater than fall concentrations (Table 4.1). Previous studies have reported similar bacterial levels in fresh dairy manure and have also noted variability in fecal indicator

concentrations among fresh manure samples. Wang et al. (2004) found that *E. coli* concentrations in fresh dairy manure averaged 7.08×10^{6} cfu g^{-1} dry manure while Muirhead et al. (2005) reported *E. coli* concentrations in fresh dairy cow fecal material ranged from 10^{5} to 10^{7} g^{-1} dry manure. Slightly higher initial concentrations of *E. coli* and enterococci observed in this study, compared with the values reported by some other investigators could be partially due to the dispersion treatment (hand shaker for 10 minutes followed by serial dilution in Tween-85 1000 mg L^{-1}) used to release cells from organic particulates and disperse bioflocculated cells. Separation of clumped cells through use of a dispersion treatment allows for greater formation of individual colonies during the membrane filtrations procedure, thus resulting in a higher number of colony forming units (cfu).

4.3.1. Seasonal Bacterial Re-growth and Die-off Trends

Cowpats were applied to field plots and monitored for *E. coli* and enterococci concentrations within 24-hours following excretion. Monitoring continued until the lower detection limit of 10^{2} cfu g^{-1} wet manure was reached or the cowpats had disintegrated to the point that they could no longer be located in the field. Figure 4.1 presents re-growth and die-off trends for *E. coli* and enterococci in cowpats applied to the field during spring, summer, fall and winter seasons. Bacterial concentrations in Figure 4.1 are presented in a dry weight basis to remove the impacts of moisture content and rainfall on decay rates.

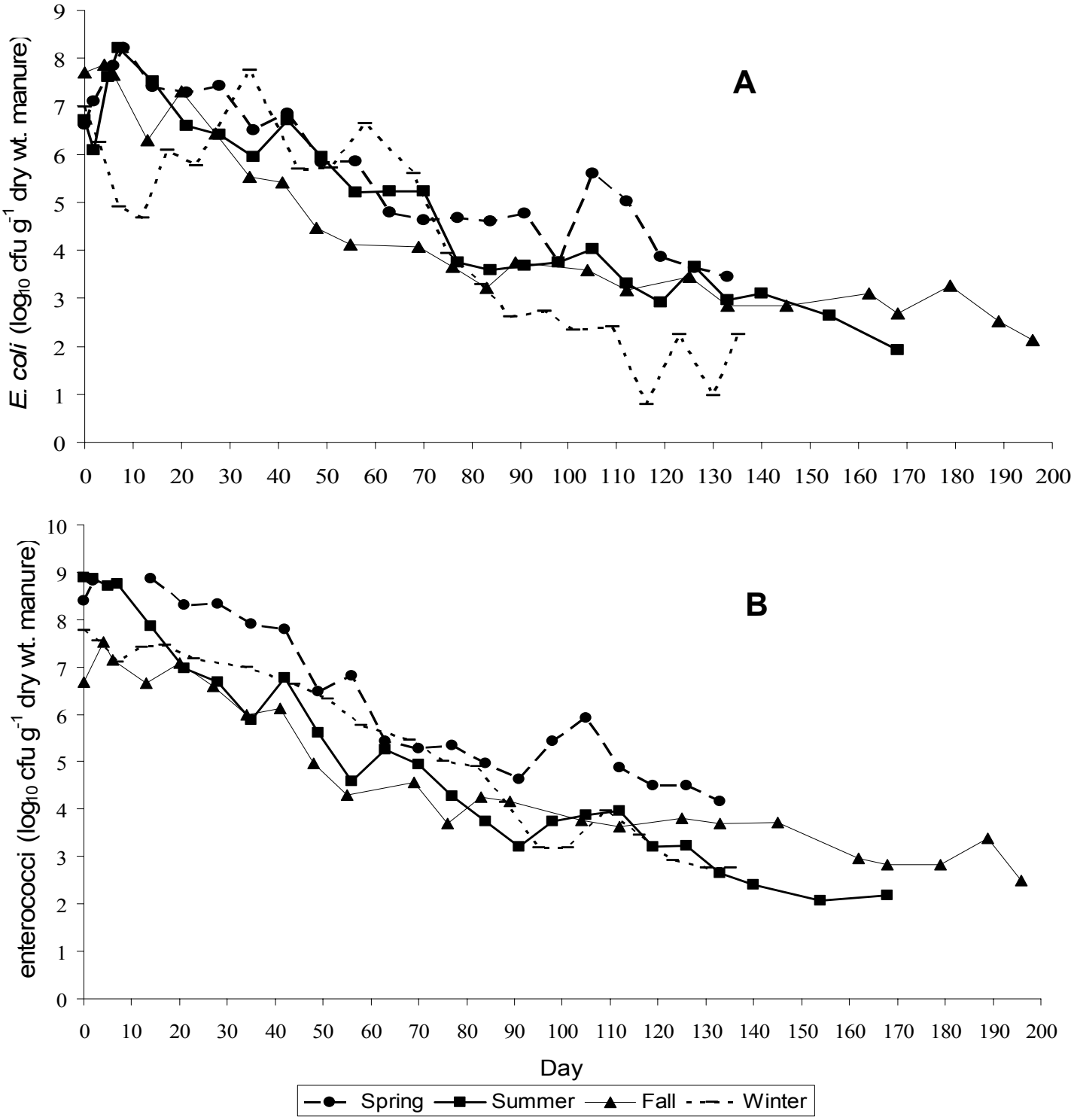

Figure 4.1. Seasonal die-off patterns of *E. coli* (A) and enterococci (B)

A fresh fecal cowpat provides an optimal environment (high moisture content, abundance of nutrients) for *E. coli* and enterococci growth and survival so it was not unexpected when both

indicators exhibited re-growth immediately or within the first few days after their land application. Re-growth appeared to vary by both indicator and season. *E. coli* concentrations peaked at days 7, 7, and 4 during spring, summer and fall sampling periods, respectively while enterococci peaked at days 13 and 4 during spring and fall sampling periods, respectively. Re-growth was not observed in enterococci concentrations monitored during the summer sampling period. Mixed results were observed from winter sampling periods as both indicators exhibited initial die-off. *E. coli* concentrations experienced about a 2log decrease following deposition; concentrations increased starting on day 12 with a peak of 5.73×10^{7} cfu g^{-1} dry wt occurring on day 34. followed by a second 2log decrease and re-growth pattern with a secondary peak of 4.43×10^{6} cfu g^{-1} dry wt (not exceeding initial freshly excreted *E. coli* concentrations) on day 58. Enterococci concentrations experienced a slight decrease between days zero and seven to $1.29\times\times10^{7}$ cfu g^{-1} dry wt followed by re-growth until day 17 to 2.91×10^{7} cfu g^{-1} dry wt, followed by gradual decay. Re-growth is rarely accounted for in bacterial fate and transport modeling (Benham et al., 2006; Jamieson et al., 2004; Pachepsky et al., 2006; Tian et al., 2002), but it has been frequently observed in laboratory and field studies. Many researchers have observed a re-growth period of just a few days (Conner and Kotrola, 1995; Himathongkham et al., 1999; Thelin and Gifford, 1983; Wang et al., 1996; Wang et al., 2004) but increased concentrations of fecal coliforms in poultry manure applied to bare plots have been observed for up to 12 days (Crane et al., 1980). In situations where heavy rainfall occurs shortly after the application of manure to the agricultural lands, the re-growth can play a major role in determining the concentration of bacteria in runoff.

Cowpats applied to the field in the spring (April) and winter (February) seasons were monitored for the shortest period of time, 133 and 135 days, respectively, while cowpats applied to the field in the fall (September) were monitored for nearly 200 days (Table 1). During the fall sampling period, the lowest average temperatures (6.22 ºC) and solar radiation (0.84 MJ) values were observed. Cool temperatures seemed to preserve both the fecal cowpat in the field and bacterial concentrations as exhibited by the longer monitoring period. Highest average temperatures (19.1 ºC) and average solar radiation values (1.16 MJ) were observed during the spring sampling period, while the maximum solar radiation reading (3.24 MJ) occurred during the winter

monitoring season (which continued into July). Quickest decay of the cowpats occurred during warm temperatures when vegetation and insects hastened the disappearance of the fecal deposits.

While collection of all seasonal cowpat samples ceased after a maximum of 200 days, *E. coli* and enterococci (in levels below the detection limit or in cowpats that could no longer be visually located) were still present in the field and could still contribute loadings to surface waters during runoff events. Kress and Gifford (1984) found that 100-day-old cowpats released fecal coliform concentrations of 4,200 cfu 100 mL^{-1} and others have concluded that cowpats could remain a source of fecal contamination even long after removal of cattle from grazed pasturelands (Howell et al., 1995; Jawson et al., 1982). End-of-study concentrations of *E. coli* ranged from 81 to 2.8×10^2 cfu g^{-1} dry wt. manure and enterococci concentrations ranged from 8.5×10^2 to 8.9×10^5 cfu g^{-1} dry wt. manure. Therefore, while this study examined fecal bacteria concentrations in cowpats for an extended period of time, it is possible that degraded cowpats or cowpats with bacterial concentrations below the lower detection limit might still remain a source of fecal contamination of surface waters. It is also possible that bacteria accumulate in the soil surrounding the cowpats and act as an additional source of fecal contamination of surface waters; however, the soils surrounding the cowpats were not sampled for this study.

4.3.2. First-order Approximations

Visual observation of die-off trends over time indicates that first order decay would not sufficiently estimate bacterial concentrations (Figure 4.1) but because this approach is commonly used by researchers, the fit of first order models was examined. The bacterial concentrations during each season were fit as a function of time by linear regression (SAS Institute, 2004) to estimate *E. coli* and enterococci die-off rate constants, and results are presented in Table 4.3. Additional model details and residual plots are available in Appendix C. First-order models do not capture re-growth, thus in some cases the statistically determined intercept overestimated the experimental initial bacterial concentration in manure during the seasons when re-growth was observed (Figure 4.1). Using these equations to estimate decay could result in lower than observed bacterial concentrations because the re-growth period is neglected. While the observed re-growth period challenges the fit of first-order decay models, the values obtained for the

coefficient of determination would lead many to classify the fit of the first order model as adequate (R^2 values range from 0.70 to 0.90).

Table 4.3. *E. coli* and enterococci seasonal decimal reduction times (*D*-values) and die-off rate coefficients.

	***D*-values (day)**	**k (day^{-1})**	**R^2**	**P-values[3]**
***E. coli*[1]**				
Spring	33	0.0748 b[2]	0.70	<.0001
Summer	29	0.0788 b	0.84	<.0001
Fall	40	0.0581 c	0.76	<.0001
Winter	26	0.0995 a	0.74	0.0799
enterococci[1]				
Spring	32	0.0759 a[2]	0.71	0.1745
Summer	24	0.0978 b	0.89	<.0001
Fall	41	0.0557 a	0.81	0.0957
Winter	27	0.0951 b	0.90	0.4946

[1]Dry weight basis
[2] k-values for each indicator followed by the same letter do not differ at the 5% level of significance.
[3]P-values ≤ 0.05 indicate statistically significant differences between initial experimentally determined bacterial concentrations and the statistically determined intercept

Comparison of *D*-values and die-off rate constants could assist in evaluating the seasonal impacts on *E. coli* and enterococci decay. The *E. coli* and enterococci *D*-values were very similar with a 10-fold reduction in both populations occurring within five days of each other. This indicates that similar on-farm management strategies to reduce indicator populations should apply to both *E. coli* and enterococci. *D*-values were greatest during the fall sampling period for both indicators but lowest during the winter sampling period for *E. coli* and the summer sampling period for enterococci. While the winter sampling period began during low-temperature conditions (February), sample collection ceased during the warmest part of the year (July), which could be partially responsible for the higher die-off rate coefficients observed during the winter monitoring period. Die-off rate constants were highest during the winter monitoring period for *E. coli* and summer monitoring period for enterococci; however, enterococci decay rates did not differ statistically during the winter and summer monitoring periods. Lowest decay rates occurred during the fall monitoring period for both indicators, but enterococci decay rates were not statistically different between the spring and fall monitoring periods.

Pasture was mowed periodically during the growing season which likely reduced the bacterial survival time, most likely through its effect on drying rates and increased exposure to solar radiation (Taylor and Burrows, 1971). Van Donsel et al. (1967) found much higher reduction times of indicator organisms when *E. coli* and *Streptococcus faecalis* cells were cultured and then poured onto outdoor soil plots. The 90% reduction times (*D*-values) varied seasonally, ranging from 3.3 days in summer to 13.4 days in fall, while the *Streptococcus faecalis* 90% reduction times ranged from 2.7 days in summer to 20.1 days in the winter (VanDonsel et al., 1967). The protective environment provided by fecal cowpats in this study greatly extends survival of indicator organisms exposed to the external environment when compared to previous studies.

Several researchers have found first-order decay to adequately describe die-off kinetics of fecal indicators from agricultural sources (Crane and Moore, 1986; Himathongkham et al., 1999; Oliver et al., 2006), however, most die-off studies have been conducted under laboratory conditions making it difficult to compare these laboratory-based findings to the field-based findings presented in this article. In a laboratory investigation, Wang et al.(2004) found that first order die-off rate coefficients sufficiently described *E. coli* decay in freshly excreted dairy cow manure maintained at three moisture contents and three temperatures, but only after day three and for the following three week period. Die-off rate coefficients increased as temperature increased with values averaging 0.11 d^{-1} at 4ºC, 0.20 d^{-1} at 27ºC, and 0.32 d^{-1} at 41ºC. *E. coli* die-off rate coefficients in freshly deposited cattle feces (steers) incubated at 15ºC averaged 0.054 d^{-1} (25% moisture) and 0.058 d^{-1} (50% moisture) over a 111 day sampling period (Oliver et al., 2006). *E. coli* O157:H7 decay rates were 0.111 d^{-1} at 4ºC (75% RH), 0.046 d^{-1} at 20ºC (50% RH) and 0.112 d^{-1} at 37ºC (30% RH) in the top layer of fresh dairy manure and 0.054 d^{-1}, 0.074 d^{-1}, and 0.279 d^{-1} at 4, 20, and 37ºC, respectively, in the middle and bottom layers of fresh dairy manure (Himathongkham et al., 1999). In another laboratory investigation, Mubiru et al. (2000) inoculated *E. coli* O157:H7 and nonpathogenic *E. coli* strains in two soil types and enumerated concentrations weekly for an 8 week period. The decay rate constants for a first order approximation ranged from 0.09 d^{-1} to 0.17 d^{-1}, varying slightly by strain and soil type, with high regression coefficients ranging from 0.89 to 0.93. A two-staged first order function improved the fit and the initial mortality rates was much higher 0.15 d^{-1} to 0.25 d^{-1} than the

second stage 0.05 d^{-1} to 0.08 d^{-1}. Mortality rates of fecal coliforms and fecal streptococci have also been adequately described by a two-stage exponential decay model (Zhai et al., 1995).

E. coli die-off rate coefficients developed under laboratory conditions at constant temperatures from freshly excreted dairy manures are generally higher (Wang et al., 2004) than the field-based seasonal die-off rate coefficients presented here. *E. coli* die-off rate coefficients observed during the fall sampling period are most comparable with results from the Oliver et al. (2006) study conducted on freshly excreted steer feces; however, average temperature conditions of 6.22ºC (Table 4.1) differ from the 15ºC incubation temperature in the laboratory study. Mostaghimi et al. (1999) monitored *E. coli* and fecal coliform concentrations in lactating, heifer and beef cowpats deposited onto grazed pastureland in late April and mid-July and determined seasonal impacts to have a greater influence over bacterial decay than cattle species. If water quality models continue to use first-order decay to predict in-field bacterial concentrations, in-field die-off rate coefficients should be developed for utilization in these models.

4.3.3. Multiple Regression Analysis to Approximate Seasonal Die-off Patterns

Multiple regression analysis was conducted to find the best approximation of die-off in dairy cowpats. Higher order and two-staged approximations were first examined before addition of weather and moisture parameters to obtain best-fit models. Difficulties in approximating indicator decay rates based on two-staged decay included estimating the break point between the end of re-growth and the beginning of decay. As shown in Figure 4.1, re-growth typically only occurred within the first seven days following excretion except during the winter monitoring period; however, a fit of the re-growth period produced poor regression coefficients ($R^2 = 0.36$ for the first stage of *E. coli* in studies beginning in spring, summer and fall, Appendix C) Because of the difficulties in establishing break points for two-staged decay and availability of insufficient data to adequately determine this break point in predictive models, higher order approximations were examined. Higher order approximations excluding weather parameters resulted in increased regression coefficients (R^2 ranging from 0.81 to 0.91 for *E. coli* and R^2 ranging from 0.74 to 0.95 for enterococci, Appendix C) and improved distribution about zero in residual plots. An F-test was used to examine if statistically significant differences existed between the statistically determined intercept and the initial bacterial concentrations in cowpats

with mixed results. Enterococci initial concentrations did not differ from the intercept (*p values* ranged from 0.1471 to 0.4946) except for the spring monitoring period (*p value* = 0.008) and *E. coli* initial concentrations were statistically different for studies beginning in the fall (*p value* = 0.0090) and winter (p value = <0.0001). While regression coefficients from first-order decay models were deemed reasonable (Table 4.3), examination of residual plots indicated that higher order approximations and the inclusion of weather data were both necessary to eliminate the trends present in residual plots and improve predictive equations. Figure 4.2 presents residual plots for *E. coli* during the spring for a) first-order decay model and the b) higher order approximation model including weather variables.

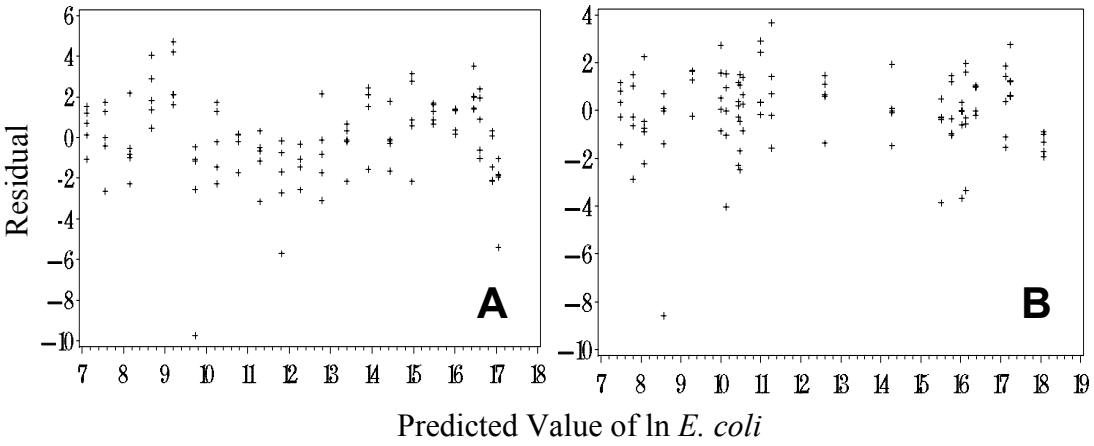

Figure 4.2. Residual plots for E. coli during the spring season for A) first-order decay and B) higher order approximations combined with weather variables.

Crane and Moore (1986) summarized past investigations of bacterial die-off and identified relationships between environmental and physical parameters to bacterial survival as the greatest need for future research. They acknowledged that variability in reported die-off rate coefficients was likely due to the impact of environmental factors on bacterial decay but also concluded that a first-order model accurately described bacterial die-off when considering all conditions. The authors were unsuccessful in correlating environmental parameters and die-off rate coefficients because many environmental factors increase decay only under extreme conditions (non-linear relationships) and oftentimes investigators do not measure certain parameters that might be responsible for variability in die-off rates.

This study monitored a range of environmental parameters in an attempt to more clearly identify which factors influence bacterial decay. Although these parameters are specific to a single field

study, they provide information on which weather variables should be considered when modeling decay of bacterial indicators in NPS models. Higher order approximations including weather variables are presented in Table 4.4 and Figure 4.3 presents an example of the predicted and observed *E. coli* and enterococci decay during the fall season. Predicted values were calculated using the equations presented in Table 4.4 for *E. coli* and enterococci decay beginning during the fall monitoring period. Residual plots associated with each model and the predicted and observed *E. coli* and enterococci decay figures for the remaining seasons are available in Appendix C. The two weather parameters consistently identified as significantly improving predictions of *E. coli* and enterococci decay by increasing regression coefficients and distribution about zero of residual plots were temperature and solar radiation. The impact of temperature on bacterial decay has been previously well documented and solar radiation has also been identified as an important factor associated with bacterial decay (Crane et al., 1983; Taylor and Burrows, 1971). Inclusion of weather parameters improved either the regression coefficient or residual plot distribution in all models except for the *E. coli* and enterococci die-off during studies beginning in the fall. It is likely that cooler temperatures and lower solar radiation recorded during the fall and winter months were not extreme enough to contribute significantly to bacterial decay.

Table 4.4. Best estimates of seasonal *E. coli* and enterococci die-off by higher order approximation and including weather parameters.

Seasonal Die-off Models: *E. coli*										
ln *E. coli* (dry wt) die-off: Spring $R^2 = 0.8116$			**ln *E. coli* (dry wt) die-off: Summer $R^2 = 0.9091$**			**ln *E. coli* (dry wt) die-off: Fall $R^2 = 0.9125$**			**ln *E. coli* (dry wt) die-off: Winter $R^2 = 0.8266$**		
Variable	**Parameter estimate**	***p* value**	**Variable**	**Parameter estimate**	***p* value**	**Variable**	**Parameter estimate**	***p* value**	**Variable**	**Parameter estimate**	***p* value**
Intercept	4.459	0.2077	Intercept	28.85	<.0001	Intercept	18.22	<.0001	Intercept	19.48	<.0001
Time	0.3933	<.0001	Time	-0.3232	<.0001	Time	-0.2226	<.0001	Time	0.4735	0.0006
$Time^2$	-0.01793	<.0001	$Time^2$	3.13×10^{-3}	0.0007	$Time^2$	1.53×10^{-2}	<.0001	$Time^2$	-7.08×10^{-3}	<.0001
$Time^3$	2.016×10^{-4}	<.0001	$Time^3$	-9.78×10^{-6}	0.0078	$Time^3$	-3.72×10^{-6}	<.0001	$Time^3$	2.796×10^{-5}	<.0001
$Time^4$	-7.075×10^{-7}	<.0001	Temp PWA	0.6495	<.0001				Temp PWA	-0.2412	0.0013
Temp PDH	-0.4917	0.0194	SR PWA	-5.918	0.0004				SR PWH	-4.991	0.0263
Temp PDA	0.6652	0.0035	RH PWA	-0.1888	<.0001				rainfall PWT	0.2848	0.0264
SR PDA	4.546	0.0013	rainfall PWT	0.2999	0.0229						
RH PDA	0.09619	0.0092									
Seasonal Die-off Models: enterococci										
ln enterococci (dry wt) die-off: Spring $R^2 = 0.8296$			**ln enterococci (dry wt) die-off: Summer $R^2 = 0.9656$**			**ln enterococci (dry wt) die-off: Fall $R^2 = 0.9062$**			**ln enterococci (dry wt) die-off: Winter $R^2 = 0.9042$**		
Variable	**Parameter estimate**	***p* value**	**Variable**	**Parameter estimate**	***p* value**	**Variable**	**Parameter estimate**	***p* value**	**Variable**	**Parameter estimate**	***p* value**
Intercept	3.405	0.3096	Intercept	20.071	<.0001	Intercept	15.375	<.0001	Intercept	17.785	<.0001
Time	0.243	0.0011	Time	-0.320	<.0001	Time	0.266	0.0021	Time	-0.0957	<.0001
$Time^2$	-9.55×10^{-3}	0.0002	Sq_time	2.99×10^{-3}	<.0001	$Time^2$	-0.0175	<.0001	rainfall PDT	0.161	0.0577
$Time^3$	1.006×10^{-4}	0.0004	Cu_time	-1.056×10^{-5}	<.0001	$Time^3$	3.197×10^{-4}	0.0002			
$Time^4$	-3.393×10^{-7}	0.0009	Temp PDH	0.139	0.0004	$Time^4$	-2.71×10^{-6}	0.0007			
Temp PDH	0.709	<.0001	SR PDA	-1.892	<.0001	$Time^5$	1.099E-8	0.0020			
Temp PDA	-0.860	<.0001				$Time^6$	-1.719×10^{-11}	0.0041			
SR PDH	1.120	0.0183									
RH PDA	0.108	0.0009									

RH, relative humidity; SR, solar radiation; PWA, previous week average; PWH, previous week high; PDT, previous daily total; PWT, previous weekly total; PDA, previous day average; PDH, previous day high

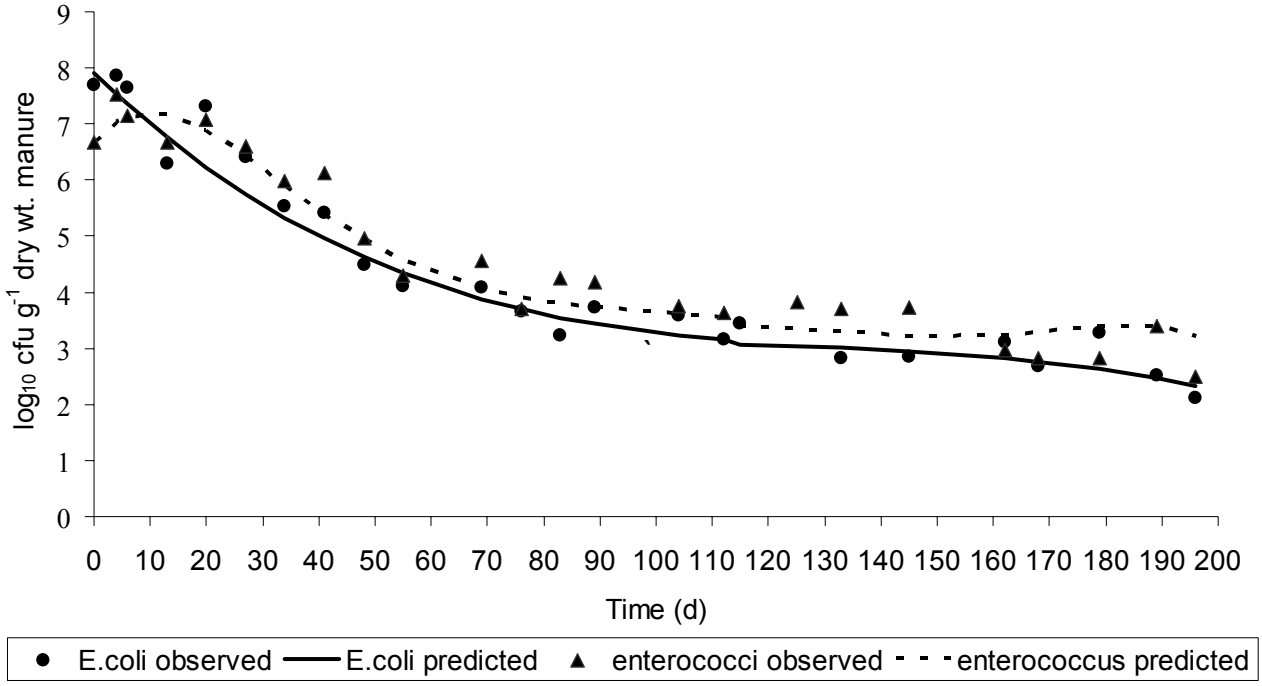

Figure 4.3. Example of predicted and observed *E. coli* and enterococci decay. Predicted values are calculated using the equations presented in Table 3 for *E. coli* and enterococci decay beginning during the fall monitoring period.

The impact of moisture content on the die-off of *E. coli* and enterococci is unclear in the literature. Some studies have found that low moisture content will promote die-off (Entry et al., 2000; Sjogren, 1994; Wang et al., 2004) while others have found little or no effect (Oliver et al., 2006; Ritchie et al., 2003; Vinten et al., 2002). In this study, moisture content of the manure was not included in the dry-weight-based decay models because it is a parameter necessary to calculate bacterial concentrations in dry-weight manures. However, two factors impacting moisture content, rainfall and relative humidity, were both included as statistically significant model parameters in most of the presented models, indirectly indicating that moisture is a factor in bacterial decay. Moisture content was included as a variable in separately developed wet-based manure die-off models (Soupir, unpublished data) and was identified as a significant parameter for inclusion in *E. coli* spring, summer and fall models (p-value = 0.0173) and enterococci spring, summer, fall and winter models (p-value = 0.0026). Kress and Gifford (1984) reported that declines in peak fecal coliform counts occurred after a second rainfall simulation, suggesting that bacteria available for transport were washed from the feces during the first simulation. While this study sampled the entire cowpat, it is

likely that the decrease in fecal indicators present in the surface crust of the cowpat following rainfall events was reflected in the total bacterial count.

This research presents a different method to capture the re-growth and die-off dynamics of *E. coli* and enterococci over an extended period of time. While a field-based study makes it difficult to assess the direct impact of individual environmental factors on bacterial decay, inclusion of temperature and solar radiation parameters consistently improved predictive capabilities of bacterial decay models during all monitoring periods except fall which covered the period of September to April. Moisture also indirectly seemed to impact bacterial decay through the inclusion of relative humidity or rainfall in most models. Clearly, higher order approximations and the inclusion of weather variables improves predictions of bacterial decay when compared to first order approximations; however, caution is advised prior to direct implementation of these procedures into NPS models unless similar field conditions are being simulated. Cowpats examined in this study were undisturbed and rotational or continuous grazing systems will often allow for repeated grazing and thus trampling of the cowpats before previous deposits disappear, likely increasing decay rates through additional environmental exposure. Additional field-based monitoring of bacterial decay and weather parameters is necessary to represent the many different fecal sources present within a watershed and to further monitor the impacts of seasonal and weather parameters over time.

4.4. Summary and Conclusions

Standard cowpats were formed and applied to mowed hayfields during spring, summer, fall and winter seasons to test the hypothesis that first order decay equations most frequently used to predict in-field bacterial decay do not adequately describe *E. coli* or enterococci die-off in seasonally monitored cowpats. First order approximations were used to determine die-off rate coefficients and decimal reduction times (*D*- values). Seasonal variations in decay patterns were assessed. Higher order approximations and weather parameters were evaluated by multiple regression analysis to identify environmental parameters impacting in-field *E. coli* and enterococci decay.

Populations of *E. coli* and enterococci both exhibited re-growth, which seemed to differ by both indicator and season, immediately or within the first few days after field application. In general, cool temperatures preserved bacterial concentrations while increased decay occurred during warm

temperatures when vegetation and insects hastened the disappearance of the fecal deposits. First order kinetics approximated *E. coli* and enterococci decay rates with regression coefficients ranging from 0.70 to 0.90; however, when indicators exhibited re-growth patterns, the first order approximations overestimated initial concentrations present in freshly excreted manures. Die-off rate constants were greatest in cowpats applied to pasture during late winter and monitored into summer months for *E. coli* ($k = 0.0995\ d^{-1}$) and applied to the field during the summer and monitored until December for enterococci ($k = 0.0978\ d^{-1}$). Decay rates were lowest in cowpats applied to the pasture during the fall and monitored over the winter ($k = 0.0581\ d^{-1}$ for *E. coli* and $k = 0.0557\ d^{-1}$ for enterococci). *E. coli* and enterococci *D*-values were very similar with a 10-fold reduction in both populations occurring within five days of each other. The *D*-values were greatest during the fall monitoring period (40 and 41 days for *E. coli* and enterococci, respectively).

Higher order approximations and addition of weather variables improved regression coefficients to values ranging from 0.81 to 0.97 and improved distribution of residual plots for both indicators was noted. The addition of weather variables improved predictability of regression equations for all seasonal studies except the fall monitoring period. It is possible that the weather conditions that occurred during the fall monitoring period were not extreme enough to contribute significantly to bacterial decay. Statistically significant variables included in the models predicting bacterial decay during the spring, summer and winter monitoring periods were temperature, solar radiation, rainfall and relative humidity.

Die-off rate coefficients previously reported in the literature are generally higher than the field-based seasonal die-off rate coefficients presented here. New die-off rate coefficients should be developed in the field for implementation in decay models if first-order decay models continue to be used to predict in-field bacterial concentrations. Comparable *E. coli* and enterococci seasonal *D*-values suggest that similar on-farm management strategies should reduce both *E. coli* and enterococci indicator populations. This study recommends higher order approximations and the inclusion of weather variables to better capture re-growth and die-off trends over extended periods of time.

Chapter 5. Attachment of Bacterial Indicators to Particulates in Runoff from Three Virginia soils

5.1. Introduction

Limited understanding of how microbes are released from fecal matter and transported along overland flow pathways results in high uncertainty in bacterial fate and transport models (Collins and Rutherford, 2004). Specifically, little is known about microbial partitioning between the freely suspended and particulate attached phases (Oliver et al., 2007b) and data on the partitioning between these two phases are not yet available (Benham et al., 2006; Collins and Rutherford, 2004; Jamieson et al., 2004). The limited research conducted on partitioning of fecal bacteria indicates that cells are transported primarily in the unattached state. Muirhead et al. (2005) studied the transport state of *E. coli* cells by placing cowpats and fecal-material-soil mixtures on a metal tray 250 mm long and 200 mm wide, and runoff was created by placing a rainfall simulator nozzle 250 cm above the soil. On average only 8% of *E. coli* cells attached to sediment particles and most cells were not bioflocculated (attached to one another to form aggregates), but instead transported in runoff as single cells. In-stream stormwater partitioning studies have determined that on average, between 20 to 35% of microorganisms are attached to sediments (Characklis et al., 2005; Jeng et al., 2005; Krometis et al., 2007). Re-suspension of bottom sediments as a source of attached bacteria (Jamieson et al., 2003) might distinguish results between stormwater partitioning studies and overland flow partitioning studies; however, suspension of sediment and bacteria from the soil surface is likely a source of attached bacteria during overland flow.

Low attachment of *E. coli* to particulates in runoff from lands receiving fecal deposits might be explained by the presence of organic matter and carbon in the fecal material or by limitations in the availability of attachment sites. Guber et al. (2007) found that the presence of manure colloids decreased attachment of fecal coliforms to clay, silt, and organic coated sand particles when compared to particulate attachment in the absence of manure particulates. Similarly, equilibrium batch experiments examining the effect of dairy manure on *E. coli* attachment to soils found increasing manure content from 0 to 40 g L^{-1} resulted in decreased attachment (Guber et al., 2005a). Guber et al. (2005b) hypothesized that the decreased bacterial attachment to particulates in the presence of manures could be caused by a variety of factors including competition between bacteria

and dissolved organic matter for attachment sites on soil, modification of soil mineral surfaces by soluble manure constituents, or modification of bacterial surfaces by dissolved organic matter.

Relationships between bacterial attachment and suspended sediments have been primarily observed in samples collected in streams, lakes and estuaries. Characklis et al. (2005) identified potential relationships between in-stream fecal coliform partitioning and particle number concentrations and Fries et al. (2006) found that concentrations of *E. coli* and enterococci increased along with particulates in suspension following storm events. Goulder (1977) evaluated the attached and unattached bacteria in an estuary with high concentrations of suspended sediment. He found that bacteria and the concentration of suspended solids were highly correlated; implying that attached bacteria concentrations might be controlled by the suspended solids concentrations. However, there were many particles on which no bacteria were attached, so there did not seem to be a shortage of attachment sites. Greater than 80% of fecal indicator organism were found to be associated with suspended sediments at two separate locations in the Chesapeake Bay as determined by centrifugation (Sayler et al., 1975). However, correlation between the suspended sediment concentrations and the bacteria associated with particulate matter was not observed. An et al. (2002) found that *E. coli* concentrations increased with depth at Lake Texoma due to the association of bacteria with sediments. A direct relationship was found between *E. coli* concentrations and gasoline sold at a marina, indicating recreational boating activity was responsible for the resuspension of sediments and thus the associated *E. coli*. Attachment of fecal bacteria to sediments allows for increased survival and potential for waterborne disease outbreak. Howell et al. (1996) found that sediment type significantly affected fecal coliform mortality, with significantly lower mortality rates in clay-sized sediments than in coarser sediments.

High amounts of sediment transport is common from grazed and trampled streambanks and thus the abundance of readily available attachment sites could result in high association of pathogen indicators with particulates. This study examines the state of *E. coli* and enterococci transport from three bare soil types receiving cowpat treatments. Relationships between bacterial partitioning and total suspended solids (TSS) phosphorous and carbon transport were examined and particles sizes to which cells preferentially associated were identified by fractional filtration and centrifugation methods. We hypothesized that the *E. coli* and enterococci partitioning would be related to the

dissolved/suspended phosphorus ratio and that the majority of cells would be associated with particles retained by the 8 μm filter.

5.2. Materials and Methods

Small, portable box plots were used to measure *E. coli* and enterococci attachment to particulates in runoff from three bare Virginia soils. Fifteen boxes were packed with soils collected from the Ap horizon. Five boxes were packed with Grosclose silt loam (Creggar et al., 1985); five boxes were packed with Levy silty clay loam (Reber et al., 1981); and the remaining five boxes were packed with Eunola loamy fine sand (Reber et al., 1981). Soils were compacted by hand tamping at saturation and leveled at least 24-hrs prior to each rainfall simulation. Box plots were left un-vegetated to create a condition where large volume of runoff and erosion are produced. 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. Soil samples were dried, sieved (2 mm) and stored prior to their analysis. Soils were analyzed for Mehlich -1 P by an inductively coupled plasma atomic emission spectrometer; organic matter was analyzed using a modified Walkley-Black method; pH was determined using a 1:1 soil to distilled water ratio and solid state pH meter and cation exchange capacity was estimated by summation (Mullins and Heckendorn, 2005).

Table 5.1. Soil properties.

Soil	Mehlich-1 P	Organic Matter	pH (1:1 water)	Cation Exchange Capacity	Particle Size Distribution		
					Sand	Silt	Clay
	mg kg^{-1}	%		meq 100 g^{-1}	----------%----------		
Eunola loamy fine sand	18	0.7	6.78	1.6	81.5	7	11.5
Grosclose silt loam	7	2.6	5.77	6.1	28.8	51.2	20
Levy silty clay loam	3	2.0	4.65	4.8	60.8	12.8	26.4

Fresh dairy cattle fecal deposits were collected at the Virginia Tech dairy facility and a single standard cowpat (Thelin and Gifford, 1983) was applied to each of the plots. Standard cowpats were formed by mixing the manure in a cement mixer for fifteen minutes. The homogenized manure was placed in molds with a diameter of 20.3 cm (8 in) and a depth of 2.54 cm (1 in) until a weight of 0.9 kg (2.0 lbs) was reached and applied to the central section of the plots. Manure samples were analyzed by the Clemson Agricultural Service Laboratory. Water soluble P, (2.06 g kg^{-1}),was determined by the method proposed by Sharpley and Moyer (2000). The pH, (5.6), was measured

potentiometrically in a 1:2 manure/water slurry (Peters et al., 2003). Average moisture content of fresh manure samples was 83.1%. *E. coli* and enterococci concentrations in fresh fecal material averaged 1.56×10^{7} cfu g^{-1} and 1.72×10^{6} cfu g^{-1}, respectively.

Figure 5.1. Application of a standard cowpat to portable box plots packed with three different Virginia soils.

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. 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 intensity averaged 9.0 cm hr^{-1}. Rainfall simulations were first conducted within 24 hours of the manure application to represent a condition where rainfall occurs soon after manure application (run 1). Simulated rain was applied until 30 minutes after the initiation of runoff (SERA-17, 2005). Grab samples were collected 10, 20, and 30 minutes after the start of runoff. Following collection of the 30 minute sample, the rainfall simulation ended. Soil boxes were placed outside on mowed pastureland and a second set of simulations (run 2) was conducted about 80 days after the first set of simulations to examine bacteria and nutrient release patterns from aged fecal deposits.

Figure 5.2. Tlaloc 3000 portable rainfall simulator was used to apply rain to the box plots and samples were collected from the base of the plots 10, 20, and 30 minutes after the onset of runoff.

5.2.1. Bacterial Partitioning and Enumeration

Collected samples were transported to the laboratory immediately following the end of the rainfall simulation and analyzed for *E. coli* and enterococci. Partitioning of pathogen indicators between attached and unattached phases was achieved by fractional filtration followed by centrifugation (Chapter 3). Fractional filtration has been used previously to identify particle sizes to which bacteria are attached (Auer and Niehaus, 1993; Schillinger and Gannon, 1985), and a filter pore size of 8 µm has been identified as a viable method to separate attached and free bacteria (Gordon et al., 2002; Henry, 2004; Mahler et al., 2000; Qualls et al., 1983). The presence of sediments and organic particles in runoff from agricultural lands makes it very likely that the filters could clog and retain free cells, resulting in a higher fraction of cells being classified as attached. To assess the retained, unattached cells, we rinsed the screens and filters with phosphate buffered water (Hach Company, Loveland, CO) and then centrifuged the re-suspended solution.

A number 35 mesh screen (Bel-Art Products, Pequannock, NJ) was used to retain particles larger than coarse sand (>500 µm) and a number 230 mesh screen was used to retain medium, fine, and very fine sand (63 - 500 µm). An 8 µm filter (Poretics, Polycarbonate, GE Water and Processes

Technologies) was used to retain fine, medium, and coarse silt particles and a 3 μm filter (Nuclepore Track – Etch Membrane Filtration Products, Whatman) was used to retain clay and very fine silt particles. Throughout the study few particulates passed thorough the 8 μm filter. Following filtration, the retained solids were re-suspended in phosphate buffered water and centrifuged (Avanti J-25I, Beckman Coulter, Fullerton, CA) at 4,700 rpm for 15 seconds (Huysman and Verstraete, 1993; Lago, 2005). The filtrate and supernatant was enumerated for *E. coli* and enterococci concentrations on Modified mTEC and mE agar (U.S. EPA, 2000) using membrane filtration (Clesceri et al., 1998) to assess the unattached bacterial concentrations. Following centrifugation, the solutions associated with each particle size were re-suspended and dispersed prior to enumeration of the total *E. coli* and enterococci concentration by treatment with a hand shaker for 10 minutes. The dispersed solution was enumerated for *E. coli* and enterococci concentrations on modified mTEC and mE agar (U.S. EPA, 2000) by membrane filtration (Clesceri et al., 1998). Additional details on the development and validation of the dispersion and partitioning method are included in Chapter 3. The large number of samples collected in this study made it likely that re-plating would be necessary. Serial dilutions in 1,000 ppm Tween 85 were not performed in addition to the 10 minute hand shaker treatment because the long term impact of Tween on cellular survival is unknown.

5.2.2. Nutrient Analysis

Runoff samples were analyzed for nutrient and suspended solids concentrations to examine potential relationships between bacterial and nutrient attachment ratios. The nutrient analysis was performed following procedures in Standard Methods for the Examination of Wastewater (Clesceri et al., 1998). Nutrient analysis included Total Dissolved Phosphorus (TDP, 0.45 μm polyethersulfone filter, Pall Life Sciences, Ann Arbor, MI), Total Phosphorus (TP), Dissolved Organic Phosphorus (DOP), Total Organic Phosphorus (TOP), Dissolved Organic Carbon (DOC), and Total Organic Carbon (TOC). Total suspended phosphorous (TSP) was calculated as the difference between TP and TDP and Suspended Organic Phosphorus (SOP) was calculated as the difference between DOP and TOP (Clesceri et al., 1998). Suspended organic carbon (SOP) was calculated as the difference between TOC and DOC. Total Suspended Solids (TSS) were analyzed (0.45 μm glass fiber filter, Pall Life Sciences, Ann Arbor, MI) as recommended by Clesceri et al. (1998).

5.2.3. Calculations and Statistical Analysis

The attached portion was assumed to be the difference between the unattached and total *E. coli* and enterococci concentrations. The partitioning coefficient was calculated using equation 5.1 and the particulate associated fraction was calculated using equation 5.2. The attached portion associated with each screen size was divided by the total suspended solids associated with each screen size to obtain the cfu (colony forming units) per gram of particulates and determine the particle sizes to which *E. coli* and enterococci preferentially attach.

$$Partitioning\ Coefficient = \frac{attached}{unattached} \quad [5.1]$$

$$Partitculate\ \ Attached\ Fraction = \frac{attached}{attached + unattached} \quad [5.2]$$

Statistical analysis of data was performed using the Statistical Analysis System (SAS Institute, 2004). Data were normalized prior to analysis and statistical significance was determined when $p \leq 0.05$. Bacterial and nutrient Total Concentration (TC), Particulate Associated Fraction (PAF), and Partitioning Coefficient (PC) were modeled as a function of soil type and simulation (run 1 and run 2) using analysis of variance (ANOVA). Least square means for bacterial and nutrient concentrations were compared using Tukey's pairwise comparison (Ott and Longnecker, 2001). Multiple regression analysis was conducted using the REG procedure in SAS and the final criteria to be included in the best model was selected based on the C_p statistic (Ott and Longnecker, 2001). Dummy variables were used to develop a full model representing bacterial PC and TC from all three soil types and a t-test was used to determine statistically significant slopes and intercepts. Statistical significance between particle sizes to which bacteria preferentially associate and soil type were also determined using the Statistical Analysis System (SAS Institute, 2004) by two-way ANOVA for run 1.

5.3. Results and Discussion

Packed bare soils were used to simulate a condition where large volume of runoff and erosion are expected. Three different soil types were used in this plot study. The PC, PAF, and TC were

calculated for *E. coli* enterococci, phosphorus, organic phosphorus, and organic carbon and are presented in Table 2 and results from individual samples are presented in Appendix D. The PC and PAF were calculated using equations 5.1 and 5.2, respectively. Attachment appeared to be influenced by both indicator organisms and soil type. The PAF 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. Percent attachment of enterococci was higher to the silty loam and silty clay loam while *E. coli* had a higher percent attached to the loamy fine sand. No statistically significant differences existed between the *E. coli* PAF values for the three soil types and only the enterococci PAF value from the silty clay loam soils was statistically higher than that for the loamy fine sand soils. Overall, the PC was highest in samples collected from the silty loam soils. The enterococci PC was greater than the *E. coli* PC in runoff samples collected from the silty loam and silty clay loam soils, while the *E. coli* PC was greater in samples collected from the loamy fine sand. Similar to the PAF results, only the enterococci PC from the silty loam soils was significantly higher than the loamy fine sand soils.

Table 5.2. Bacteria and nutrient partitioning coefficients (PC), particulate associated fractions (PAF) and total concentrations (TC) present in runoff from bare soils dominated by three different particle sizes and with a single cowpat.

	E. coli PC[1] Mean (SD)		*E. coli* PAF[1] Mean % Attached (SD)		*E. coli* TC[1] cfu 100mL^{-1} Mean (SD)	
	Run 1	Run 2	Run 1	Run 2	Run 1 a[2]	Run 2 b
loamy fine sand	0.50 (0.40) a[2]	NA	43 (0.19) a	NA	2.57×10^8 a	487 (806) a
silty loam soils	0.54 (0.44) a	NA	30 (0.18) a	NA	1.21×10^9 b	66 (258) b
silty clay loam	0.38 (0.34) a	NA	42 (0.27) a	NA	6.50×10^7 c	320 (1157) b
	enterococci PC Mean (SD)		**enterococci PAF Mean % Attached (SD)**		**enterococci TC cfu 100mL^{-1} Mean (SD)**	
	Run 1	Run 2	Run 1	Run 2	Run 1 a	Run 2 b
loamy fine sand	0.42 (0.22) a	NA	28 (0.11) a	NA	3.57×10^6 a	553 (568) a
silty loam soils	1.79 (2.49) b	NA	49 (0.23) b	NA	3.65×10^6 a	0 (0) b
silty clay loam	0.86 (0.53) ab	NA	43 (0.15) ab	NA	1.83×10^7 b	1620 (2421) a
	Phosphorus PC[3] Mean (SD)		**Phosphorus PAF[3] Mean % Attached (SD)**		**Phosphorus TC mg L^{-1} Mean (SD)**	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
loamy fine sand	1.19 (0.54) a	0.81 (0.35) a	52 (12) a	42 (14) a	16.67 (4.11) a	6.37 (0.77) a
silty loam soils	1.65 (0.69) a	1.72 (0.51) b	52 (12) a	62 (7) b	10.07 (2.35) b	8.18 (1.78) b
silty clay loam	3.02 (1.23) b	0.24 (0.21) c	73 (10) b	18 (12) c	12.47 (2.70) b	3.29 (0.83) c
	Organic Phosphorus PC Mean (SD)		**Organic Phosphorus PAF Mean % Attached (SD)**		**Organic Phosphorus TC mg L^{-1} Mean (SD)**	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
loamy fine sand	1.16 (0.47) a	0.87 (0.36) a	52 (11) a	44 (14) a	8.26 (1.97) a	3.28 (0.39) a
silty loam soils	1.65 (0.80) a	1.77 (0.50) b	59 (13) a	63 (7) b	5.10 (1.13) b	4.15 (0.88) b
silty clay loam	4.73 (7.35) b	0.38 (0.36) c	73 (12) b	25 (13) c	6.16 (1.38) c	1.80 (0.62) c
	Organic Carbon PC Mean (SD)		**Organic Carbon PAF Mean % Attached (SD)**		**Organic Carbon TC mg L^{-1} Mean (SD)**	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
loamy fine sand	0.50 (0.15) a	0.12 (0.08) ab	33 (7) a	10 (6) a	23.97 (2.46) a	3.77 (0.87) a
silty loam soils	0.16 (0.12) b	0.06 (0.04) b	13 (9) b	5 (3) a	16.39 (2.31) b	3.34 (0.91) a
silty clay loam	0.27 (0.30) b	0.15 (0.08) a	18 (13) ab	12 (6) a	21.32 (5.68) a	7.27 (2.55) b

[1]PC (partitioning coefficient), PAF (particulate attached fraction), TC (total concentration), NA (not applicable)
[2]Values followed by the same letter do not differ statistically between soils or run 1 and run 2 according to Tukey's pairwise comparison.
[3]Phosphouus, Organic Phosphorus and Organic Carbon PC and PAF were calculated using the dissolved, total, and suspended concentrations. The suspended concentrations were calculated as the difference between total and dissolved concentrations.

Statistically significant decreases in *E. coli* and enterococci TC were observed between run 1 and run 2 ($p \leq 0.05$). The time between simulations averaged 80 days. While the cowpats were exposed to environmental factors likely to encourage die-off of pathogens and indicator organisms, cowpats were completely undisturbed during the almost three month period and no degradation of the exterior of the cowpat was observed. Temperature averaged 21.4 ºC (70.5 ºF) with a maximum of 32.8ºC (91ºF); solar radiation averaged 1.19 MJ with a maximum of 3.11 MJ; relative humidity averaged 80.8% and precipitation totaled 41.2 cm (16.2 in) during the 80 day period. Of the total

precipitation, 25.7 cm occurred in June, approximately 18 cm above average. Previously Thelin and Gifford (1983) reported fecal coliform concentrations of 40,000 MPN $100mL^{-1}$ thirty days after cowpat deposition and fecal coliform concentrations peaked at 4,200 MPN $100mL^{-1}$ 100 days following deposition of standard cowpats (Kress and Gifford, 1984). Kress and Gifford (1984) found that declines in peak fecal coliform counts occurred after a second rainfall simulation, suggesting that bacteria available for transport were washed from the feces during the first simulation. While warm temperatures and high solar radiation are likely to have contributed to death of pathogen indicators in the outer crust of the cowpats during this experiment, it is also likely that the first rainfall simulation, combined with the above average rainfall in June, rinsed readily available pathogen indicators from the surface of the cowpat. Extended monitoring of *E. coli* and enterococci in cowpats has found viable cells up to 195 days following deposition (Chapter 4). However, bacteria would be unlikely to move from the protected, moist interior environment of a cowpat to the moisture limited exterior crust and therefore surviving cells remain unavailable for release and transport during subsequent runoff events. Common grazing practices allow cowpats to be trampled, which would expose the interiors of the cowpat, and thus presents a fresh supply of indicator organisms for future release and transport; however, trampling would also likely hasten die-off by increasing environmental exposure.

5.3.1. Impact of Soil Type on Attachment

In general, the *E. coli* cells attached at a higher rate to sediments in runoff from the loamy fine sand soils (43%) and enterococci attached at a higher rate to the silty loam (49%) and silty clay loam (43%) soils (Table 5.2); however, only the enterococci PC and PAF in runoff from the silty clay loam was significantly higher than the enterococci PC and PAF in runoff from the loamy fine sand ($P = 0.0137$ and $P = 0.0074$, respectively). The higher attachment of enterococci to the silty loam and silty clay loam soils might be associated with the higher cation exchange capacity and organic matter contents of these soils (Table 5.1). Guber et al. (2005a) found that the addition of manure particulates to solution increased ionic strength and pH when compared with bacteria-water suspensions but decreased attachment overall. They attributed decreased *E. coli* attachment to competition between manure particulates and bacteria; however, a less significant decrease in attachment was observed from soils with a higher organic matter content. Competition between attachment sites might help to explain differences between laboratory-based attachment studies and

field observations; however, attachment is high from all three soil types in this study, compared to previous edge-of-field (Chapter 6) and stormwater partitioning studies (Characklis et al., 2005; Krometis et al., 2007). Organic matter content was certainly higher for the silty loam (2.6%) and silty clay loam (2.0%) soils but significantly lower attachment ratios to loamy fine sand did not occur consistently for either *E. coli* nor for enterococci; thus, soil organic matter does not seem to be the primary factor contributing to the higher attachment rates observed in this study.

E. coli attachment to sand-dominated soils has been observed previously (Henry, 2004). It is likely that *E. coli* are able to associate with a broader range of particle sizes because the motility and rod shape of the *E. coli* makes them more able to attach to different angles or faces of the particles (Jeng et al., 2005). Laboratory-based attachment studies have identified particle size as a significant factor influencing attachment (Fontes et al., 1991, Ling et al., 2002); however, greater variability exists in the sorption properties of wild strains of *E. coli* used in this study (Lago, 2005; Muirhead et al., 2005). It appears that a combination of factors including (but not limited to) soil type and organic content of soils, carbon content and organic composition of fecal material, and cellular properties of indicator organisms are likely necessary to explain attachment of pathogen indicators to particulates.

5.3.2. Bacterial Attachment Related to TSS and Nutrient Transport

The average total suspended solids (TSS) concentrations in runoff samples collected from silty loam, loamy finesand and silty clay loam soils were 4.1 g $L^{-1,}$ 4.0 g L^{-1}, and 2.3 g L^{-1} , respectively, during Run 1. High sediment transport rates were expected based on the experimental design: no vegetation to prevent sediment transport, short distance between detachment sites and sample collection, high intensity rainfall to promote detachment and erosion by raindrops, and dispersion of the cowpats all contributed to the high suspended solids concentrations. High TSS concentrations combined with higher bacterial attachment rates than previously observed indicates that attachment sites are not limited; however, microscopic analysis was not conducted to confirm this assumption. The presence of organic matter and fecal material in bacterial sediment suspensions has been reported to decrease bacterial attachment (Guber et al., 2005a; Johnson and Logan, 1996) and was cited as a factor contributing to low attachment rates in runoff from large (18.3-m long by 3-m wide), highly vegetated plots (Chapter 6). Total organic carbon concentrations in runoff from vegetated plots averaged 15.67 mg L^{-1} and TSS concentrations averaged 152 mg L^{-1}. The TOC

concentration in runoff from this study averaged 20.56 mg L^{-1} among all three soil types. Comparison of the average TOC and TSS concentrations in runoff from this study (bare soil boxes) to the TOC and TSS concentrations in runoff from the vegetated plot study in Chapter 6 reveals that TOC and TSS concentrations increased by 24% and 96%, respectively, in runoff from this study. The higher bacterial attachment in runoff from the box plot study combined with higher TSS concentrations suggests that perhaps a threshold concentration exists where bacterial attachment is no longer decreased by competition with organic carbon and limited by availability of attachment sites. Findings from this study which simulates highly erodable soils indicate that attachment is likely a significant bacterial transport mechanism.

5.3.3. Multiple Regression Analysis

Relationships between bacterial indicators and nutrient partitioning were examined by regression models in an effort to predict bacterial partitioning ratios. Log transformation of the *E. coli* and enterococci partitioning coefficients did not improve normality of the data or the fit of the model. Two dummy variables, z1 and z2, were used to develop a single model for all soil types that can then be simplified into three separate models to predict partitioning ratios specifically from each soil. Attempts were made to model *E. coli* and enterococci PC and PAF with two sets of independent variables TSS, TP, TDP,TOP, DOP, TOC, DOC and TSS (Group 1), TP, P PC, TOP, organic P PC, TOC, and organic C PC (Group 2). Dummy variables are defines as follows: if z1 = 1 and z2 = 0 then responses from the silty loam soil are modeled; if z1 = 0 and z2 = 1 then responses from the loamy fine sand soil are modeled; and if z1 = 0 and z2 = 0 then responses from the silty clay loam soil are modeled. A t-test was used to test for statistical significance between intercepts and slopes and only those statistically different from the different soils were included in the full model. The presence of dummy variables in the full model indicates that statistically significant differences existed between equations which best predicted the partitioning coefficient from the three soil types. For example, in the *E. coli* PC full model, the z1 intercept is included because the silty loam soil intercept was statistically different from the silty clay loam and loamy fine sand soil intercepts. Similarly, the DP×z1 and DP×z2 slopes are both included in the full model because the DP slope was determined to be a significantly significant variable based on a t-test and also required significantly different values to predict the *E. coli* PC in runoff from the silty loam versus the loamy fine sand soils. The strongest relationships to predict *E. coli* and enterococci partitioning

coefficients are presented in Table 3 (additional details in Appendix E) and substitution of dummy variables will produce reduced models specific to each soil type. In runoff from silty clay loam soils (z1 = 0 and z2 = 0), the partial model for the *E. coli* PC = -11.76438 + 0.81134 [DOC (mg L^{-1})] - 0.06723 [TOC (mg L^{-1})]. The coefficient of determination, R^2, represents the proportional reduction in the squared error of the response corresponding to the addition of independent variables (Ott and Longnecker, 2001). Variance inflation and residual plots were examined for all models and deemed acceptable.

Organic carbon was the only significant independent variable necessary to predict *E. coli* PC in runoff from all three soil types. Phosphorus and organic phosphorus are added to the model to predict *E. coli* PC in runoff from the loamy fine sand and silty loam soils while including TSS concentrations only improved predictions in runoff from the silty loam soils. Total suspended solids were included in the prediction of enterococci PC for all three soil types. Phosphorous and carbon were both added to the model to improve enterococci PC predictions in runoff from the silty loam soils. In each of the reduced models containing the TSS variable, the slope is positive, indicating a positive relationship between indicator attachment and increased concentrations of suspended solids. Organic carbon, phosphorous, and organic phosphorous were not consistently a positive or negative slope for all models.

Table 5.3. Regression equations to predict *E. coli* and enterococci partitioning coefficients (PC) and total concentrations (TC) in runoff from three Virginia soils.

Partitioning Coefficient Models: *E. coli* and enterococci						**Total Concentration Models: *E. coli* and enterococci**					
***E. coli* partitioning coefficient: full model**			**enterococci partitioning coefficient: full model**			**ln *E. coli* total concentration: full model**			**ln enterococci total concentration: full model**		
Variable	**Parameter estimate**	***p* value**	**Variable**	**Parameter estimate**	***p* value**	**Variable**	**Parameter estimate**	***p* value**	**Variable**	**Parameter estimate**	***p* value**
Intercept	-11.76438	0.0012	Intercept	-0.94187	0.0216	Intercept	-6.92961	<.0001	Intercept	0.97240	0.1044
DOC (mg L^{-1})	0.81134	0.0006	TSS (mg L^{-1})	0.00077745	<.0001	TP (mg L^{-1})	0.32350	0.0019	DOC (mg L^{-1})	0.90990	<.0001
TOC (mg L^{-1})	-0.06723	0.0003	z1	18.57816	<.0001	DOC (mg L^{-1})	1.19672	<.0001	z1	-4.65642	<.0001
z1	21.11521	<.0001	z2	1.26955	0.0135	z2	5.33296	<.0001	TP×z2 (mg L^{-1})	-3.40860	0.0151
TSS×z1 (mg L^{-1})	0.00015222	0.0035	TSS×z1 (mg L^{-1})	-0.00063635	0.0008	TP×z2 (mg L^{-1})	-0.22609	0.0506	TOP×z2 (mg L^{-1})	6.82788	0.0204
TP×z1 (mg L^{-1})	-0.48864	0.0019	TSS×z2 (mg L^{-1})	-0.00075304	0.0002	TOC×z1 (mg L^{-1})	0.42762	<.0001	DOP×z2 (mg L^{-1})	1.21106	0.0535
DP×z1 (mg L^{-1})	-0.27794	0.0656	TP×z1 (mg L^{-1})	-1.01389	<.0001				TOC×z1 (mg L^{-1})	0.33689	<.0001
DP×z2 (mg L^{-1})	-3.73534	0.0070	P_ PC ×z1	-1.37963	0.0179				TOC×z2 (mg L^{-1})	0.50089	0.0059
TOP×z1(mg L^{-1})	0.45554	0.0567	TOP×z1 (mg L^{-1})	0.76631	0.0055				DOC×z2 (mg L^{-1})	-1.10629	0.0006
TOP ×z2 (mg L^{-1})	-0.31962	0.0007	Organic_P_ PC ×z1	2.36197	0.0002	$R^2 = 0.9308$					
DOP×z2 (mg L^{-1})	8.39783	0.0051	TOC×z1 (mg L^{-1})	-0.90573	<.0001				$R^2 = 0.9311$		
DOC×z1 (mg L^{-1})	-1.15476	<.0001	Organic_C_PC×z1	14.44640	<.0001						
$R^2 = 0.6755$			$R^2 = 0.8964$								

Total organic phosphorus and TOC concentrations significantly decreased between run 1 and run 2, but the TP concentrations in runoff from the silty loam soils were not significantly reduced (Table 5.2). While most nutrient concentrations did significantly decrease between runs as did the bacterial TC, the presence of phosphorus and carbon in runoff during run 2 questions the ability of nutrients to predict bacterial concentrations from an aged fecal source. Bacterial partitioning data was not available for run 2 (because of very low bacterial concentrations), so only the total concentrations were examined by regression models. Nutrient and TSS concentrations from run1 and run 2 were both included in an effort to predict bacterial concentrations from a fresh and aged fecal source in a single model. Log transformation of the *E. coli* and enterococci TC was necessary to achieve normal distribution of the data. The full models are presented in Table 5.3 (additional details in Appendix E) and substitution of dummy variables as described previously will generate reduced models specific to each soil type. Prediction of *E. coli* TC in runoff from all soils required phosphorous and organic carbon variables while the enterococci TC models specific to loamy fine sand also required the addition of organic phosphorous as an independent variable. Again it is difficult to draw conclusions based on the role of organic carbon, phosphorous, and organic phosphorous in predicting concentrations as a positive or negative slope was not consistently identified for all models. Interestingly, organic carbon exhibited a positive slope for all *E. coli* models but DOC had a negative slope in the enterococci TC model for loamy fine sand soils.

The most notable difference between regression equations developed to predict PC versus TC is the exclusion of TSS concentrations from the TC models. This again emphasizes the importance of attachment site availability in determining the fraction of attached cells; however, the presence of high TSS concentrations does not appear to be a factor in predicting total concentrations. Therefore, while high TSS concentrations appear to influence the state in which *E. coli* and enterococci are transported to surface waters, availability of attachment sites does not increase survival on the land to the point that overall concentrations are influenced by TSS. However, the state in which the cells are transported likely impacts long-term in-stream cellular survival and selection of management practices to reduce indicator transport.

5.3.4. Preferential Attachment to Particulates

Both fecal indicators preferentially attached to sediments retained by an 8μm filter (Table 5.4, Appendix D). At least 50% of the attached *E. coli* and enterococci were associated with sediment and organic particles passing through the 63μm filter. No samples resulted in measurable solids retained by the 3 μm filter even though the silty clay loam soils contained approximately 26% clay (Table 5.1). We assumed that the clay sized particles present in runoff were most likely transported as aggregates.

Table 5.4. Particle sizes to which *E. coli* and enterococci preferentially attach in runoff samples collected from bare soil box plots.

	Particle Size[1] μm	Average TSS (mg/L)	% TSS associated with each particle category	*E. coli*		enterococci	
				cfu/mg solids	% attached	cfu/mg solids	% attached
Grosclose silt loam	>500	4,363	24	9,038	2	28	3
	63 - 499	9,256	50	51,007	10	209	20
	8 - 62	4,930	26	435,668	88	800	77
Eunola loamy fine sand	>500	3,209	32	8,596	0.6	38	0.2
	63 - 499	5,306	52	168,640	12	507	15
	8 - 62	1,621	16	1,254,993	88	2,971	85
Levy silty clay loam	>500	536	25	67,321	2	248	3
	63 - 499	517	24	1,151,284	34	3,204	37
	8 - 62	1,096	51	2,134,764	64	5,179	60

[1]3 μm filter did not retain measurable TSS concentrations.

The distribution of TSS present in runoff samples did not correspond to the distribution of *E. coli* or enterococci attachment to particle sizes. Both the silt loam (74% retained by the 63 μm filter) and loamy fine sand (84% retained by the 63 μm filter) soils had higher concentrations of sediments classified as sand than silt (retained by the 8 μm filter), but greater concentrations of *E. coli* and enterococci preferentially attached to the silt-sized particles retained by the 8 μm filter. The silty clay loam soils had the greatest percentage of TSS particles in runoff retained by the 8 μm filter (51%) and similar to the silt loam and loamy fine sand soils, *E. coli* and enterococci both preferentially attached to these particulates. The higher surface area associated with finer particles allows for more attachment sites and thus greater bacterial attachment. 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). Auer and Niehaus (1993) found that fecal coliforms were primarily sorbed to particle classes 0.45 – 1 μm and 6 – 10 μm during storm overflow events. On average 90.5% of

the attached fecal coliform bacteria were found to be sorbed to particle sizes ranging from 0.45 – 10 µm. Mitchell and Chamberlin (1978) also found that clays tend to adsorb coliforms more than silts or sands. The soils used in this study generally had low clay content (Table 1) and thus the majority of particulates present in runoff were classified by screen filtration as sand or silt. Similar to findings from previous studies, *E. coli* and enterococci both preferentially attached to the smallest particles present in runoff (silt in this study) even when the particle distribution in runoff was dominated by sand.

A two-way ANOVA was used to study the effects of soil type and particle size and any interactions between particle size and soil type on the attached bacteria (cfu/mg soil). *E. coli* and enterococci data were normalized by natural log transformation prior to analysis and soil type and particle size both significantly impacted attached bacteria (Table 5.5). Interactions between soil type and particle size were not significant for *E. coli* attachment but were significant for enterococci attachment ($P = 0.02$). Least square means for *E. coli* and enterococci attachment between soil type and particle size were compared using Tukey's pairwise comparison (Ott and Longnecker, 2001) and results are presented in Table 5.5.

Table 5.5. *P* values showing statistically significant differences between *E. coli* and enterococci associated with sediments retained by the three particle size categories, >500 µm, 63 - 499 µm, and 8 - 62 µm (for each soil type).

Soil type	Particle Size µm	*E. coli* *P* value	enterococci *P* value
Eunola loamy fine sand	>500 - 63	< 0.0001	< 0.0001
	63 - 8	< 0.0001	0.0001
	8 - 500	< 0.0001	< 0.0001
Grosclose silt loam	>500 - 63	0.0002	< 0.0001
	63 - 8	0.0001	0.0235
	8 - 500	< 0.0001	< 0.0001
Levy silty clay loam	>500 - 63	< 0.0001	< 0.0001
	63 - 8	0.3844	0.9994
	8 - 500	< 0.0001	< 0.0001

Statistically significant differences were noted in bacterial attachment between all three particle size categories for all soils except the 63µm and 8 µm size category retaining silty clay loam soils; however, when comparing bacterial attachment between soils for each particle size category, less statistical differences were observed (Table E.1). Bacterial attachment did not

differ between loamy fine sand and silt loam soils classified in the 500 μm and 63 μm categories. In addition, *E. coli* attachment did not differ between loamy fine sand and silt loam soils (p = 0.1027) and loamy fine sand and silty clay loam soils (p = 0.9340) classified as silt (8 μm) while enterococci attachment did not differ statistically between loamy fine sand and silty clay loam soils (p = 0.8874) also classified as silt (8 μm). According to the particle size analysis (Table 5.1), the silt loam and loamy fine sand soils varied greatly in particle composition; yet few statistically significant differences in attachment were noted between two soils, regardless of the particle size.

From this portion of the study we are able to conclude that particle size in runoff greatly influences attachment, as at least 50% of all *E. coli* and enterococci were associated with sediment and organic particles retained by the 8 μm filter, the smallest particle category examined. No clear trends emerged when examining attachment of *E. coli* and enterococci to particulates in runoff related to the soil properties (organic matter and cation exchange capacity) and distribution of the particle sizes within the soil matrix. Comparison of *E. coli* and enterococci preferential attachment to particle size categories within the soil matrix and the resulting preferential attachment to particle size categories present in runoff would further clarify whether or not relationships exist between soil matrix particle size distribution and indicator preferential attachment; however, the author is not aware of any such study. More screen sizes would have provided additional insight into distribution of indicator attachment among particle sizes and is recommended for future study, especially if examining attachment to soils with higher clay content than the soils used in the study.

5.4. Summary and Conclusions

A soil box study was conducted to examine the state of *E. coli* and enterococci transport from three bare soil types receiving cowpat treatments and develop relationships between bacterial partitioning and phosphorous and carbon transport. Particles sizes to which cells preferentially associated were also identified. Soil boxes (100-cm × 20-cm × 7.5-cm) were packed with three different Virginia soils, loamy fine sand, silty loam and silty clay loam. A rainfall simulation was conducted 24-hours after application of a standard cowpat, followed by a second rainfall simulation approximately 80 days later. Runoff samples were analyzed for *E. coli*, enterococci,

TSS, phosphorous, organic phosphorous and organic carbon. *E. coli* and enterococci partitioning coefficient (PC) and particulate associated fraction (PAF) were calculated to compare fecal indicator attachment in runoff from the different soil types and between two pathogen indicators, *E. coli* and enterococci, and fractional filtration followed by centrifugation identified particle sizes to which indicators preferentially attached. Regression analysis was conducted to examine potential relationships to utilize nutrient and TSS data to predict *E. coli* and enterococci PC and total concentration (TC).

Percent of *E. coli* and enterococci attached to particulates in runoff ranged from 28% to 49%. In general, the *E. coli* cells attached at a higher rate to sediments in runoff from the loamy fine sand box plots and enterococci attached at a higher rate to the silty loam and silty clay loam soils. Enterococci appeared associate with soils with a higher cation exchange capacity and organic matter content while *E. coli* had higher association with loamy fine sand soils, which has a lower cation exchange capacity and organic matter content. We hypothesized that the majority of cells would be associated with particles retained by the 8 μm filter and at least 50% of all attached cells were associated with particles less than 63 μm in size. The larger surface area of the smaller particles corresponds to a higher number of sites available for bacterial attachment. While particle size in runoff greatly influenced attachment, we were unable to establish a particular particle size range present in the soil matrix as a dominant factor statistically impacting overall attachment. We hypothesized that the *E. coli* and enterococci partitioning would be related to the dissolved/suspended phosphorus ratio, but a direct linear relationship was not present. Regression equations were developed to predict *E. coli* and enterococci PC ($R^2 = 0.54$ and $R^2 = 0.86$, respectively) and *E. coli* and enterococci TC ($R^2 = 0.93$ $R^2 = 0.92$, respectively). A single regression model was capable of predicting *E. coli* and enterococci TC in runoff from both a fresh and aged fecal source. TSS concentrations were only included as independent variables in regression equations developed to predict PC, emphasizing the importance of attachment sites in predicting the fraction of attached cells.

Based on this study and previous findings, it appears that a combination of factors influence attachment of *E. coli* and enterococci to particulates in runoff; including soil type and organic content of soils, carbon content and organic composition of fecal material, and cellular properties

of indicator organisms. Partitioning coefficients and PAF developed in this study can be incorporated into the initial release equations in non-point source models, typically described by a linear partitioning relationship (Equation 2.4), to improve prediction of in-stream bacterial concentrations from highly erobible soils. In addition the regression equations developed in the study could improve predictive capabilities of current NPS models when only nutrient data is available. Partitioning coefficients and PAF developed in this study are not meant to represent bacterial attachment during overland flow events or at the edge-of-the-field because of the small plot size and short distance between the fecal source and sample collection point. Future study is recommended to assess bacterial attachment from fecal sources other than cowpats. It could be possible that soils with higher clay content than the Levy soils used in this study (29% clay) would have even higher attachment rates due to increased availability of attachment sites. Higher attachment associated with highly erodible soils as used in this study (when compared to previous edge-of-field and stormwater studies) indicates that lower concentrations of total suspended solids could be limiting bacterial attachment and thus PC and PAF from other landuses could be much lower. Settling of particulates prior to release of runoff to surface waters by best management practices such as detention basins or vegetative filter strips might be an appropriate method of reducing bacterial loadings by as much as 50% in the presence of high sediment loads.

Chapter 6. *E. coli* and Enterococci Attachment to Particles and Loading Rates in Pastureland Runoff

6.1. Introduction

Pathogens are the leading cause of water quality impairments in many parts of the United States. 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 (U.S. EPA, 2003). The three most common pathogen indicators in the United States include fecal coliforms, *E. coli*, and enterococci (U.S. EPA, 1986). Although fecal coliform have been traditionally used as an indicator 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 gastrointestinal illnesses (U.S. EPA, 1986) and are therefore currently the recommended indicator organisms (U.S. EPA, 1998; U.S. EPA, 2002b). In an attempt to reduce pollutant loading to the nation's water bodies, Total Maximum Daily Loads (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.

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 and most currently-used NPS models simulate bacterial transport to surface waters as an unattached or dissolved pollutant (Paul et al., 2004). Cell surface properties such as hydrophobicity of the cell (Fattom and Shilo, 1984; Kinoshita et al., 1993) and the electrostatic nature of the cell envelope (Jamieson et al., 2004) and external factors including availability of attachment sites (Characklis et al., 2005; Fries et al., 2006), ionic strength, and pH of the carrying solution (Jewett et al., 1995; Scholl and Harvey,

1992), and size of the particulate matter (Fontes et al., 1991) have been listed as factors that influence bacterial attachment to soils. Previous studies have determined that fecal bacteria preferentially attached to particulates (Auer and Niehaus, 1993; Henry, 2004; Ling et al., 2002) and statistically indistinguishable release rates between manure particulates and fecal coliforms have been observed through stony soils (Shelton et al., 2003). Very little data is available on bacterial partitioning between the attached and unattached phases during movement along overland transport pathways (Jamieson et al., 2004).

Many researchers and practitioners recognize the 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 flow, particulates, and water quality indicators is needed. Many models already partition between nutrient phases; thus, identifying correlations between bacterial and nutrient partitioning might improve predictive capabilities of bacterial transport models by modification of existing nutrient overland transport process algorithms. In addition, if attachment is a significant edge-of-field transport factor, design and selection of management practices could be improved to encourage settling of particulates and the attached fecal indicators for reduction of pathogen transport to surface waters.

The goal of this study was to investigate the partitioning of *E. coli* and enterococci between the unattached and particulate-attached phases during overland flow from pasturelands. The objectives of this study were to examine correlations between bacterial and nutrient partitioning ratios and loading rates for potential relationships and to use multiple regression analysis to develop equations to predict *E. coli* and enterococci partitioning between the attached and unattached phases with suspended solids and nutrient data. The next objective was to employ the separation technique described in Chapter 3 to partition between the unattached and attached phases of *E. coli* and enterococci at the edge-of-the-field and to identify the particle sizes to which the attached bacteria preferentially associated. Similar to the box plot study presented in Chapter 5, we hypothesized that the *E. coli* and enterococci partitioning would be related to the

dissolved/suspended phosphorus ratio and that the majority of cells would be associated with particles retained by the 8 μm filter.

6.2. Materials and Methods

Plots were constructed on newly established vegetation on an area that had not received any manure applications of any kind in the previous three years. A seedbed of Kentucky 31 Tall Fescue was prepared the fall prior to plot construction. The existing vegetation was sprayed twice with Roundup™, plowed twice, limed, fertilized and broadcast with Kentucky 31 Tall Fescue. The area was irrigated weekly until vegetation emerged. The following spring five field plots 3-m (9.8-ft) wide by 18.3-m (60-ft) long were constructed on a Groseclose silt loam hayfield (35% sand, 60% silt, and 5% clay) on an approximate 9-percent slope dominated by a dense stand of Kentucky 31 Tall Fescue. A "V" shaped outlet at the down-slope end of each plot directed runoff into a 0.15-m (6-inch) H-flume equipped with a stilling well and a stage recorder for flow measurement (Figure 6.1). The stage recorder did not function properly on the control plot, so runoff rates and flow volumes from the control plot in Chapter 7 were used to calculate the flow weighted concentrations (FWC) and loads presented in the results and discussion section. The plot cover and rainfall application rates were similar between the Chapter 6 and Chapter 7 simulations. Surface soil samples (0 - 8 cm depth) were collected with a soil probe from each transport plot. The samples were sieved (2 mm), and stored prior to analysis. Soils were analyzed for Mehlich -1 P, organic matter by a modified Walkley-Black method and pH by 1:1 soil to distilled water ratio and solid state pH meter (Donohue and Heckendorn, 1994).

Fresh dairy cattle fecal deposits were collected at the Virginia Tech dairy facility over a 24 hour period. Standard cowpats (Thelin and Gifford, 1983) were formed by mixing the manure in a cement mixer for fifteen minutes. The homogenized manure was placed in molds with a diameter of 20.3 cm (8 in) and a depth of 2.54 cm (1 in) until a weight of 0.9 kg (2.0 lbs) was reached. Manure samples were collected prior to land application and analyzed by the Clemson Agricultural Service Laboratory. Water soluble P was determined by the method proposed by Sharpley and Moyer (2000). The pH was measured potentiometrically in a 1:2 manure/water slurry (Peters et al., 2003). Approximately 106 cowpats were applied to four of the five plots to represent grazed pastureland. The plot length was divided into 0.91 meter (3 ft) segments and

five cowpats were randomly applied to each section. Six cowpats were applied to the "V" shaped outlet at the down-slope end of each plot. One plot received no treatment and was used as a control.

Due to the unreliability of natural precipitation for short-term field research, a rainfall simulator (Dillaha et al., 1988) generated a uniform rainfall event (2.8 cm/h) to all plots (Figure 6.1) twenty hours after application of manure to the plots. After the beginning of runoff, discreet grab samples were collected at the outfall of the flumes. Samples were collected at the onset of runoff, at ten minute intervals during the storm event, immediately following the end of the storm event, and four minutes after the precipitation ceased. Three samples were collected during each sampling event, one for bacterial partitioning studies, one for total *E. coli* and enterococci concentration analysis, and one for nutrient analysis. The rainfall event continued until runoff from all plots reached steady state (three hours and 20 minutes) and the longest runoff event lasted 90 minutes (plot 2).

Figure 6.1. Portable rainfall simulator was used to apply rain to the transport plots and samples were collected from the base of the plots every 10 minutes after the onset of runoff.

6.2.1. Bacterial Partitioning and Enumeration

Collected samples were transported to the laboratory immediately following the end of the rainfall simulation and analyzed for *E. coli* and enterococci. Partitioning of pathogen indicators between attached and unattached phases was achieved by fractional filtration followed by centrifugation. Fractional filtration has been used previously to identify particle sizes to which bacteria are attached (Auer and Niehaus, 1993; Schillinger and Gannon, 1985), and a filter pore

size of 8 μm has been identified as a viable method to separate attached and free bacteria (Gordon et al., 2002; Henry, 2004; Mahler et al., 2000; Qualls et al., 1983). The presence of sediments and organic particles in runoff from agricultural lands makes it very likely that the filters could clog and retain free cells, resulting in a higher fraction of cells being classified as attached. To assess the retained, unattached cells, we rinsed the screens and filters with phosphate buffered water (Hach Company, Loveland, CO) and then centrifuged the re-suspended solution. This technique combines the benefits of fractional filtration, identifying particle sizes to which cells attach, with the more common practice of centrifugation (Chapter 3).

The number 35 mesh screen (Bel-Art Products, Pequannock, NJ) was used to retain particles larger than coarse sand (>500 μm) and number 230 mesh screen was used to retain medium, fine, and very fine sand (63 - 500 μm). An 8 μm filter (Poretics, Polycarbonate, GE Water and Processes Technologies) was used to retain fine, medium, and coarse silt particles and a 3 μm filter (Nuclepore Track – Etch Membrane Filtration Products, Whatman) was used to retain clay and very fine silt particles. Throughout the study no measurable particulates passed thorough the 8 μm filter. Following filtration, the retained solids were re-suspended in phosphate buffered water and centrifuged (Avanti J-25I, Beckman Coulter, Fullerton, CA) at 4,700 rpm for 15 seconds (Huysman and Verstraete, 1993; Lago, 2005). The filtrate and supernatant were enumerated for *E. coli* and enterococci concentrations on modified mTEC and mE agar (U.S. EPA, 2000) using membrane filtration (Clesceri et al., 1998) to assess the unattached bacterial concentrations. Following centrifugation, the solutions associated with each particle size were re-suspended and dispersed prior to enumeration of the total *E. coli* and enterococci concentration by treatment with a hand shaker for 10 minutes (Chapter 4). The dispersed solution was enumerated for *E. coli* and enterococci concentrations on modified mTEC and mE agar (U.S. EPA, 2000) by membrane filtration (Clesceri et al., 1998).

6.2.2. Nutrient Analysis

Runoff samples were analyzed for nutrient and suspended solids concentrations to examine potential relationships between bacterial and nutrient attachment ratios. Nutrient analysis was performed following procedures in Standard Methods for the Examination of Wastewater (Clesceri et al., 1998). Nutrient analysis included Total Dissolved Phosphorus (TDP, 0.45 μm

polyethersulfone filter, Pall Life Sciences, Ann Arbor, MI), Total Phosphorus (TP), Dissolved Organic Phosphorus (DOP), Total Organic Phosphorus (TOP), Dissolved Organic Carbon (DOC), and Total Organic Carbon (TOC). Total suspended phosphorous (TSP) was calculated as the difference between TP and TDP and Suspended Organic Phosphorus (SOP) was calculated as the difference between DOP and TOP (Clesceri et al., 1998). Nutrient analysis included total and organic forms of phosphorus to account for inorganic residual from fall fertilizer application and the organic forms present in fresh cowpats. Suspended organic carbon (SOP) was calculated as the difference between TOC and DOC. Total Suspended Solids (TSS) were analyzed (0.45 μm glass fiber filter, Pall Life Sciences, Ann Arbor, MI) as recommended by Clesceri et al. (1998).

6.2.3. Calculations and Statistical Analysis

The attached cells were assumed to be the difference between the unattached and total *E. coli* and enterococci concentrations. The partitioning coefficient was calculated using Equation 6.1 and the particulate associated fraction was calculated using Equation 6.2. The attached portion associated with each screen size was divided by the TSS associated with respective screen size to obtain the colony forming units per gram of particulates and determine the particle sizes to which *E. coli* and enterococci preferentially attach.

$$Partitioning\ Coefficient = \frac{attached}{planktonic} \qquad [6.1]$$

$$Partitculate\ Attached\ Fraction = \frac{attached}{attached + planktonic} \qquad [6.2]$$

Statistical analysis of data was performed using the Statistical Analysis System (SAS Institute, 2004). The nonparametric Kruskal-Wallis rank test was used to test for significant differences between partitioning ratios, particulate associated fractions, and total concentrations during the rising, peak, and receding limbs of the runoff hydrograph. Pearson correlation coefficients between bacteria partitioning and concentrations and runoff, TSS, and nutrients were determined using PROC CORR and a *p*-test was performed to test for statistical significance (SAS Institute, 2004). Multiple regression analysis was conducted using the REG procedure in SAS and

analysis of variance (ANOVA) was used to determine statistical significance of the model. Statistical significance between particle sizes to which bacteria preferentially associate were also determined by ANOVA. Data were normalized prior to analysis and statistical significance was determined when $p \leq 0.05$.

6.3. Results and Discussion

Soils were analyzed for Mehlich -1 P (11 mg kg^{-1}), organic matter (2.6%) and pH (6.22) prior to land application of manure. Fresh manure samples were also analyzed prior to land application. Water soluble P was 2.02 g kg^{-1}, pH was 5.6, and average moisture content of fresh manure samples was 83.61%.

The average *E. coli*, enterococci, and nutrient flow-weighted concentrations and loads are presented in Table 6.1 and the results associated with each sample are available in Appendix F. Flow-weighted concentrations were calculated by multiplying the sample concentrations by the subsequent runoff volume and then dividing by the total runoff volume from each plot. Bacterial and nutrient loads were calculated by multiplying the sample concentrations by the subsequent runoff volume and converting the plot area to a per hectare basis. Plot 3 was excluded from bacterial load calculations because of missing data points. High standard deviations in load calculations are to be expected because of the differences in total runoff volumes from each plot (ranging from 0.12 to 0.50 m^3).

One plot received no treatment and was used as a control. Background *E. coli* concentrations flow-weighted concentrations were 72.4 cfu 100 mL^{-1} and enterococci concentrations were 0.0 cfu 100 mL^{-1}. The control plot *E. coli* load was 8.87×10^5 cfu ha^{-1} and enterococci load was 0.0 cfu ha^{-1}. Control plot bacteria samples were not partitioned between the attached and unattached phases because of the low cell counts. The background *E. coli* was only detected in two of the ten samples collected during the runoff event and is most likely attributed to wildlife (Doran et al., 1981; Patni et al., 1985).

Table 6.1. Average *E. coli*, enterococci, total suspended solids, and nutrient flow-weighted concentrations and loads

	Treatment Plot Mean FWC[1]	Control Plot FWC	units		Treatment Plot Mean Load (SD)	Control Plot Load	units
E. coli	6.96×10^{5}	176	cfu 100 mL^{-1}	*E. coli* attached	1.63×10^{10} (1.40×10^{10})	NA	cfu ha^{-1}
enterococci	3.63×10^{5}	0.00	cfu 100 mL^{-1}	*E. coli* unattached	4.16×10^{11} (5.29×10^{11})	NA	cfu ha^{-1}
TDP	2.67	0.210	mg L^{-1}	enterococci attached	6.97×10^{9} (7.72×10^{9})	NA	cfu ha^{-1}
TSP	1.41	0.002	mg L^{-1}	enterococci unattached	2.71×10^{11} (4.47×10^{11})	NA	cfu ha^{-1}
TP	4.08	0.212	mg L^{-1}	TDP	0.11 (0.09)	6.40×10^{-3}	kg ha^{-1}
DOP	1.36	0.103	mg L^{-1}	TSP	0.049 (0.030)	6.97×10^{-5}	kg ha^{-1}
SOP	0.75	0.003	mg L^{-1}	DOP	0.056 (0.046)	3.14×10^{-3}	kg ha^{-1}
TOP	2.11	0.106	mg L^{-1}	SOP	0.025 (0.018)	8.62×10^{-5}	kg ha^{-1}
DOC	14.65	9.801	mg L^{-1}	DOC	0.604 (0.462)	2.95×10^{-1}	kg ha^{-1}
SOC	1.02	1.05	mg L^{-1}	SOC	0.038 (0.020)	3.92×10^{-2}	kg ha^{-1}
TOC	15.67	10.85	mg L^{-1}	TSS	544 (273)	0.951	kg ha^{-1}
TSS	152.22	19.81	mg L^{-1}				

[1]Flow weighted concentration (FWC), Standard Deviation (SD), Total Dissolved Phosphorus (TDP), Total suspended phosphorous (TSP), Total Phosphorus (TP), Dissolved Organic Phosphorus (DOP), Suspended Organic Phosphorus (SOP), Total Organic Phosphorus (TOP), Dissolved Organic Carbon (DOC), Suspended organic carbon (SOP), and Total Organic Carbon (TOC), Total Suspended Solids (TSS)

6.3.1. Bacterial Partitioning Related to Flow Regime

As indicated previously, following the onset of runoff from the plots, samples were collected at ten minute increments from the outfall of the flume. Bacterial partitioning might be impacted by flow velocities (Guber et al., 2005b; Krometis et al., 2007) so bacterial and nutrient partitioning was separated into rising, peak, and receding limbs of the overland flow hydrograph (Figure 6.2). The number of samples collected from each plot varied due to different beginning of runoff times but ranged from seven to eleven samples. Nineteen samples were included in the rising limb analysis, ten samples in the peak limb analysis, and 6 samples in the falling limb analysis. Partitioning coefficients and PAF are presented as box and whisker plots illustrating 10^{th}, 25^{th}, 75^{th}, and 90^{th} percentiles. The *E. coli* PC and PAF medians are both slightly lower during the rising and falling limbs of the hydrograph following the same pattern as TSS concentrations (Figure 6.3). Enterococci, however followed the opposite trend; the mean PC and PAF increased during the rising and falling limbs of the runoff hydrograph. The total bacterial concentrations decreased as the runoff hydrograph progressed (Figure 6.3), similar to what you would expect from a first flush effect (Novotny, 2003).

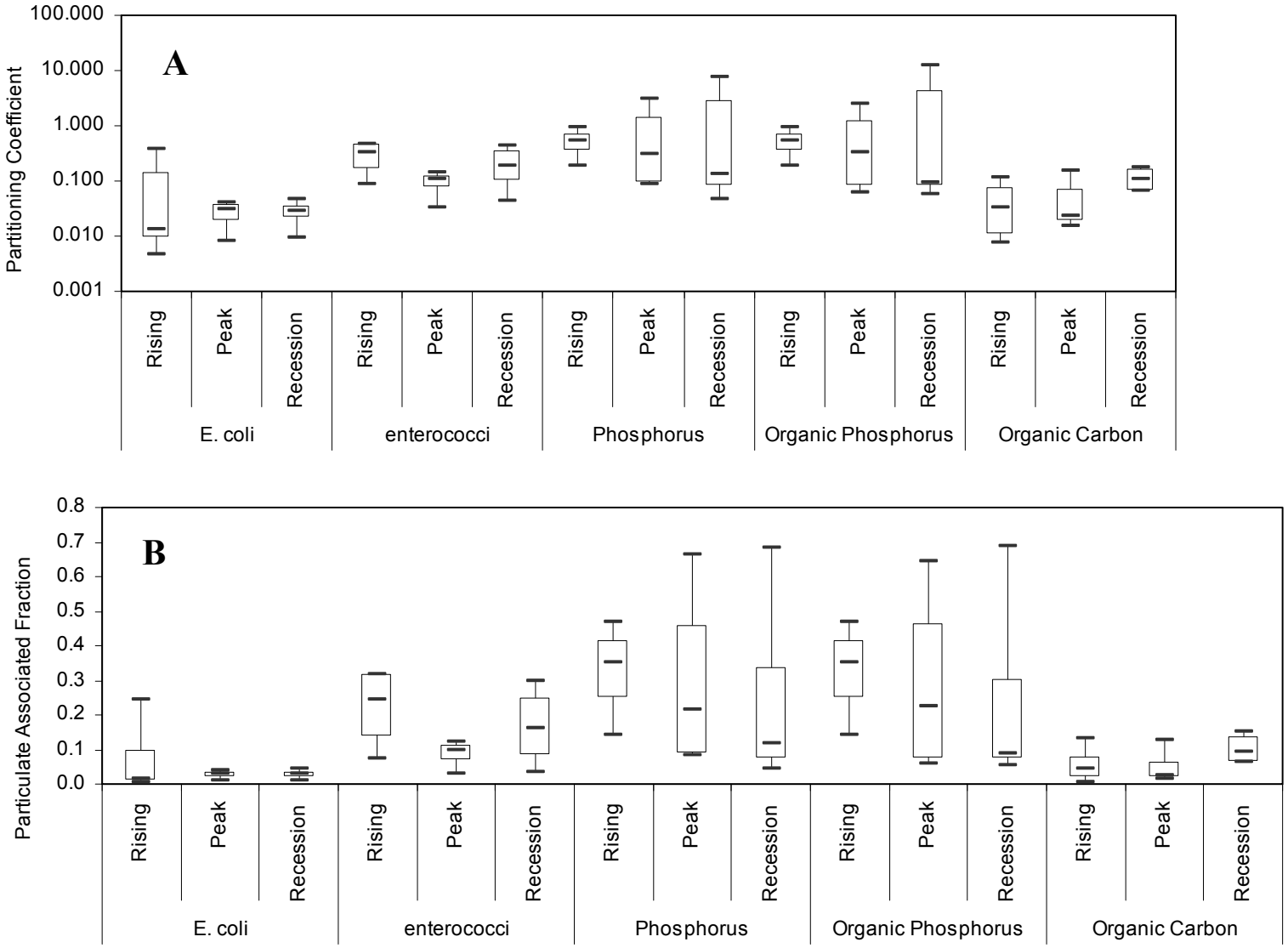

Figure 6.2. Partitioning coefficients (a) and particulate associated fractions (b) of *E. coli*, enterococci, phosphorus, organic phosphorus, and organic carbon in runoff from pasturelands during the rising, peak, and recession limbs of a runoff hydrograph.

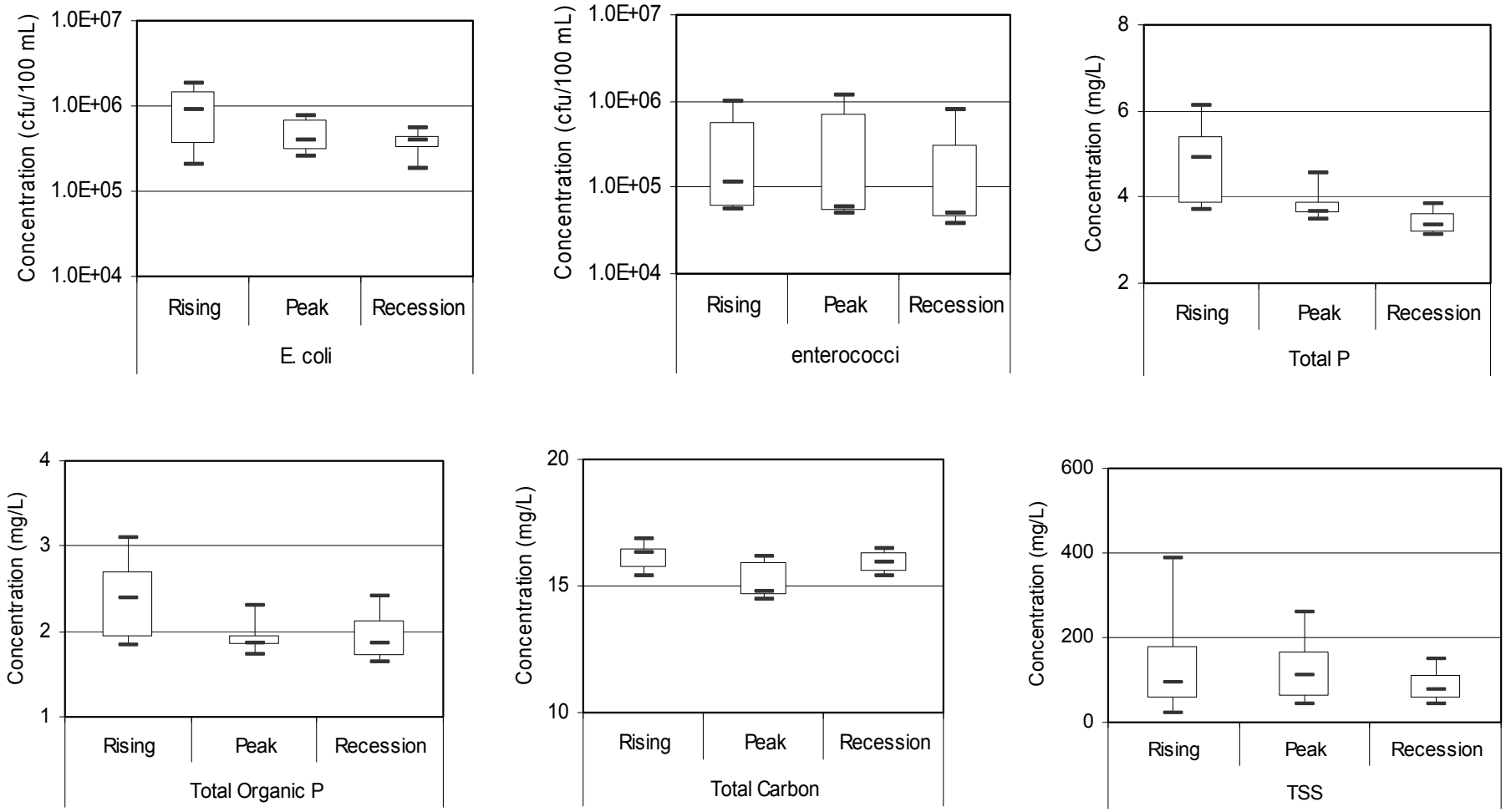

Figure 6.3. Bacterial and nutrient concentrations in runoff from pasturelands during the rising, peak, and recession limbs of a runoff hydrograph for (a) *E. coli*, (b) enterococci, (c) total phosphorus, (d) total organic phosphorus, (e) total carbon, and (f) total suspended solids.

The nonparametric Kruskal-Wallis rank test was used to identify statistically significant differences between PC, PAF, and TC during the rising, peak, and recession phases of the runoff hydrograph (Appendix G). Neither *E. coli* nor enterococci PC, PAF, or TC were significantly different between the rising, peak, and recession limbs of the runoff hydrograph. Only organic carbon exhibited statistically significant differences among the partitioning coefficients ($p \leq 0.0300$) and particulate associated fractions ($p \leq 0.0300$). Concentrations of TP, TOP, and TOC all significantly differed between phases of the runoff hydrograph ($p \leq 0.0003$, $p \leq 0.0187$, and $p \leq 0.0045$, respectively). A Wilcoxon pairwise comparison found the concentration of TP to differ among all three phases while TOP concentrations only differed between the peak and rising limbs of the hydrograph. Total organic carbon differed between the peak and the rising limb and the peak and recession limb while the PC and PAF differed between the peak and recession and rising and recession limbs.

The average *E. coli* PC for all samples collected was 0.06 which corresponded to a PAF of 4.8% and the average PC for enterococci was 0.18 with corresponding PAF of 13%. Partitioning coefficients and PAF were calculated using Equations 6.1 and 6.2. Characklis et al. (2005) found *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, enterococci cells have a tendency to occur in pairs or short chains during the exponential or log phase of the growth curve (Holt et al., 1993). Enterococci were likely in the active growth stage since the rainfall simulation occurred within 24 hours after deposition and several decay studies have reported that bacteria increase in fresh fecal deposits for up to two weeks before die-off begins (Conner and Kotrola, 1995; Crane et al., 1980; Muirhead et al., 2005; Wang et al., 1996; Wang et al., 2004). It is possible that these chains could have been retained by the filters and removed from suspension during centrifugation.

These rates of *E. coli* and enterococci attachment are lower than the majority of studies that have focused on in-stream background and storm event partitioning. 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 an average attachment ranging from 20% to 35% in grab samples collected during storm events. Muirhead et al. (2005) found that on average 8% of *E. coli* cells attached to sediment particles and most cells were not bioflocculated in runoff from cowpats and fecal-material–soil mixtures placed on metal trays. Differences between this study and previous findings are likely due to the differences in landuses contributing runoff. The high concentration of manure particulates in runoff from a fresh fecal source (Guber et al., 2005a, Guber et al., 2005b) and the different methods used to partition between unattached and attached phases might also help to explain the lower attachment rates observed in this study.

Different time periods between introduction of the fecal sources into the environment and sample collection could also partially explain differences in bacterial partitioning rates. Cells exposed to an oligotrophic (nutrient limited) environment are more likely to attach to particulates in an effort to obtain nutrients and increase survival (Morita, 1997). The short time (24 hours) between manure application and runoff in our study could help to explain the low bacterial attachment rates. Bacteria present in runoff from lands treated with a fresh manure source are unlikely to be stressed since nutrients and moisture are in abundant supply. Sources from which the fecal indicators originated might also explain differences between the results of our study and previous studies. The source of *E. coli* from stormwater samples (Characklis et al., 2005; Jeng et al., 2005; Krometis et al., 2007) is unknown and 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). Nevertheless, many different environmental strains of dairy cow *E. coli* were also applied to the plots in this study and attachment rates also differ among strains from the same source (Muirhead et al., 2005). Therefore, even though the source species in the stormwater studies are unknown, it is difficult to attribute lower attachment ratios to differences in environmental strains without knowledge of the strains present in each study.

6.3.2. Bacterial Attachment and TSS Concentrations

Raindrops detached fecal material from cowpats but the thick vegetation aided to reduce particulate transport. The TSS flow-weighted concentration of 152 mg L^{-1} (Table 6.1) is similar to TSS concentrations in runoff from previous pastureland studies and slightly higher than

simulated pastureland plots receiving liquid dairy and poultry litter applications (Soupir et al., 2006a). A correlation analysis found no significant linear relationships between bacterial concentrations, PC, or PAF and TSS concentrations. Characklis et al. (2005) identified potential linkages between in-stream fecal coliform partitioning and particle number concentrations (R^2 = 0.51), and Fries et al. (2006) found that concentrations of *E. coli* and enterococci increased along with particulates in suspension following storm events. After being transported into the stream and prolonged exposure to the external environment, bacteria attachment might increase to enhance survival (Morita, 1997). The exposed cell surface of the attached cell is decreased and the attached portion of the cell does not participate in substrate uptake. In addition to surface attachment, bacteria also bioflocculate, usually when substrates are depleted and bacteria are stressed for nutrients (Morita, 1997). Therefore, once in- stream, the availability of attachment sites could influence attachment. If in fact attachment is limited by availability of attachment sites, bacterial attachment from poorly managed pasturelands with lower vegetative cover and erosive soils might be higher than the PAF presented in this study.

6.3.3. Bacterial Attachment and Nutrient Partitioning

NPS pollution models assess sources of microbial loadings in watersheds and identify reductions necessary for these sources to meet water quality standards. These models already have mechanisms in place to partition between dissolved and suspended forms of nutrients, so relationships developed between bacterial partitioning and nutrient partitioning could be easily incorporated in these models. Partitioning coefficients and PAF were calculated for phosphorus, organic phosphorus, and organic carbon to compare trends between the pathogen indicator and nutrient fractions. A correlation analysis was conducted to investigate the potential linear relationships between bacterial and nutrient partitioning. Data were normalized and statistically significant ($p \leq 0.05$) correlation coefficients are presented in Table 6.2. The correlation analysis provides an initial indication as to which nutrient variables could predict bacterial partitioning by water quality models. This analysis indicates that *E. coli* PC is most closely related to TP PC (r = 0.50800); correlations also exist between TP PAF (r = 0.48819), TOP PC (r = 0.49983), and TOP PAF (r = 0.49100). Correlations between enterococci partitioning and phosphorous and organic carbon were not as strong, but TOP PC (r = 0.37557) and TOC (r = 0.42220 and r = 0.41358) might both aid in prediction of enterococci partitioning at the edge-of-the-field.

Nutrients and nutrient fractions not significantly correlated with bacteria PC or PAF included TP, TP PAF, DOC, TOC PC, and TOC PAF. Total concentrations of *E. coli* and enterococci also were not significantly correlated with nutrient TC, PC or PAF. While it appears that some statistically significant correlations exist, these relationships are relatively weak and lower than the Pearson correlation coefficient value of 0.6 which is a minimum value necessary to identify linear relationships between parameters (Ott and Longnecker, 2001). Additional research and data collection is needed prior to implementation of these linear relationships into NPS models.

Table 6.2. Pearson correlation coefficients identify statistically significant relationships between nutrient parameters and bacterial partitioning.

	TDP TC	TP PC	TP PAF	DOP	TOP	TOP PC	TOP PAF	TOC
E. coli PC	-0.40074[1]	0.50800	0.49235	-0.40593	NS	0.49983	0.49541	0.37521
E. coli PAF	-0.39995	0.50414	0.48819	-0.40493	NS	0.49622	0.49100	0.36824
enterococci PC	NS	0.38811	0.39793	NS	0.43041	0.37557	0.37895	0.42220
enterococci PAF	NS	0.39312	0.40209	NS	0.41113	0.37808	0.37996	0.41358

[1]All values presented significant at the $p \leq 0.05$ level, data normalized prior to analysis

6.3.4. Bacterial and Nutrient Loading Rates

Loading curves describing the bacterial partitioning over the duration of a storm event from a single plot are presented in Figure 6.4. Plot 2 was selected because runoff first began from this plot, resulting in the highest number of samples. The unattached fraction consistently exceeded the attached fraction by more than an order of magnitude. The overwhelming loading of indicators in the unattached state suggests that management practices should focus on removal of unattached cells to improve water quality from agricultural landuses with dense vegetation that receive high applications of animal waste.

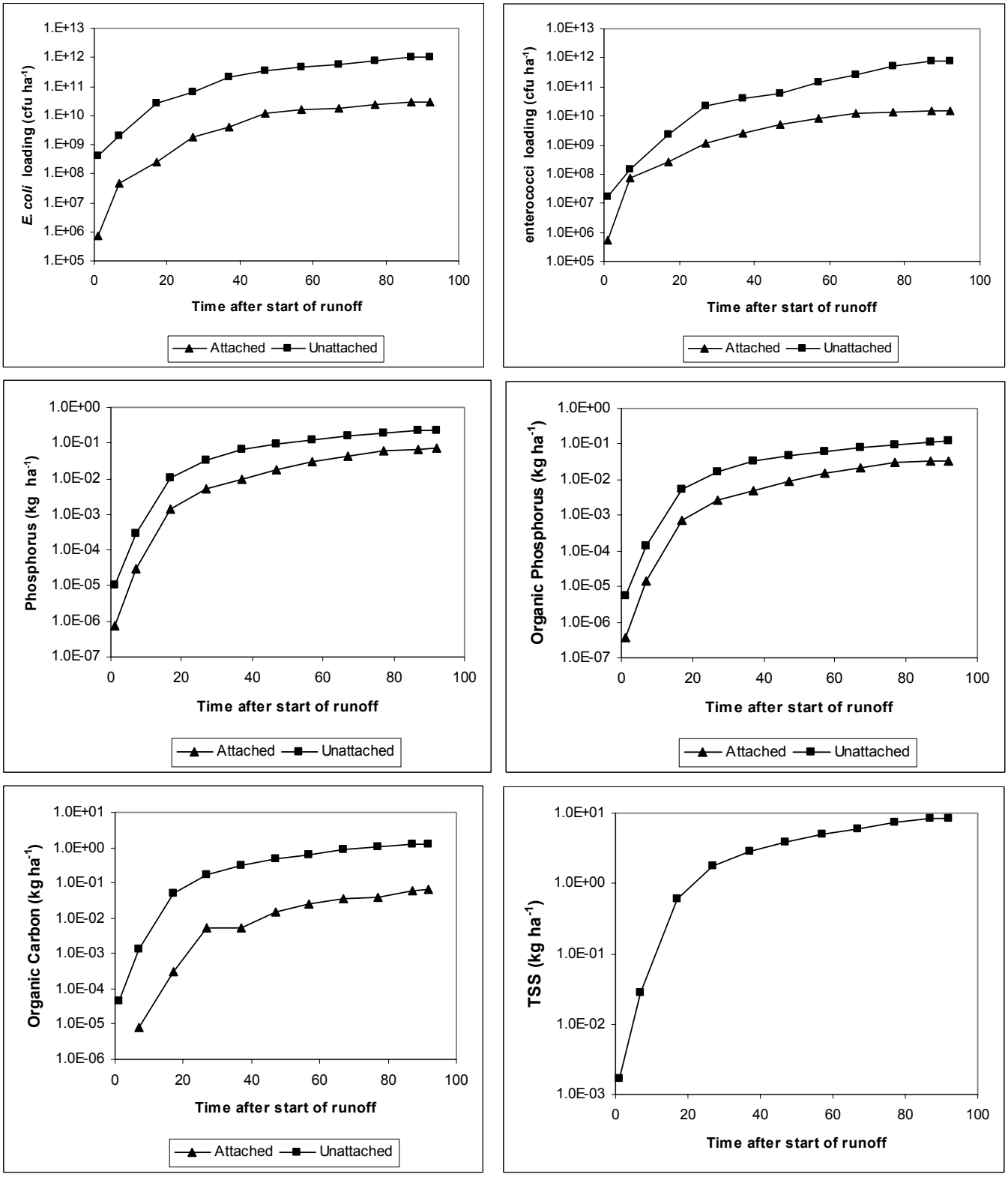

Figure 6.4. ***E. coli*** **and enterococci loading rates from a single plot (plot 2) treated with cowpats during an overland flow event.**

Because loading curves appeared to follow similar trends as nutrients, a second correlation analysis was conducted to investigate the potential linear relationships between bacterial and nutrient loading rates. Again, data were normalized and statistically significant ($p \leq 0.05$) correlation coefficients are presented in Table 6.3. The correlation analysis provides an initial indication as to which nutrient variables could predict bacterial partitioning by water quality models. Significant correlations were not found when relating ratios of partitioned bacteria and nutrient phases. A different approach could be to model separately the attached and unattached phases *of E. coli* and enterococci. Attached *E. coli* loads were significantly correlated with SOP (r = 0.84682). Unattached *E. coli* (r = 0.93166) and both attached and unattached enterococci loading curves (r = 0.92257 and 0.87567, respectively) were most closely related to DOC.

Table 6.3. Pearson correlation coefficients identify statistically significant relationships between bacteria and nutrient partitioning loads

	TSP	TDP	P ratio	SOP	DOP	Organic P ratio	TSC	DOC	Organic C ratio	TSS
E. coli attached	0.83974[1]	0.74154	NS	0.84682	0.74581	NS	0.69583	0.83832	NS	0.74991
E. coli unattached	0.82664	0.88770	0.46104	0.82786	0.88980	0.39711	0.82757	0.93166	NS	0.83984
E. coli ratio	0.60040	0.35792	NS	0.60993	0.36235	NS	NS	0.45832	NS	0.41334
enterococci attached	0.87319	0.89986	0.38204	0.87499	0.90161	NS	0.76396	0.92257	NS	0.85686
enterococci unattached	0.77906	0.84105	0.46726	0.77963	0.84248	0.39569	0.74988	0.87567	NS	0.77922
enterococci ratio	NS	NS	NS	NS	NS	NS	-0.37S046	NS	NS	NS

[1]All values presented significant at the $p \leq 0.05$ level, data normalized prior to analysis

6.3.5. Regression Equations for Predicting Partitioning Ratios and Loading Curves

Relationships between bacterial indicators and nutrient partition were examined by regression models in an effort to predict bacterial PC and loading rates. Attempts were made to model *E. coli* and enterococci PC, PAF, and TC with two sets of independent variables: flow, TSS, TP, TDP,TOP, DOP, TOC, DOC, and flow, TSS, TP, P ratio, TOP, organic P ratio, TOC, and organic C ratio (Appendix G). The strongest relationships to predict *E. coli* and enterococci ratios are as follows:

$$\textit{E. coli}\ \text{PC (ln)} = -12.765 - [0.981 \times \text{TDP (mg L}^{-1})] + [0.648 \times \text{TOC (mg L}^{-1})] \quad [6.3]$$

$$\text{enterococci PC (ln)} = -17.088 - [10.59 \times \text{runoff volume (m}^3)] + [0.949 \times \text{TOC (mg L}^{-1})] \quad [6.4]$$

Both models were statistically significant ($p = 0.01$ and $p = 0.01$, respectively) and all slopes and intercepts were statistically significant as well ($p \leq 0.05$) except TOC in the *E. coli* ratio model ($p = 0.07$). While it appears that TOC, TDP, and runoff volume might explain some trends in the dataset, correlations were relatively weak (adjusted $R^2 = 0.22$ and $R^2 = 0.25$, respectively). The enterococci PAF also correlated with runoff volume and TOC ($R^2 = 0.23$) while the *E. coli* PAF correlated best with DOP and TOC ($R^2 = 0.22$).

Similarities between the loading curves indicated that a greater potential might exist to model that unattached and attached bacterial loading rates separately. Regression models examined for potential to predict *E. coli* and enterococci loading rates with two sets of independent variables. Group 1 included TSS, TP, TDP,TOP, DOP, TOC, DOC and group 2 included TSS, TP, P loading ratio, TOP, organic P loading ratio, TOC, and organic C loading ratio as independent variables. *E. coli* and enterococci loading ratios (attached loading rate/unattached loading rate) were modeled as a function of TSS, TP, P loading ratio, TOP, organic P loading ratio, TOC, and organic C loading ratio. The strongest relationships for predicting *E. coli* and enterococci loading rates are as follows:

E. coli unattached (ln kg ha^{-1}) =

$$21.20 + [182.7 \times TP\ (kg\ ha^{-1})] - [25.88 \times DOC\ (kg\ ha^{-1})] - [1349 \times TP^2\ (kg\ ha^{-1})] + [63.90 \times DOC^2\ (kg\ ha^{-1})] \quad [6.5]$$

Adjusted $R^2 = 0.85$

E. coli attached (ln kg ha^{-1}) =

$$15.87 + [3.647 \times TSS\ (kg\ ha^{-1})] + [4.473 \times P\ ratio] - [0.8014 \times TSS^2\ (kg\ ha^{-1})] - [1.045 \times P\ ratio^2 + [17.99 \times TOC^2\ (kg\ ha^{-1})] \quad [6.6]$$

Adjusted $R^2 = 0.86$

enterococci unattached (ln kg ha^{-1}) =

$$17.79 + [3.973 \times TSS\ (kg\ ha^{-1})] + [2.403 \times OP\ ratio] - [0.8577 \times TSS^2\ (kg\ ha^{-1})] - [0.6558 \times OP\ ratio^2\ (kg\ ha^{-1})] + [19.78 \times TOC^2\ (kg\ ha^{-1})] \quad [6.7]$$

Adjusted $R^2 = 0.90$

enterococci attached (ln kg ha^{-1}) =

$$17.69 + [55.05 \times [TP\ (kg\ ha^{-1})] - [123.4 \times TP^2\ (kg\ ha^{-1})] \quad [6.8]$$

Adjusted $R^2 = 0.77$

Equations 6.5, 6.6, 6.7, and 6.8 are all statistically significant ($p \leq 0.0001$) and all intercepts and slopes are also statistically significant ($p \leq 0.01$) except for DOC in equation 6.5 ($p \leq 0.07$). Attempts to predict *E. coli* and enterococci loading ratios resulted in lower coefficient of determinations (adjusted $R^2 = 0.58$ and $R^2 = 0.47$, respectively) and not all slopes were statistically significant (Appendix G). Results of this study indicate that prediction of indicator loading rates in the attached and unattached phases at the edge-of-the-field is possible with combinations of phosphorous, carbon and TSS. While these equations are the best that we were able to develop from this limited dataset, the applications of these equations are limited. Most notably, the attached enterococci loading rate equation did not include TSS as a statistically significant independent variable. These equations are only applicable to the specific scenario simulated in this study and because of the limited dataset they have not been validated.

After *E. coli* and enterococci enter into the stream, literature suggests that partitioning between phases might be predicted by availability of attachment sites (Characklis et al., 2005; Fries et al., 2006), resuspension of bottom sediments (An et al., 2002; Burton et al., 1987; Jamieson et al., 2003; Sayler et al., 1975), and changes in flow regime (Guber et al., 2005a; Krometis et al., 2007); however, relationships between *E. coli* and enterococci in-stream partitioning and phosphorous partitioning has not yet been examined to the best of our knowledge.

6.3.6. Preferential Attachment to Particulates

The TSS associated with each particle size category were weighed and used to identify the particle sizes to which fecal bacteria preferentially attach. Total suspended solids associated with a screen size weighing less than 1 mg 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. 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 as presented in Table 6.4 and Appendix D. The larger surface area of the smaller particles corresponds to a higher number of sites available for bacterial attachment. 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

which 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 6.4. Particle sizes to which *E. coli* and enterococci preferentially attach in samples collected during an overland flow event.

		E. coli		**enterococci**	
Particle Size	Mean TSS (mg/L)	Mean cfu/mg solids	% attached	Mean cfu/mg solids	% attached
>500 μm	43.9	851ab[1]	28%	240b	13%
63 - 499 μm	70.7	433b	14%	549a	29%
8 - 62 μm	171.9	1,766a	58%	1,095a	58%

[1]Means followed by the same letter do not differ at the 5% level of significance according to Tukey's pairwise comparison.

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 unattached 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.

While the majority of attached *E. coli* and enterococci cells were associated with particulates retained by an 8 μm screen, the overall low attachment ratios indicate that reducing particulate transport will not sufficiently reduce transport of pathogen indicators to surface waters. Between 87 and 95% of all fecal indicators transported along overland flow pathways from a fresh manure source are not attached to either sediments or manure particulates. It is possible that attachment might be higher in runoff from different sources or land management scenarios. Thick vegetative cover, present in our study, reduced TSS concentrations when compared to levels that might be observed from pasturelands or different agricultural landuses. In that case, it is possible that attachment to particulates could be a significant transport mechanism and management practices would need to focus on retention of the smallest particulates (8 μm in this study) to reduce the bacterial loadings and improve water quality.

6.4. Summary and Conclusions

A field study was conducted to evaluate the partitioning of *E. coli* and enterococci between the unattached and attached phases in runoff from virgin pasturelands and to identify the particle sizes to which the fecal indicators preferentially attach. Field plots were constructed on simulated pastureland with high vegetative cover to determine partitioning ratios of *E. coli* and enterococci in runoff samples collected at the edge-of-the-field. The goal of this study was to investigate the partitioning of *E. coli* and enterococci between the unattached and particulate-attached phases during overland flow from pasturelands by examining correlations between bacterial and nutrient partitioning ratios and loading rates and developing relationships to predict *E. coli* and enterococci partitioning between the attached and unattached phases with suspended solids and nutrient data. The particle sizes to which the attached bacteria preferentially associated were identified. *E. coli* and enterococci partitioning were not clearly related to the dissolved/suspended phosphorus ratio as hypothesized since all linear relationships between bacterial and nutrient ratios were weak. The majority of cells were associated with particles retained by the 8 μm filter.

Results indicate that the majority of bacterial indicator organisms are transported from a fresh manure source in the unattached state. Average PC for *E. coli* was 0.06 which corresponds to 4.8% attachment and 0.18 for enterococci corresponding to 13% attachment. Low attachment rates might be best explained by the low TSS concentrations (relative to poorly managed and other agricultural landuses) and competition for attachment sites between fecal indicators and organic carbon. Linear correlations existed between *E. coli* loading rates and SOP while unattached *E. coli* and both attached and unattached enterococci loading rates were most closely related to DOC. Regression models to predict unattached and attached indicator loading rates separately as a function of phosphorous and organic carbon were developed; however, application of these models to landuse scenarios other than highly vegetated pastureland is not recommended. Comparison of unattached and attached indicator loading rates found that the unattached fraction exceeded the attached fraction by at least two orders of magnitude. Fifty-eight percent of all attached cells were associated with particles between 8 - 62 μm in diameter.

Partitioning ratios developed from this study can be incorporated into NPS models that allow for partitioning between the attached and unattached phases. The majority of cells were transported in the unattached state from pasturelands with high vegetative cover receiving fresh fecal deposits and therefore, development of best management practices for well-managed pastureland scenarios should focus on reduction of unattached pathogen indicators. Future study is recommended to determine partitioning of indicators from different landuses with higher transport of suspended solids and from aged fecal sources.

Chapter 7. *E. coli* and Enterococci Attachment to Particles during Runoff from High and Low Vegetative Cover Pastureland

7.1. Introduction

Runoff from grazed pasturelands often contributes bacterial loadings resulting in downstream water quality impairments (Doran and Linn, 1979; Doran et al., 1981; Moore et al., 1982). Flow-weighted concentrations of *E. coli*, fecal coliform, and enterococci in runoff from simulated pasturelands receiving cowpat applications were 1.37×10^5, 1.65×10^5 and 1.19×10^5, respectively (Soupir et al., 2006b). Stream bank fencing is often recommended as a management practice to reduce pollutant loadings to surface waters (Line, 2003) when cattle have direct access to streams; however, other management practices, in addition to stream fencing, are likely necessary to reduce fecal loadings to the streams from grazed lands (Oliver et al., 2007b). Vegetated buffer strips are often promoted as a practice to reduce pollution transport to surface waters, but their effectiveness in reducing pathogen indicators has produced mixed results (Coyne et al., 1995; Entry et al., 2000; Larsen et al., 1994; Lim et al., 1998). Meals (2001) found that the combination of buffers, riparian fencing and protected stream crossings were necessary for significant reduction of bacterial counts in an agricultural watershed in Vermont.

Limited understanding of how microbes are released from fecal matter and transported along overland flow pathways results in high uncertainty in bacterial fate and transport models (Collins and Rutherford, 2004). Specifically, little is known about microbial partitioning between the freely suspended and particulate attached phases (Oliver et al., 2007b) and data on the partitioning between these two phases are not yet available (Benham et al., 2006; Collins and Rutherford, 2004; Jamieson et al., 2004). Previous chapters in this dissertation have investigated attachment rates present in runoff from different pastureland management scenarios and found low attachment rates averaging 4.8% attached for *E. coli* and 13% attached for enterococci when cowpats were applied to simulated pasturelands. These low attachment rates might be explained by the low TSS concentrations present in runoff, but linear correlations between attachment rates and TSS concentrations were not observed. The application of a single cowpat to bare soil box plots resulted in much higher attachment rates ranging from 28% to 49% attached for both *E. coli* and enterococci. While the bare soil box plot study indicated that increased particulate

transport might allow for increased attachment because of increased availability of attachment sites, results from the box plot study are not representative of edge-of-field partitioning that would be observed during an overland flow event.

The goal of this study was to investigate the partitioning of *E. coli* and enterococci between the unattached and particulate-attached phases during overland flow from pasturelands. The objectives were to examine partitioning of *E. coli* and enterococci to sediments and organic particles from different pastureland vegetative cover scenarios during overland flow and to identify the particle sizes to which the attached bacteria preferentially associate. Bacterial partitioning was examined for relation to flow regime, TSS, and nutrients. Similar to the previous two chapters, we hypothesized that the *E. coli* and enterococci partitioning would be related to the dissolved/suspended phosphorus ratio and that the majority of cells would be associated with particles retained by the 8 μm filter.

7.2. Materials and Methods

The five field plots described in Chapter 6 were used to conduct a second rainfall simulation. Of the five field plots, vegetation was removed from two of the plots with a dethatcher and string trimmer to represent varying soil cover that might be correlated with overgrazed or poorly-managed pasture conditions. The dethatcher was first used to remove approximately 50% of all established vegetation. Three equally sized bare areas were created in the top, middle, and lower third of the plots using the string trimmer. Areas void of all vegetation accounted for an average of 28.4% of the total plot area. Plots with vegetation removed are referred to as low vegetation plots and the plots without removal of vegetation are referred to as high vegetation plots (Figure 7.1). There were two replicates of each treatment and one control.

Fresh dairy cattle fecal deposits were collected at the Virginia Tech dairy facility over a 24 hour period. Standard cowpats (Thelin and Gifford, 1983) were formed by mixing the manure in a cement mixer for fifteen minutes. The homogenized manure was placed in molds with a diameter of 20.3 cm (8 in) and a depth of 2.54 cm (1 in) until a weight of 0.9 kg (2.0 lbs) was reached. Manure samples were collected prior to land application and analyzed by the Clemson Agricultural Service Laboratory. Water soluble P, (2.02 g kg^{-1}),was determined by the method

proposed by Sharpley and Moyer (2000). The pH, (5.6), was measured potentiometrically in a 1:2 manure/water slurry (Peters et al., 2003). Average moisture content of fresh manure samples was 83.61%. Approximately 106 cowpats were applied to four of the five plots to represent a heavily grazed area. The plots receiving manure applications had received a previous, similar application of fresh fecal cowpats approximately four months prior to this application (Chapter 6). Previous cowpats had disintegrated due to warm temperatures, thick vegetation and frequent mowing; however, it is likely that a fraction of the total concentrations of fecal indicators was due to the previous manure applications (Kress and Gifford, 1984). One plot received no treatment and was used as a control.

Figure 7.1. Poorly-managed and well-managed pasturelands received cowpat applications

Due to the unreliability of natural precipitation for short-term field research, a rainfall simulator (Dillaha et al., 1988) generated a uniform rainfall event (2.8 cm/h) to all plots. After the beginning of runoff, discreet grab samples were collected at the outfall of the flumes (Figure 6.1). Samples were collected at the onset of runoff, at ten minute intervals during the storm event, at the end of the storm event, and four minutes after the precipitation ceased. Three types of samples were collected during each sampling event, one for bacterial partitioning studies, one for total *E. coli* and enterococci concentration analysis, and one for nutrient analysis. The rainfall event continued until runoff from all plots reached steady state (four hours and 11 minutes) and the longest runoff event lasted 105 minutes (plot 2). Steady-state occurred when flow at the outfall of the flumes remained constant as determined by the stage recorder.

7.2.1. Bacterial Partitioning and Enumeration

Collected samples were transported to the laboratory immediately following the end of the rainfall simulation and analyzed for *E. coli* and enterococci within 24 hours. Partitioning of pathogen indicators between attached and unattached phases was achieved by fractional filtration followed by centrifugation. Fractional filtration has been used previously to identify particle sizes to which bacteria are attached (Auer and Niehaus, 1993; Schillinger and Gannon, 1985), and a filter pore size of 8 μm has been identified as a viable method to separate attached and free bacteria (Gordon et al., 2002; Henry, 2004; Mahler et al., 2000; Qualls et al., 1983). The presence of sediments and organic particles in runoff from agricultural lands makes it very likely that the filters could clog and retain free cells, resulting in a higher fraction of cells being classified as attached. To assess the retained, unattached cells, we rinsed the screens and filters with phosphate buffered water (Hach Company, Loveland, CO) and then centrifuged the re-suspended solution. This technique combines the benefits of fractional filtration, identifying particle sizes to which cells attach, with the more common practice of centrifugation (Chapter 3).

The number 35 mesh screen (Bel-Art Products, Pequannock, NJ) was used to retain particles larger than coarse sand (>500 μm) and number 230 mesh screen was used to retain medium, fine, and very fine sand (63 - 500 μm). An 8 μm filter (Poretics, Polycarbonate, GE Water and Processes Technologies) was used to retain fine, medium, and coarse silt particles and a 3 μm filter (Nuclepore Track – Etch Membrane Filtration Products, Whatman) was used to retain clay and very fine silt particles. Throughout the study no measurable particulates passed thorough the 8 μm filter. Following filtration, the retained solids were re-suspended in phosphate buffered water and centrifuged (Avanti J-25I, Beckman Coulter, Fullerton, CA) at 4,700 rpm for 15 seconds (Huysman and Verstraete, 1993; Lago, 2005). The filtrate and supernatant was enumerated for *E. coli* and enterococci concentrations on Modified mTEC and mE agar (U.S. EPA, 2000) using membrane filtration (Clesceri et al., 1998) to assess the unattached bacterial concentrations. Following centrifugation, the solutions associated with each particle size were re-suspended and dispersed prior to enumeration of the total *E. coli* and enterococci concentration by treatment with a hand shaker for 10 minutes (Chapter 4). The dispersed solution was enumerated for *E. coli* and enterococci concentrations on Modified mTEC and mE agar (U.S. EPA, 2000) by membrane filtration (Clesceri et al., 1998).

7.2.2. Nutrient Analysis

Runoff samples were analyzed for nutrient and suspended solids concentrations to examine potential relationships between bacterial and nutrient attachment ratios. The nutrient analysis was performed following procedures in Standard Methods for the Examination of Wastewater (Clesceri et al., 1998). Nutrient analysis included total dissolved phosphorous (TDP, 0.45 μm polyethersulfone filter, Pall Life Sciences, Ann Arbor, MI), total phosphorous (TP), dissolved organic phosphorous (DOP), total organic phosphorous (TOP), dissolved organic carbon (DOC), and total organic carbon (TOC). Total suspended phosphorous (TSP) was calculated as the difference between TP and TDP and suspended organic phosphorous (SOP) was calculated as the difference between DOP and TOP (Clesceri et al., 1998). Suspended organic carbon was calculated as the difference between TOC and DOC. Total Suspended Solids were analyzed (0.45 μm glass fiber filter, Pall Life Sciences, Ann Arbor, MI) as recommended by Clescersi et al. (1998).

7.2.3. Calculations and Statistical Analysis

The attached cells were assumed to be the difference between the unattached and total *E. coli* and enterococci concentrations. The partitioning coefficient was calculated using Equation 7.1 and the particulate associated fraction was calculated using Equation 7.2. The attached portion associated with each screen size was divided by the TSS associated with respective screen size to obtain the colony forming units per gram of particulates and determine the particle sizes to which *E. coli* and enterococci preferentially attach.

$$Partitioning\ Coefficient = \frac{attached}{planktonic} \qquad [7.1]$$

$$Partitculate\ Attached\ Fraction = \frac{attached}{attached + planktonic} \qquad [7.2]$$

Statistical analysis of data was performed using the Statistical Analysis System (SAS Institute, 2004). A 2-way ANOVA and Tukey's pairwise comparison were used to test for significant

differences between partitioning coefficients and total concentrations during the rising, peak, and receding limbs of the runoff hydrograph and between high and low vegetative cover plots using the MIXED procedure in SAS. Multiple regression analysis was conducted using the REG procedure in SAS and analysis of variance (ANOVA) was used to determine statistical significance of the models. Statistical significance between particle sizes to which bacteria preferentially associate was also determined by ANOVA and Tukey's pairwise comparison. When necessary, data were normalized prior to analysis and statistical significance was determined when $p \leq 0.05$.

7.3. Results and Discussion

The average *E. coli*, enterococci, and nutrient flow-weighted concentrations (FWC) are presented in Table 7.1 and individual samples are presented in Appendix H. Flow-weighted concentrations were calculated by multiplying the sample concentrations by the subsequent runoff volume, summing the multiplied terms, and then dividing by the total runoff volume from each plot. Nutrient analysis included total and organic forms of phosphorus to account for inorganic residual from fall fertilizer application and the organic forms present in fresh cowpats and the cowpats from the previous simulation. The most notable difference in pollutant transport between the high and low vegetative cover plots was in the TSS FWC. Thick vegetation prevented particulate transport with average FWC of 51.02 mg L^{-1} while removal of vegetation increased TSS concentrations by an order of magnitude to 670.2 mg L^{-1}.

One plot received no treatment and was used as a control. Background *E. coli* concentrations flow-weighted concentrations were 2.52×10^{2} cfu 100 mL^{-1} and enterococci concentrations were 4.02×10^{3} cfu 100 mL^{-1}. The background *E. coli* was detected in five of the twelve samples collected during the runoff event while the enterococci was detected in all samples. Background bacterial loading are most likely attributed to wildlife (Doran et al., 1981; Patni et al., 1985).

Table 7.1. Average *E. coli*, enterococci, total suspended solids, and nutrient flow-weighted concentrations (FWC).

	FWC High-vegetation	FWC Low-vegetation	FWC Control	units
E. coli	2.73×10^{4}	2.00×10^{4}	2.52×10^{2}	cfu 100 mL^{-1}
enterococci	1.51×10^{4}	1.36×10^{4}	4.02×10^{3}	cfu 100 mL^{-1}
Total dissolved phosphorous	4.48	4.66	0.115	mg L^{-1}
Total suspended phosphorous	0.78	1.17	5.21×10^{-3}	mg L^{-1}
Total phosphorous	5.26	5.83	0.12	mg L^{-1}
Dissolved organic phosphorous	2.20	2.32	4.95×10^{-2}	mg L^{-1}
Suspended organic phosphorous	0.55	0.71	2.51×10^{-3}	mg L^{-1}
Total organic phosphorous	2.74	3.03	5.13×10^{-2}	mg L^{-1}
Dissolved organic carbon	15.54	14.09	10.0	mg L^{-1}
Suspended organic carbon	1.54	1.25	0.89	mg L^{-1}
Total organic carbon	17.08	15.35	10.89	mg L^{-1}
Total suspended solids	51.02	670.20	23.15	mg L^{-1}

7.3.1. Bacterial Partitioning Related to Flow Regime and Vegetative Cover

Previous research has determined that bacterial partitioning might be impacted by flow velocities (Guber et al., 2005b; Krometis et al., 2007) so bacterial and nutrient partitioning coefficients were separated into rising, peak, and receding limbs of the overland flow hydrograph (Figure 7.2). The number of samples collected from each plot varied due to different beginning of runoff times but ranged from seven to twelve samples. Typically, 11 samples were included in the rising limb analysis, 6 samples in the peak limb analysis, and 2 samples in the falling limb analysis. Partitioning coefficients are presented as box and whisker plots illustrating 10^{th}, 25^{th}, 75^{th}, and 90^{th} percentiles.

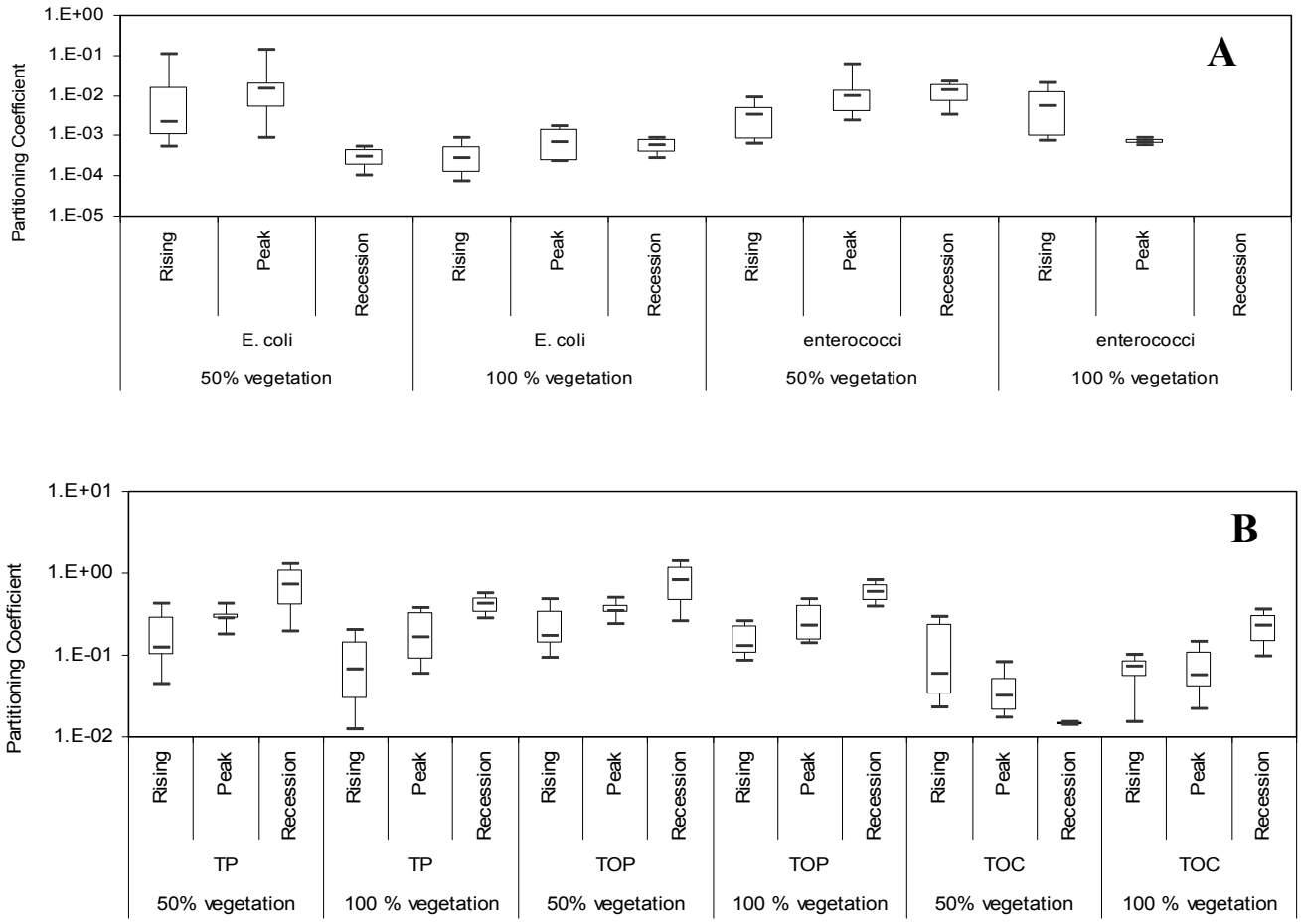

Figure 7.2. Partitioning coefficients of a) *E. coli*, enterococci, and b) phosphorus, organic phosphorus, and organic carbon in runoff from pasturelands during the rising, peak, and recession limbs of a runoff hydrograph from high and low vegetation plots.

A 2-way ANOVA with Tukey's pairwise comparison was used to test for significant differences between partitioning coefficients and total concentrations during the rising, peak, and receding limbs of the runoff hydrograph and between well managed and poorly managed pasture plots (Appendix I). The *E. coli* PC did not differ statistically between the rising, peak or recession limbs of the runoff hydrograph from either the well managed or poorly managed plots. Among treatments, the *E. coli* PC from plots with poorly managed pasture was significantly higher than the *E. coli* PC from well managed plots. Interactions between plot treatment and hydrograph stage (rising, peak and recession) were not found to be statistically significant and the reduced model determined that statistically significant differences in *E. coli* PC existed between the two

plot vegetative covers (*p-value* = 0.0002). Interactions between plot treatment and hydrograph stage were found to be statistically significant in enterococci PC but no differences existed between PC in runoff from the two treatments or runoff stages.

Similar analysis was conducted on phosphorous and carbon PC in runoff (Appendix I). The full model found no statistically significant differences between plot treatment or hydrograph stage; however, interactions were not significant and the reduced model determined that the TP PC was statistically lower from the well managed pasture plots (p-value = 0.0489) as compared with the poorly managed pasture plots. Flow appeared to impact TOP PC more than plot treatment. Again, interactions among treatment and stage were not significant and the only statistically significant difference occurred between the TOP PC in runoff from the rising and receding limbs of the runoff hydrograph (p-value = 0.0313). The DOC PC differed between the two plot treatments (p-value = 0.0397).

Figure 7.3 presents total bacterial concentrations during the rising, peak, and receding limbs of the overland flow hydrograph. The total *E. coli* and enterococci concentrations were not impacted by vegetative cover; the only statistically significant differences (p-value = 0.0387 for *E. coli* and p-value = 0.0007 for enterococci) occurred between the rising and peak limbs of the runoff hydrographs. Similar analysis was conducted on total TSS, phosphorous and carbon concentrations (Appendix I). Well-managed pasture significantly reduced TSS concentrations (p-value < 0.0001) and increased TOC TC (p-value = 0.0217) while the TP and TOP TC were only impacted by the hydrograph stage in runoff from the high vegetation plots. The rising limb was statistically higher than the peak and receding limbs for both forms of phosphorous.

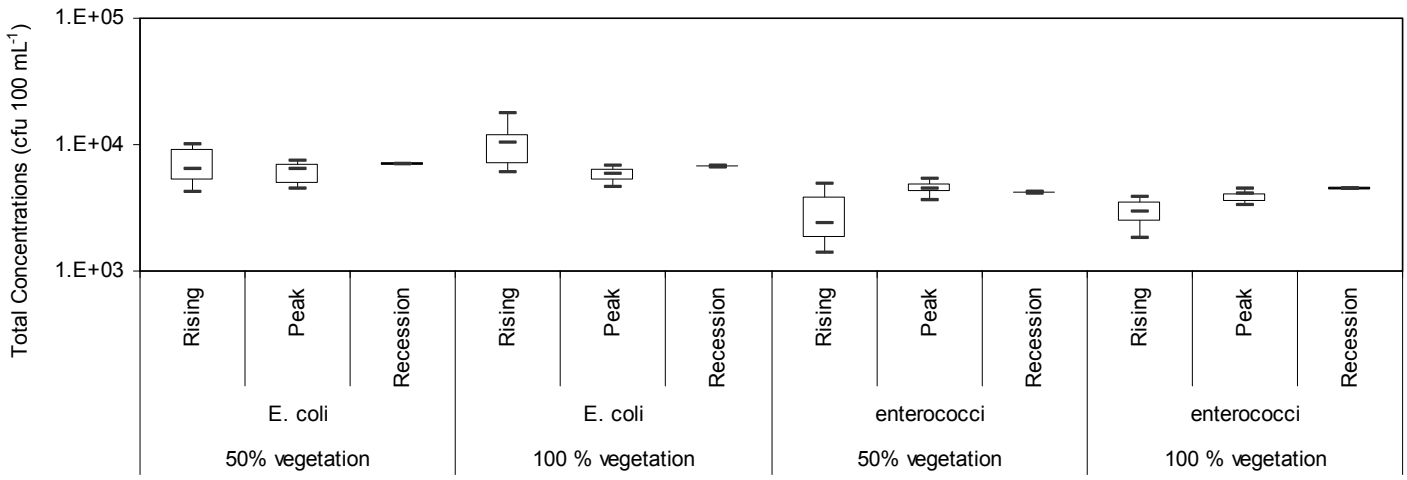

Figure 7.3. Bacterial total concentrations in runoff from pasturelands during the rising, peak, and recession limbs of a runoff hydrograph for *E. coli* and enterocci from pasturelands with 100 and 50% vegetative cover.

The average *E. coli* PC for all samples collected was 0.0006 from plots with high vegetative cover and 0.029 from plots with low vegetative cover, which corresponded to a particulate attached fraction (PAF) of 0.06% and 2.8%, respectively. The average PC for enterococci was 0.0103 from well managed plots and 0.0132 from poorly managed plots, which corresponded to a PAF of 0.98% and 1.23%, respectively. Partitioning coefficients and PAF were calculated using Equations 7.1 and 7.2. In general, attachment from both high and low vegetative cover plots was low with less than 3% of all *E. coli* and enterococci classified as attached. Among the two fecal indicators, only *E. coli* attachment was statistically impacted by vegetative cover and hydrograph stage did not impact attachment of either indicator. The overall low attachment rates and the relatively small impact of the reduced vegetation (and greater TSS transport) were both unexpected.

7.3.2. Bacterial Attachment and TSS Concentrations

The increase in TSS FWC between the high and low vegetative plot treatments was 619.2 mg L^{-1}. While thick vegetation efficiently reduced particulate transport, no reductions in *E. coli* or enterococci FWC were observed (Table 7.1), as concentrations from high vegetation plots were slightly higher than those resulted from low vegetation plots. Similarly, differences in PC and PAF between the two plot treatments were also very minor. This was not the case, however, when comparing the attachment rates among the three rainfall simulation studies. The previous

edge-of-field partitioning transport study (Chapter 6) examined *E. coli* and enterococci partitioning in runoff from highly vegetated plots treated with cowpats. This study found average *E. coli* PC for all samples collected was 0.06 which corresponded to a PAF of 4.8% and the average PC for enterococci was 0.18 with corresponding PAF of 13%. The average PAF was higher for enterococci (13%) than *E. coli* (4.8%). While only highly vegetated plots were examined in the previous study, the average TSS FWC was 152 mg L^{-1}; 101.2 mg L^{-1} higher than the average TSS FWC from the highly vegetated plots in this study. Denser vegetation present at the end of the summer explains the reduced TSS FWC in the second study and the reduced TSS FWC also could explain the lower attachment rates observed during the second rainfall simulation. TSS concentrations present in runoff from a bare plot soil box study (also using Grosclose silty loam soils) averaged 4.1 g L^{-1}, while *E. coli* and enterococci attachment averaged 30 and 49%, respectively (Chapter 5). Particulate transport from the box plot study was much higher than this study because of the experimental design: no vegetation to prevent sediment transport, a short distance between detachment sites and sample collection, and a high intensity rainfall application rate. Even though the experimental set-up differed, the key factor distinguishing attachment rates between the studies is the greater particulate transport. The combined results from these three simulations indicate that vegetation is very effective in filtering out particulates along with the associated pollutants and greater particulate transport corresponds to higher attachment rates. High particulate transport might be necessary to allow for sufficient attachment sites when competition exists between carbon, organic particulates, and bacteria for free attachment sites.

7.3.3. Bacterial Attachment and Nutrient Concentrations

NPS pollution models are used to assess sources of microbial loadings in watersheds and identify reductions necessary from these sources to meet water quality standards. These models already have mechanisms in place to partition between dissolved and suspended forms of nutrients, so relationships developed between bacterial partitioning and nutrients could be easily incorporated in these models. Relationships between bacterial indicators and nutrients were examined by regression models in an effort to predict bacterial PC and PAF. A single model was developed for both high and low vegetation treatments. Attempts were made to model *E. coli* and enterococci PC, PAF, and TC with two sets of independent variables: flow, TSS, TP, TDP,TOP,

DOP, TOC, DOC; and flow, TSS, TP, P ratio, TOP, organic P ratio, TOC, and organic C ratio. A t-test was used to test for statistical significance between intercepts and slopes and the coefficient of determination, R^2, represents the proportional reduction in the squared error of the response corresponding to the addition of independent variables (Ott and Longnecker, 2001). Variance inflation and residual plots were examined for all models and deemed acceptable. The strongest relationships to predict *E. coli* PC during high and low vegetation are presented in Table 7.2. Attempts to predict enterococci PC produced poor results with the highest R^2 value equal to 0.16 and the only variable influencing enterococci ratios was TSS; however, this parameter was not statistically significant. Predictions of *E. coli* and enterococci TC produced $R^2 = 0.54$ and $R^2 = 0.47$, respectively (Appendix I).

Table 7.2. Regression equations to predict *E. coli* partitioning coefficients (PC) in runoff from plots with high and low vegetative cover.

ln *E. coli* partitioning coefficient: high vegetation			ln *E. coli* partitioning coefficient: low vegetation		
Variable	**Parameter estimate**	***p* value**	**Variable**	**Parameter estimate**	***p* value**
Intercept	-0.750	0.8124	Intercept	-0.750	0.8124
TSS (mg L^{-1})	2.33×10^{-3}	<.0001	TSS (mg L^{-1})	2.33×10^{-3}	<.0001
DOC (mg L^{-1})	-0.479	0.0258	DOC (mg L^{-1})	-0.479	0.0258
			TP (mg L^{-1})	-9.32	0.0832
			DP (mg L^{-1})	1.46	0.0005
			TOP (mg L^{-1})	15.8	0.1249
		$R^2 = 0.7286$			

Both models presented in Table 7.2 were statistically significant ($p \leq 0.0001$). Based on these models, *E. coli* PC in runoff from pasturelands with high vegetation are best predicted with TSS and DOC while the addition of phosphorous is necessary to predict *E. coli* PC in runoff from pasturelands with low vegetative cover.

7.3.4. Bacterial and Nutrient Loading Rates

Loading curves describing the bacterial partitioning over the duration of a storm event from high and low vegetation plots are presented in Figure 7.4. Plots with the longest runoff event and therefore highest number of samples were used to create the figures. The *E. coli* unattached load was greatest from plots with high vegetative cover while the *E. coli* attached load was higher from plots with low vegetative cover. The unattached enterococci load was similar between the two plot treatments but the attached fraction was consistently higher in runoff from plots with low vegetative treatment. While the higher loading of the attached indicators from low

vegetation plots is to be expected, the overwhelming loading of both indicators in the unattached state suggests that management practices should focus on removal of cells transported in the free or unattached state to reduce bacterial transport from freshly grazed pasturelands, regardless of vegetative cover. Nutrient loading rates followed similar patterns as bacterial indicators but with less distinction between the dissolved and suspended phases (Figure 7.4). The highest loading rates were of dissolved phosphorous, organic phosphorous and organic carbon from the plots with high vegetative cover followed by the dissolved nutrients in runoff from the plots with low vegetative cover. Phosphorous and organic phosphorus transported in the suspended phase were indistinguishable between the two plot treatments while SOC loading rate was slightly higher from the plots with high vegetation.

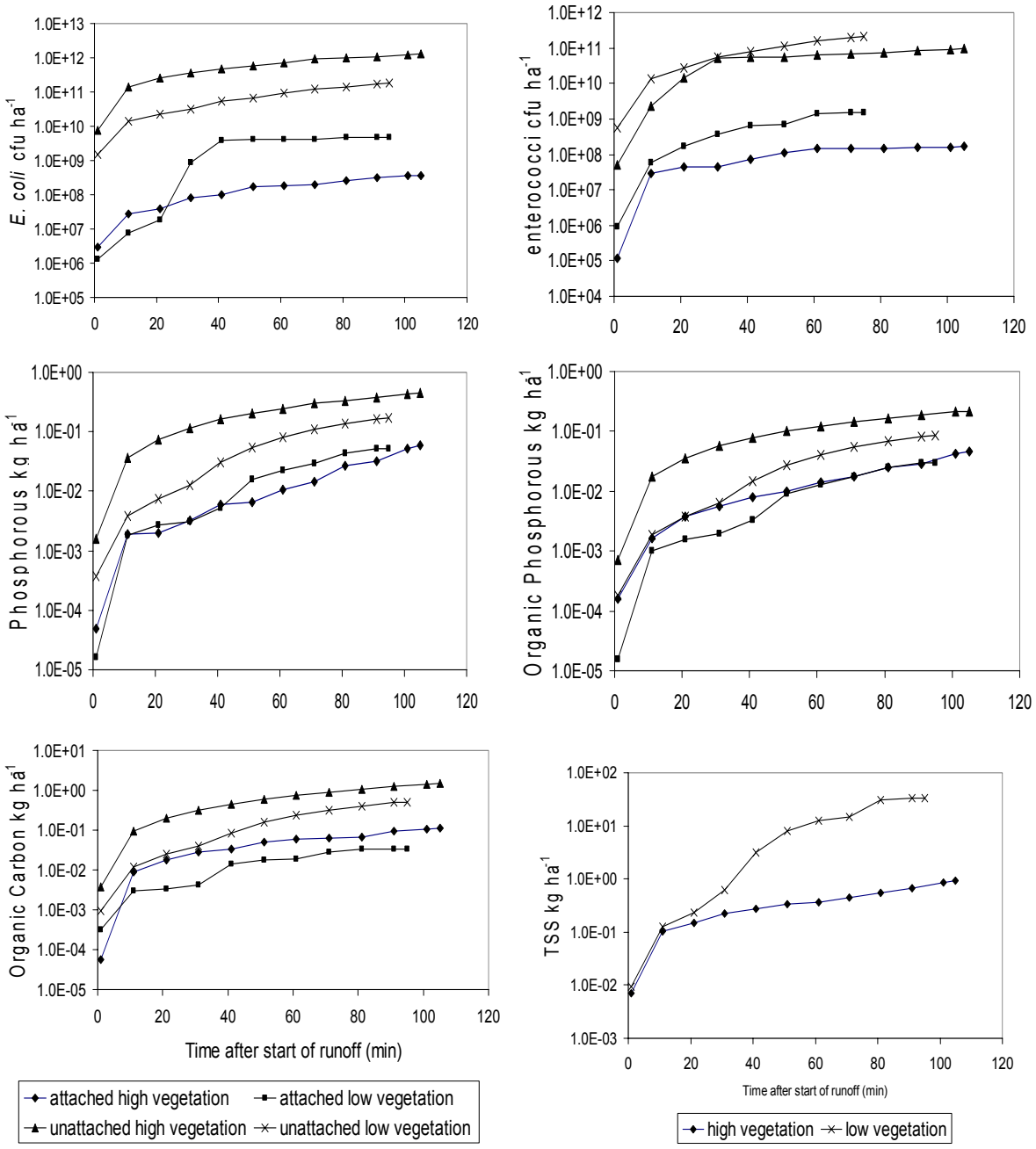

Figure 7.4. *E. coli*, enterococci, phosphorous, organic carbon, and TSS loading rates from plots with high and low vegetative cover treated with cowpats during an overland flow event.

Relationships between bacterial and nutrient loads were examined by regression models in an effort to predict bacterial loading rates. Regression models were examined for potential to predict *E. coli* and enterococci loading rates using TSS, TP, TDP, TOP, DOP, TOC, and DOC

loading rates (kg ha^{-1}) as independent variables. The strongest relationships for predicting *E. coli* and enterococci loading rates are presented in Table 7.3. A dummy variable, z, was used to develop a full model for both high and low vegetation treatments. The presence of the dummy variables in Table 7.3 indicates that statistically significant differences existed between equations which best predicted the bacterial loading rates from the two vegetative treatments. Reduced models representing only one type of vegetative cover are calculated from the full model by substitution of 1 or 0 in for the dummy variable. If z = 1, the responses from the plots with low vegetative cover are modeled and if z = 0 the responses from the plots with high vegetative cover are modeled. Additional model details and residual plots are presented in Appendix I.

Table 7.3. Regression equations to predict *E. coli* and enterococci loading rates (attached and unattached) in runoff from high and low vegetation plots.

Loading Rate Models: *E. coli* and enterococci					
***E. coli* unattached: full model**			**enterococci unattached: full model**		
Variable	**Parameter estimate**	***p* value**	**Variable**	**Parameter estimate**	***p* value**
Intercept	1.52×10^{10}	0.0083	Intercept	-4.06×10^{8}	0.8209
TSS (kg ha^{-1})	-4.68×10^{10}	0.0232	TP (kg ha^{-1})	-1.29×10^{13}	<0.0001
TDP (kg ha^{-1})	1.84×10^{13}	0.0086	TOP (kg ha^{-1})	2.71×10^{13}	<0.0001
DOP (kg ha^{-1})	-2.97×10^{13}	0.0307	TOCP (kg ha^{-1})	9.34×10^{11}	0.0156
TOC (kg ha^{-1})	-3.99×10^{12}	0.0005	DOC (kg ha^{-1})	-1.31×10^{12}	0.0015
DOC (kg ha^{-1})	3.95×10^{12}	0.0007	TOP×z1 (kg ha^{-1})	-2.73×10^{12}	<0.0001
TSS×z (kg ha^{-1})	4.77×10^{10}	0.0212	TOC×z (kg ha^{-1})	-7.25×10^{11}	0.0864
TDP×z (kg ha^{-1})	-4.53×10^{13}	0.0153	DOC×z (kg ha^{-1})	1.35×10^{12}	0.0028
DOP×z (kg ha^{-1})	8.31×10^{13}	0.0300			
TOC ×z (kg ha^{-1})	4.24×10^{12}	0.0025			
DOC×z (kg ha^{-1})	-3.87×10^{12}	0.0067			
$R^2 = 0.9988$			$R^2 = 9896$		
Loading Rate Models: *E. coli* and enterococci					
***E. coli* attached: full model**			**enterococci attached: full model**		
Variable	**Parameter estimate**	***p* value**	**Variable**	**Parameter estimate**	***p* value**
Intercept	-5.86×10^{7}	0.8581	Intercept	-3.46×10^{7}	0.5219
TSS (kg ha^{-1})	4.18×10^{8}	<0.0001	DOP (kg ha^{-1})	1.11×10^{9}	0.0774
z	1.69×10^{9}	0.0167	TP×z (kg ha^{-1})	2.00×10^{9}	0.0007
TP×z (kg ha^{-1})	-2.45×10^{12}	<0.0001	TDP×z (kg ha^{-1})	-1.99×10^{9}	0.0044
TOP×z (kg ha^{-1})	4.64×10^{12}	<0.0001			
$R^2 = 0.9424$			$R^2 = 0.8319$		

All full models were statistically significant with P-value < 0.001. This regression analysis indicates that prediction of indicator organism loading rates at the edge-of-the-field might require a combination of TSS, phosphorous and carbon loading rates for best prediction. Interestingly, TSS was not identified as a significant independent variable when predicting enterococci loading

rates but was a statistically significant independent variable when predicting *E. coli* loading rates (Table 7.3). The TSS variable slope was not consistently negative or positive in both equations to indicate either a positive or negative relationship between particulates and *E. coli* loading rates. Therefore, while these equations are the best that we were able to develop from this limited dataset, the application of these equations is limited. These equations are only applicable to the specific scenario simulated in this study and because of the limited dataset they have not been validated.

7.3.5. Preferential Attachment to Particulates

Table 7.4 and Appendix H present the distribution of attached *E. coli* and enterococci to three particle size categories. The TSS associated with each screen size were weighed and used to identify the particle sizes to which fecal bacteria preferentially attach. TSS associated with a screen size weighing less than 1 mg were considered to be negligible and in that case it was assumed that all cells retained by that screen size either remained in suspension or were bioflocculated but not attached to particulates. Very few solids passed through the 8 µm filter and no samples had measurable solids retained by the 3 µm screen, likely due to the particle size distribution of the Grosclose silt loam soils which contained only 5% clay. It is also possible 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 7.4. Particle sizes to which *E. coli* and enterococci preferentially attach in samples collected during an overland flow event.

			E. coli		enterococci	
Particle Size	**Mean TSS (mg/L)**	**% TSS**	**Mean cfu/mg solids[1]**	**% attachment**	**Mean cfu/mg solids**	**% attachment**
High Vegetative Cover						
>500 µm	28.8	21%	76a	44%	120a	76%
63 - 499 µm	34.0	24%	25a	15%	23a	15%
8 - 62 µm	76.4	55%	70a	41%	15a	9%
Low Vegetative Cover						
>500 µm	413.5	32%	18a	2%	34a	33%
63 - 499 µm	512.9	39%	49a	6%	32a	31%
8 - 62 µm	374.0	29%	726b	92%	37a	36%

[1]Means followed by the same letter do not differ at the 5% level of significance according to Tukey's pairwise comparison; data normalized by ln transformation

The majority of sediments from the plots with high vegetative cover were retained on the 8 µm screen while the majority of sediments from the plots with low vegetative cover were retained by

the 63 μm screen. These results indicate that high vegetation is either effective in filtering larger soil particles or preventing detachment of these particles. No statistically significant differences were found between the *E. coli* and enterococci concentrations associated with different particle sizes in runoff from the high vegetation plots (Appendix I). From the low vegetation plots, only the *E. coli* associated with the 8 μm screen (92%) was statistically higher than the *E. coli* associated with particles retained by the 63 μm (6%) and 500 μm (2%) screens. Typically, the larger surface area of the smaller particles corresponds to a higher number of sites available for bacterial attachment and previous studies using this same separation technique found that nearly 60% of all attached cells were associated with particles passing through a 63 μm screen (Chapter 5). The broad distribution of *E. coli* and enterococci across particles sizes might be due to their extremely low attachment rates. Only a very small fraction of the total indicator populations was classified as attached and therefore only a small sample of attached cells were available for classification. The broad distribution of enterococci among particle categories in runoff from plots with low vegetative cover is similar to the TSS distribution. *E. coli* attachment to a broad range of suspended particle diameters has been previously observed (Jeng et al., 2005). The authors attributed *E. coli* association with a broader range of particle sizes to the motility and rod shape of the cell and their ability to attach to different angles or faces of the particles (edge-to-edge or face-to-edge associations).

7.4. Summary and Conclusions

The goal of this study was to evaluate the partitioning of *E. coli* and enterococci between the unattached and attached phases in runoff from high vegetation and low vegetation pasturelands and to identify the particle sizes to which the fecal indicators preferentially attach. Field plots 3-m (9.8-ft) wide by 18.3-m (60-ft) long were constructed on two pastureland conditions to determine partitioning ratios of *E. coli* and enterococci in runoff samples collected at the edge-of-the-field. *E. coli* and enterococci partitioning was not clearly related to the dissolved/suspended phosphorus ratio as hypothesized and only the attached *E. coli* and enterococci in runoff from the low vegetative cover plots had a majority of cells associated with particles retained by the 8 μm filter.

Results indicate that the majority of bacterial indicator organisms are transported from the fresh manure source in the unattached state. The average *E. coli* partitioning coefficient (PC) for all samples collected was 0.0006 from plots with high vegetative cover and 0.029 from plots with low vegetative cover which corresponded to 0.06% and 2.8% attached to particulates, respectively. The average PC for enterococci was 0.0103 from plots with high vegetative cover and 0.0132 from plots with low vegetative cover which corresponded to 0.98% and 1.23% attached to particulates, respectively. Low attachment rates might be best explained by the moist, nutrient-rich cowpat environment and competition for attachment sites between fecal indicators and organic carbon. Attempts to predict *E. coli* and enteorocci PC as a function of flow, TSS, and nutrient concentrations produced fair results with R^2 values equal to 0.73 and 0.16, respectively. Regression models to predict unattached and attached indicator loading rates as a function of TSS, phosphorous and organic carbon had R^2 values ranging from 0.86 to 0.99 for *E. coli* and from 0.91 to 0.99 for enterococci. Only the *E. coli* present in runoff from the plots with low vegetative cover had statistically higher association with the 8 μm particle size category (92%) than the 63 μm (6%) and 500 μm (2%) particle size categories. The larger surface area of 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 unattached phases; however, the partitioning coefficients developed in this study are much lower than those previously identified in the literature so caution is recommended before direct implementation of these values. It appears that even slight changes in conditions could potentially result in great variation in attached fractions and the conditions causing these changes have not been clearly identified. The majority of cells in this study were transported to the edge-of-the-field in the unattached state from pasturelands receiving fresh fecal deposits regardless of vegetative cover, and therefore, development of best management practices from all pastureland scenarios should focus on reduction of unattached pathogen indicators. Future study is recommended to determine partitioning of indicators from different landuses with higher transport of suspended solids and from aged fecal sources with potentially stressed fecal indicators.

Chapter 8. Summary and Conclusions

The goal of this study was to improve understanding of the fate and transport mechanisms of two pathogen indicators, *E. coli* and enterococci, from grazed pasturelands. First, dispersion and separation techniques were compared and examined in the laboratory to identify the best method to disperse attached and bioflocculated cells and to separate cells transported to surface water bodies in the attached and unattached phases. Secondly, cowpats applied to pasturelands were monitored over four seasons to assess re-growth and die-off patterns of the two indicators and to examine the impacts of environmental factors on long-term indicator survival. Lastly, two separate field studies were conducted to evaluate overland transport processes of *E. coli* and enterococci. A small box plot study examined *E. coli* and enterococci attachment to particulates in runoff from three bare soils receiving cowpat treatments while a larger plot study examined the partitioning of *E. coli* and enterococci from two pastureland management scenarios during overland flow. The following sections summarize the methods and significant findings from each of the four study objectives.

8.1. Objective 1: Method development

Evaluate various laboratory procedures for dispersing and separating *E. coli* and enterococci into attached and unattached phases and to identify particle size ranges to which *E. coli* and enterococci preferentially attach

> *Hypothesis: Sonification is the best method of recovering attached bacteria from sediments.*

Many factors can contribute to cellular attachment; however, there is currently no consistency among laboratory techniques used to disperse and separate unattached and attached *E. coli* in runoff or surface water samples. Both physical and chemical dispersion techniques were evaluated. Selected chemical surfactant treatments included Tween-80 and Tween-85 at concentrations of 10, 100, 1,000, and 10,000 mg L^{-1} and sodium pyrophosphate (NaPP) combined with 1% glycerol, 1% peptone, and DI water(Trevors and Cook, 1992). Three physical dispersion methods: hand shaker treatment for 10 min, ultrasonic bath treatment for 30 sec, 2

min, 6 min and 10 min, and a one minute vortex were also compared. Physical and chemical dispersion techniques were evaluated separately and the most promising techniques were combined. The best and most consistent results were obtained using a combined treatment with a hand shaker for 10 minutes followed by dilutions in 1,000 mg L^{-1} of Tween-85. The hand shaker treatment followed by dilutions in Tween-85 significantly increased total *E. coli* concentrations by 31% (*P* value = 0.0028) and enterococci concentrations by 17% (not significant) when compared to a control.

To separate unattached from attached fractions, two commonly used techniques, fractional filtration and centrifugation, were combined and tested. A number 35 mesh screen was used to retain particles larger than coarse sand (>500 μm) and a number 230 mesh screen was used to retain medium, fine and very fine sand (63 - 500 μm). An 8 μm polycarbonate filter was used to retain fine, medium, and coarse silt particles and a 3 μm filter was used to retain clay and very fine silt particles. Centrifugation of suspended, unattached *E. coli* DH2 1030 at 4,700 rpm for 15 seconds did not reduce unattached concentrations. A mass balance was conducted to evaluate the impacts of the filtration and centrifugation treatments on total counts when compared to a control. The filtration and centrifugation treatment did not reduce *E. coli* concentrations when compared to a control but instead an increase of 22% was observed; although, this increase was not statistically significant (*P* value = 0.97). The mass balance found a 37% decrease in average enterococci counts when compared to the control, but this decrease was also not statistically significant (*P* value = 0.83). One possible reason for the differences in *E. coli* and enterococci concentrations when compared to the controls could be compounded sample variability as a result of summing the bacteria associated with each particle size range. The combination of these procedures resulted in an easy-to-replicate technique that could be applied to runoff and stormwater samples. This method is useful for determining partitioning coefficients for NPS models and identifying the particle sizes to which *E. coli* and enterococci preferentially attach.

8.2. Objective 2: Die-off study

Assess *E. coli* and enterococci re-growth and decay patterns in cowpats applied to pasturelands.

Hypothesis: First order decay does not adequately describe die-off rates for E. coli or enterococci in cowpats.

Standard cowpats were formed and applied to pastureland during spring, summer, fall and winter seasons to assess *E. coli* and enterococci re-growth and decay in cowpats. Approximately 100 cowpats were formed and applied to a mowed hay field in a randomly distributed pattern to represent a rotational grazing system during each season. Cowpats were applied to four separate field plots with no history of previous manure application and monitored until cowpats could no longer be detected or the lower bacterial detection level of 10^2 cfu g^{-1} was reached. Cowpats applied during the spring, summer, fall, and winter were monitored for 133, 175, 196, and 135 days, respectively. Seasonal variations in decay patterns were assessed using the decimal reduction times (*D*-values) and first order decay coefficients. Higher order approximations and weather parameters were evaluated by multiple regression analysis to identify parameters impacting in-field decay and to identify possible techniques to improve predictions of *E. coli* and enterococci decay in cowpats.

Populations of *E. coli* and enterococci both exhibited re-growth, which seemed to differ by both indicator and season. Re-growth occurred immediately or within the first few days after field application of cowpats. In general, cool temperatures preserved bacterial concentrations while increased decay occurred during warm temperatures when vegetation and insects hastened the disappearance of the fecal deposits. First order kinetics approximated *E. coli* and enterococci decay rates in cowpats with regression coefficients ranging from 0.70 to 0.90; however, when indicators exhibited re-growth patterns, the first order approximations overestimated initial concentrations present in freshly excreted manures. Die-off rate constants were greatest in cowpats applied to pasture during late winter and monitored into summer months for *E. coli* ($k = 0.0995\ d^{-1}$) and applied to the field during the summer and monitored until December for enterococci ($k = 0.0978\ d^{-1}$). Decay rates were lowest in cowpats applied to the pasture during the fall and monitored over the winter ($k = 0.0581\ d^{-1}$ for *E. coli* and $k = 0.0557\ d^{-1}$ for enterococci). *E. coli* and enterococci *D*-values were very similar with a 10-fold reduction in both populations occurring within five days of each other. The *D*-values were greatest during the fall monitoring period (40 and 41 days for *E. coli* and enterococci, respectively).

Higher order approximations and addition of weather variables improved regression coefficients of die-off rate equations to values ranging from 0.81 to 0.97, from 0.70 and 0.80, respectively, and improved distribution of residual plots for both indicators was noted. The addition of weather variables including solar radiation, temperature, relative humidity and rainfall improved predictability of regression equations for all seasonal studies, except for the fall monitoring period. It is possible that the average temperature and solar radiation that occurred during the fall monitoring period (average temperature = 6.22 ºC and average solar radiation = 0.84 MJ) did not contribute significantly to bacterial decay. Statistically significant variables included in the models predicting bacterial decay during the spring, summer and winter monitoring periods were temperature, solar radiation, rainfall and relative humidity.

Die-off rate coefficients representing actual field conditions should be developed for implementation in decay models when first order approximations are used to predict in-field bacterial concentrations. Comparable *E. coli* and enterococci seasonal cowpat *D*-values suggest that similar on-farm management strategies should reduce both *E. coli* and enterococci indicator populations. This study recommends using higher order approximations and the inclusion of weather variables to better capture re-growth and die-off trends over extended periods of time.

8.3. Objective 3: Box Plot Study

Quantify partitioning of *E. coli* and enterococci between the attached and unattached phase in runoff from three bare Virginia soils.

> *Hypothesis: E. coli and enterococci partitioning during runoff is related to the dissolved phosphorus/suspended phosphorus ratio and the majority of cells are associated with particles retained by the 8 μm filter.*

A field study was conducted to examine the state of *E. coli* and enterococci transport from three bare soil types receiving cowpat treatments and develop relationships between bacterial partitioning and phosphorous and carbon transport. Particles sizes to which cells preferentially associated were also identified. Soil boxes (100-cm × 20-cm × 7.5-cm) were packed with three

Virginia soils, loamy fine sand, silty loam and silty clay loam, to simulate runoff from bare soils. A Tlaloc 3000 portable rainfall simulation was conducted 24-hours after application of a standard cowpat, followed by a second rainfall simulation approximately 80 days later. Runoff samples were analyzed for *E. coli*, enterococci, TSS, phosphorous, organic phosphorous and organic carbon. *E. coli* and enterococci partitioning coefficient (PC) and particulate associated fraction (PAF) were calculated to compare fecal indicator attachment in runoff from the different soil types and between two pathogen indicators, *E. coli* and enterococci. Fractional filtration followed by centrifugations identified particle sizes to which indicators preferentially attached. Regression analysis was conducted to examine potential relationships to utilize nutrient and TSS data to predict *E. coli* and enterococci PC and total concentration (TC).

Percent of *E. coli* and enterococci attached to particulates in runoff as determined by fractional filtration and centrifugation ranged from 28% to 49%. In general, the *E. coli* cells attached at a higher rate to sediments in runoff from the loamy fine sand and enterococci attached at a higher rate to the silty loam and silty clay loam soils. Enterococci appeared to be attached at a higher rate to soils with a higher cation exchange capacity (CEC) and organic matter content while *E. coli* had higher attachment to loamy fine sand soils, which has a lower CEC and organic matter content. At least 50% of all attached cells were associated with particles less than 63 μm in diameter. The larger surface area of the smaller particles corresponds to a higher number of sites available for bacterial attachment. While particle size in runoff greatly influenced attachment, we were unable to establish a particular particle size range present in the soil matrix as a dominant factor statistically impacting attachment in runoff. Regression equations were developed to predict *E. coli* and enterococci PC ($R^2 = 0.54$ and $R^2 = 0.86$, respectively) and *E. coli* and enterococci TC ($R^2 = 0.93$ $R^2 = 0.92$, respectively) as a function of TSS, phosphorous and carbon. A single regression model was capable of predicting *E. coli* and enterococci TC in runoff from both a fresh and aged fecal source. TSS concentrations were only included as an independent variable in regression equations developed to predict PC, emphasizing the importance of attachment sites in predicting the fraction of attached cells.

A combination of factors influence attachment of *E. coli* and enterococci to particulates in runoff; including soil type and organic content of soils, carbon content and organic composition

of fecal material, and cellular properties of indicator organisms. Partitioning coefficients and PAF developed in this portion of the study are not meant to represent bacterial attachment during overland flow events or at the edge-of-the-field because of the small plot size and short distance between the fecal source and sample collection point. Future studies are recommended to assess bacterial attachment from fecal sources other than cowpats. It could be possible that soils with higher clay content than the Levy soils used in this study (29% clay) would have even higher attachment rates due to increased availability of attachment sites. Higher attachment associated with highly erodible soils as used in this study (when compared to previous edge-of-field and stormwater studies) indicates that lower concentrations of total suspended solids could be limiting bacterial attachment and thus PC and PAF values for other landuses such as well-managed pastureland could be much lower.

8.4. Objective 4: Transport Plot Study

Quantify partitioning of *E. coli* and enterococci between the attached and unattached phase from pastureland with high and low vegetative cover during overland flow.

> *Hypothesis: E. coli and enterococci partitioning during overland flow is related to the dissolved phosphorus/suspended phosphorus ratio and the majority of cells are associated with particles retained by the 8 µm filter.*

Two field studies were conducted to evaluate the partitioning of *E. coli* and enterococci between the unattached and attached phases in runoff from pasturelands and to identify the particle sizes to which the fecal indicators preferentially attach at the edge-of-the-field. A set of field plots 3-m (9.8-ft) wide by 18.3-m (60-ft) long were constructed on Groseclose silt loam pasturelands dominated by a dense stand of Kentucky 31 Tall Fescue. Runoff was directed into an H-flume equipped with a stilling well and a stage recorder for flow measurement. Approximately 106 standard cowpats were applied to four of the plots to represent a rotational grazing system. One plot received no cowpat treatment and was used as a control to monitor background bacteria and nutrient concentrations and loads. A rainfall simulator was used to generate a uniform rainfall event (2.8 cm/h) to all plots and discreet grab samples were collected at the outfall of the flumes.

Runoff samples were analyzed for *E. coli*, enterococci, TSS, phosphorous, organic phosphorous and organic carbon.

High vegetative cover plots were used to approximate well managed pasturelands during the first study which was conducted in April. A second rainfall simulation comparing high and low vegetative cover pasturelands was conducted in August of the same year. Prior to re-application of the cowpats for the second study, vegetation was removed from two of the plots (previously receiving cowpat treatments) with a dethatcher and string trimmer to represent poorly-managed pasture conditions. The dethatcher removed approximately 50% of all established vegetation. Three equally-sized bare areas accounting for 28.4% of the total plot area were created in the top, middle, and lower third of the two plots using the string trimmer. High vegetative cover, representing well managed pastureland on the remaining two plots receiving cowpat treatments and the control plot, was left undisturbed. The control plot did not receive a cowpat treatment.

First Transport Plot Study: High Vegetative Cover Pastureland

Results from the high vegetative cover pastureland study conducted in April indicate that the majority of *E. coli* and enterococci are transported from a fresh manure source in the unattached state. Average PC for *E. coli* was 0.06 which corresponds to 4.8% attachment to particulates and 0.18 for enterococci corresponding to 13% attachment to particulates. Low attachment rates might be best explained by the low TSS concentrations (relative to poorly-managed and other agricultural landuses) and competition for attachment sites between fecal indicators and organic carbon. Linear correlations existed between *E. coli* loading rates and suspended organic phosphorous (SOP) while unattached *E. coli* and both attached and unattached enterococci loading rates were most closely related to dissolved organic carbon (DOC). Comparison of unattached and attached indicator loading rates indicated that the unattached fraction exceeded the attached fraction by at least two orders of magnitude. Fifty-eight percent of all attached cells were associated with particles smaller than 63 μm in size.

Second Transport Plot Study: Comparison of High and Low Vegetative Cover Pastureland

Results from the second study comparing high and low vegetative cover pasturelands also indicated that that the majority of *E. coli* and enterococci are transported from the fresh manure

source in the unattached state. The average *E. coli* PC for all samples collected was 0.0006 from plots with well-managed pastureland and 0.029 from poorly-managed pastureland which corresponded to 0.06% and 2.8% of *E. coli* being attached in runoff, respectively. The average PC for enterococci was 0.0103 from plots with well-managed pastureland and 0.0132 from plots with poorly-managed pastureland which corresponded to 0.98% and 1.23% of enterococci being attached in runoff, respectively. Low attachment rates might be best explained by the moist, nutrient-rich cowpat environment and competition for attachment sites between fecal indicators and organic carbon. Attempts to predict *E. coli* and enteorocci PC as a function of flow, TSS, and nutrient concentrations produced fair results with R^2 values equal to 0.73 and 0.16, respectively. Regression models to predict unattached and attached indicator loading rates as a function of TSS, phosphorous and organic carbon had R^2 values ranging from 0.94 to 0.99 for *E. coli* and from 0.83 to 0.99 for enterococci (Table 7.3).

Screen filtration to identify particle size categories to which cells preferentially attach produced different results than the first transport plot study. Only the *E. coli* present in runoff from the poorly-managed pastureland plots had statistically higher association with the 8 μm particle size range (92%) than the 63 μm (6%) and 500 μm (2%) particle size ranges. The larger surface area of smaller particles corresponds to a higher number of sites available for bacterial attachment.

Partitioning ratios developed in this study can be incorporated into NPS models that allow for partitioning between the attached and unattached phases; however, the partitioning coefficients developed in the second portion of this study are much lower than those identified in the first portion so caution is recommended prior to direct implementation of these values into models. It appears that even slight changes in conditions could potentially result in great variation in attached fractions and the conditions causing these changes have not been identified. The majority of cells were transported in the unattached state from pasturelands receiving fresh fecal deposits regardless of vegetative cover, and therefore, the results of this study indicate that best management practices to reduce bacterial transport from pasturelands should focus on reduction of unattached pathogen indicators.

8.5. Implications of the Study

This study developed an easy-to-replicate method to disperse cells from particulates, partition between attached and unattached phases and identified particle size ranges to which cells preferentially attach. This method was developed using samples with high sediment and organic matter concentrations and thus could be applied to runoff samples collected from a variety of agricultural landuses including feedlots, poor pastureland or well-managed pastureland as is demonstrated in various chapters of this dissertation. While the focus of this study is on runoff from agricultural landuses, it is possible that this method could also be applied to non-agricultural runoff samples such as urban stormwater samples.

The die-off equations developed through this research will improve predictions of bacterial concentrations in surface runoff. In-field die-off rate coefficients developed in this study are representative of field conditions and should be implemented in first-order decay models rather than the currently used laboratory-developed die-off rate coefficients. The inclusion of environmental parameters and higher-order approximations better simulated die-off and re-growth trends over an extended period of time. In situations where heavy rainfall occurs shortly after the application of manure to agricultural lands, the re-growth can play a major role in determining the concentration of bacteria in runoff and thus simulating this re-growth is necessary to improve predictions of in-stream bacterial concentrations.

Partitioning coefficients developed through this research will assist in development of new management practices and/or refinement of existing practices implemented to reduce the movement of bacteria from agricultural lands to downstream water bodies. Many NPS models already partition different phases of other water quality indicators such as phosphorous, so relationships between these parameters and bacterial transport partitioning could allow for a simple adaptation to existing models. The results of this study indicate that bacterial transport from high vegetation and low vegetation areas is primarily in the unattached phase during overland flow conditions; however, in runoff from highly erosive areas as much as 50% of *E. coli* and enterococci could be transported in the attached phase. Management practices should consider particulate loadings and soil type when designing for the removal of pathogen indicators. Identification of particulate size ranges to which bacteria preferentially attach can

also be used to improve selection and design of best management practices when particulate transport is high.

8.6. Limitations of the Study and Future Research Recommendations

The method used to disperse and partition *E. coli* and enterococci between the attached and unattached phases was tested and validated on a single soil type (Grosclose silt loam) and portions of the method development were conducted on a single strain of dairy cow *E. coli* (DH2 1030). It is recommended that the dispersion technique be evaluated prior to application to samples collected from different soil types to ensure that the optimal dispersion technique is selected for soils with different particle size distributions. Additional screen sizes would be useful to identify a finer distribution of the particle size ranges to which *E. coli* and enterococci preferentially attach, especially in samples collected from areas with high clay content.

Originally, this study proposed a mass balance of the fate and transport of fecal indicators applied to the land surface during intensive grazing conditions. Lysimeters designed to capture infiltrating bacterial concentrations at 30 and 60-cm failed and attempts to assess bacterial concentrations in sediments surrounding cowpats proved to be too extensive of a task to be performed on the large vegetated plots. A future mass balance study is recommended on much smaller plots that would allow for destructive sampling.

While this study focused on fate and transport of *E. coli* and enterococci from grazed pasturelands, future studies are also recommended to determine partitioning of indicators from different landuses, especially those with higher transport of suspended solids. Fresh cowpats were applied to plots prior to each rainfall simulation so the potential for increased attachment of *E. coli* and enterococci to particulates when the fecal source is aged and fecal indicators are stressed was not evaluated. Improvements in predicting *E. coli* and enterococci fate in cowpats through the use of higher order approximations and environmental parameters appears promising and similar efforts should be attempted on other landuses serving as a source of fecal indicators to surface waters.

Evaluating the fate and transport of fecal indicators after delivery to the stream would provide valuable information as to when attachment occurs. Current in-stream partitioning studies (primarily collected during storm events) generally have identified higher attachment rates than the edge-of-field attachment rates identified in this study. Evaluation of in-stream processes through a flume simulation is recommended to determine if these higher attachment rates are a function of different or aged fecal sources in a watershed; the cellular response to environmental stress and limited substrates over time; or the resuspension of stable populations of fecal indicators surviving in bottom sediments.

Chapter 9. References

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Appendix A. Additional Data for Dispersion and Separation Technique Development

Table A.1. Preliminary comparison of chemical dispersion treatments applied to runoff samples collected from a dairy cowpat on pastureland.

	E. coli		enterococci	
Chemical dispersion treatments	**Concentration (cfu mL^{-1})**	**Average % change from control[1]**	**Concentration (cfu mL^{-1})**	**Average % change from control**
Control (phosphate buffer water)	7.83×10^3 $7.0 – 9.2\times10^3$[2]	---	1.40×10^5 $1.31– 1.44\times10^5$	---
Tween 85 (10 ppm)	5.17×10^3 $4.2 – 6.2\times10^3$	-34%	5.00×10^4 $2.0 – 10.0\times10^4$	-64%
Tween 85 (100 ppm)	5.33×10^3 $4.8 – 5.7\times10^3$	-32%	1.29×10^5 $1.15 – 1.47\times10^5$	-8%
Tween 85 (1,000 ppm)	1.06×10^4 $8.8 – 11.8\times10^3$	36%	1.68×10^5 $1.33 – 2.27\times10^5$	21%
Tween 85 (10,000 ppm)	5.60×10^3 $4.6 – 6.2\times10^3$	-29%	1.35×10^5 $1.12 – 1.56\times10^5$	-4%
Control (phosphate buffer water)	7.03×10^4 $6.4 – 7.3\times10^4$	---	5.00×10^2 $4.0 – 6.0\times10^2$	---
Tween 80 (10 ppm)	6.80×10^4 $5.7 – 7.9\times10^4$	-3%	4.00×10^2 $3.0 – 5.0\times10^2$	-20%
Tween 80 (100 ppm)	6.60×10^4 $5.9 – 7.7\times10^4$	-6%	8.00×10^2 $6.0 – 10.0\times10^2$	60%
Tween 80 (1,000 ppm)	5.73×10^4 $4.9 – 7.1\times10^4$	-18%	5.33×10^2 $1.0 – 11.0\times10^2$	7%
Tween 80 (10,000 ppm)	5.87×10^4 $5.4 – 6.4\times10^4$	-17%	6.00×10^2 $6.0 – 6.0\times10^2$	20%
Control (phosphate buffer water)	1.95×10^5 $1.90 – 2.04\times10^5$	---	2.97×10^4 $2.3 – 3.5\times10^4$	---
0.1% NaPP + DI water	4.90×10^4 $4.0 – 5.6\times10^4$	-75%	2.40×10^4 $2.1 – 2.8\times10^4$	-19%
0.1% NaPP + 1% glycerol + DI water	4.20×10^4 $3.3 – 5.8\times10^4$	-78%	3.37×10^4 $2.4 – 4.0\times10^4$	13%
0.1% NaPP + 1% peptone + DI water	6.90×10^4 $6.3 – 7.4\times10^4$	-65%	3.07×10^4 $2.0 – 3.8\times10^4$	3%

[1] A negative value indicates a decrease in cells when compared to the control
[2]Range of values

Table A.2. Preliminary comparison of physical dispersion treatments applied to runoff samples collected from a dairy cowpat on pastureland.

	E. coli		enterococci	
Physical dispersion treatments	**Concentration (cfu mL^{-1})**	**Average % change from control[1]**	**Concentration (cfu mL^{-1})**	**Average % change from control**
Control (Phosphate buffer water)	2.77×10^4 $2.5 – 3.0\times10^4$[1]	---	5.33×10^2 $3.0 – 9.0\times10^2$	---
Ultrasonic bath treatment 30 sec	8.63×10^4 $7.1 – 9.5\times10^4$	212%	1.50×10^3 $1.3 – 1.8\times10^3$	181%
Ultrasonic bath treatment 2 min	7.40×10^4 $6.7 – 8.4\times10^4$	167%	1.87×10^3 $1.6 – 2.0\times10^3$	250%
Ultrasonic bath treatment 6 min	6.40×10^4 $6.2 – 6.6\times10^4$	131%	2.23×10^3 $2.0 – 2.5\times10^3$	319%
Ultrasonic bath treatment 10 min	5.93×10^4 $5.5 – 6.6\times10^4$	114%	2.20×10^3 $1.9 – 2.4\times10^3$	313%
Control (Phosphate buffer water)	3.17×10^4 $2.5 – 4.3\times10^4$	---	6.00×10^2 $5.0 – 7.0\times10^2$	---
Vortex 1 minutes	2.13×10^4 $1.9 – 2.5\times10^4$	-33%	5.67×10^2 $5.0 – 6.0\times10^2$	-6%
Control (Phosphate buffer water)	3.50×10^4 $2.7 – 4.0\times10^4$	---	6.00×10^2 $5.0 – 7.0\times10^2$	---
Hand Shaker 10 minutes	8.70×10^4 $8.2 – 9.6\times10^4$	149%	8.00×10^2 $7.0 – 10.0\times10^2$	33%

[1]A negative value indicates a decrease in cells when compared to the control
[2]Range of values

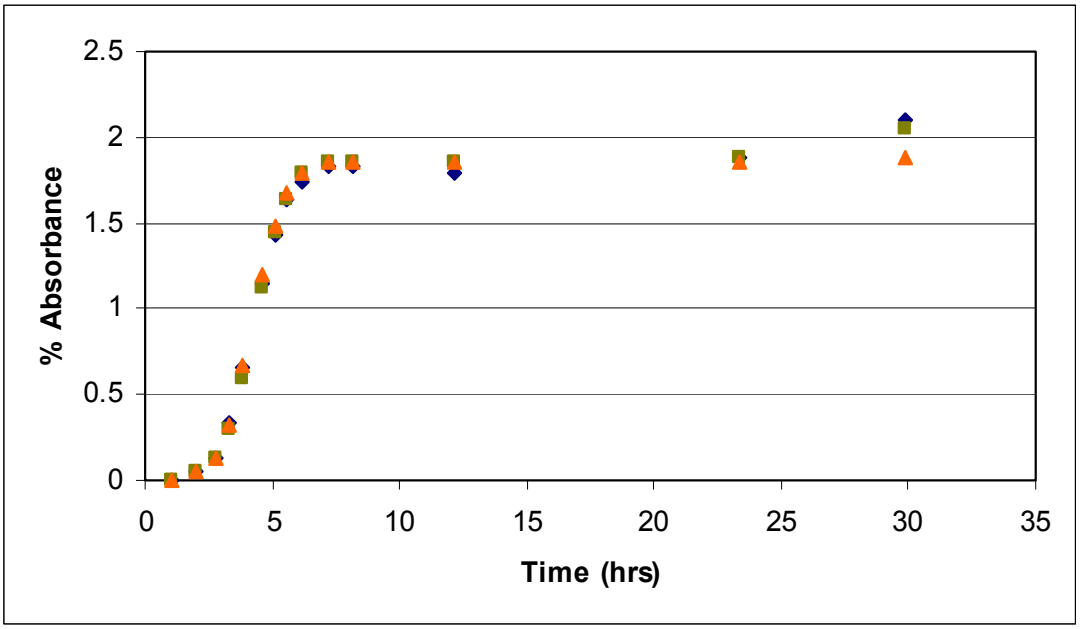


Figure A.1. Growth curve of *E. coli* DH2 1030 in Tryptic Soy nutrient broth

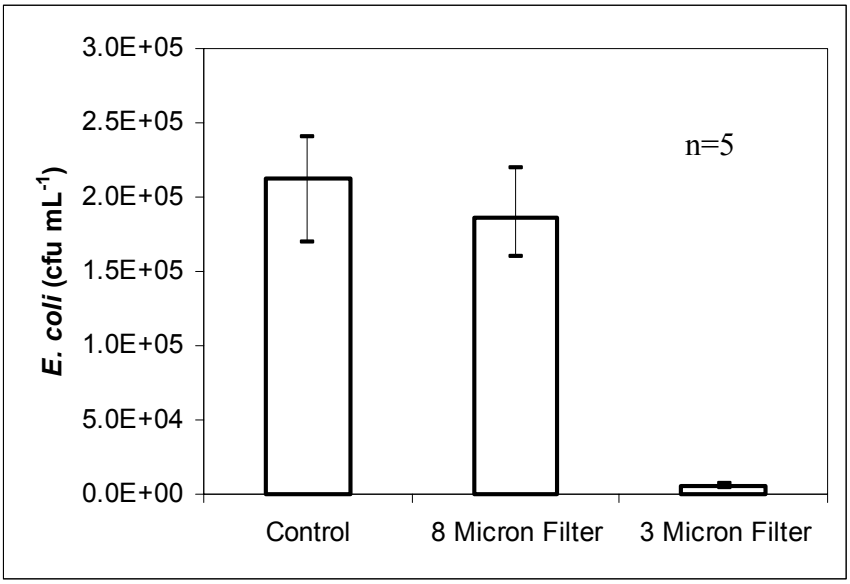


Figure A.2. ***E. coli*** **DH2 1030 concentrations were reduced by filtering cells through 8 µm and 3 µm filters, when compared to a control.**

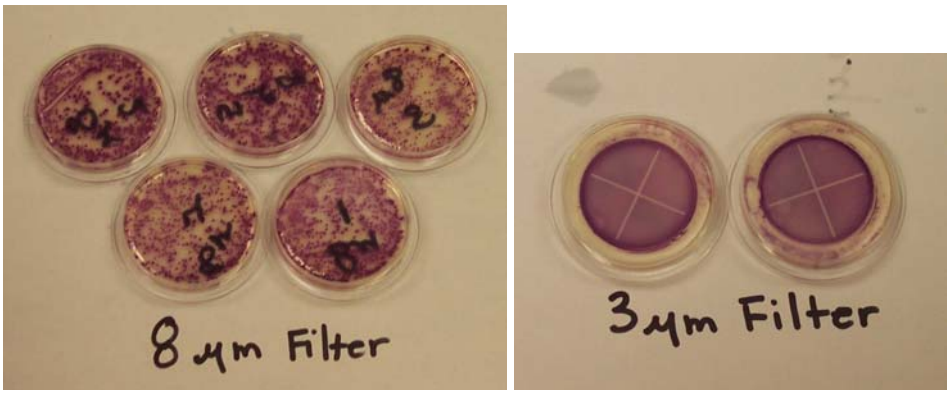


Figure A.3. ***E. coli*** **DH2 1030 cells captured on the 8 µm and 3 µm filters when 10 mL of 10^5 E. coli cfu mL^{-1}.**

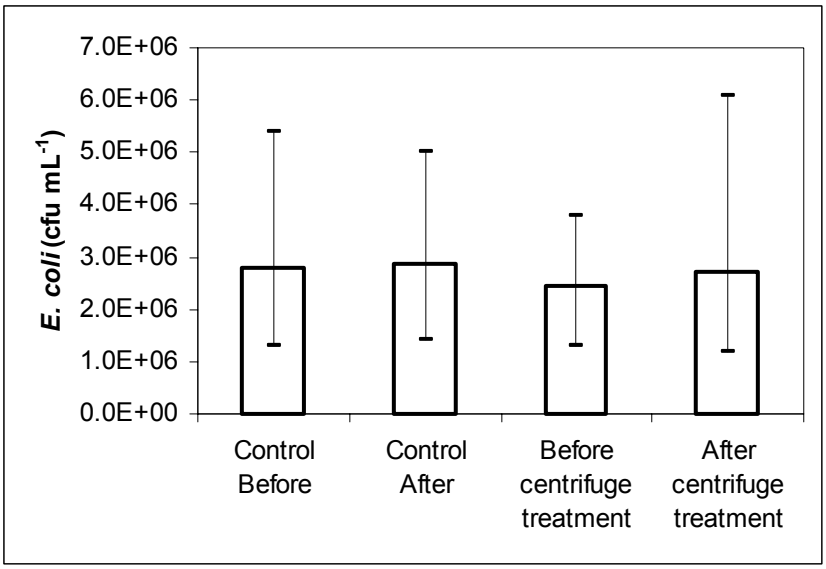


Figure A.4. ***E. coli*** **DH2 1030 concentrations collected from the stationary phase of the growth curve before and after a 10 minute centrifugation treatment at 1,200 × g (4700 rpm) and 4°C (n=12).**

Appendix B. Die-off Monitoring Data

Table B.1. Weather Data and Indicator Concentrations Collected during Die-off Monitoring

Sample No.	Season	Sample date	Time (d)	*E. coli* (cfu g^{-1}) wet wt.	*E. coli* (cfu g^{-1}) dry wt.	ENT (cfu g^{-1}) wet wt.	ENT (cfu g^{-1}) dry wt.	MC (%)	Temp PDH (ºC)	Temp PWH (ºC)	Temp PDA (ºC)	Temp PWA (ºC)	SR PDH (MJ)	SR PDA (MJ)	SR PWH (MJ)	SR PWA (MJ)	RH PDA (%)	RH PWA (%)	rainfall PDT (cm)	Rainfall PWT (cm)
M1	spring	4/19/2006	0	1.70E+06	8.99E+06	6.92E+07	3.66E+08	0.81	23.9	23.9	13.9	13.9	2.881	1.69			61.08		0.00	
M2	spring	4/19/2006	0	2.00E+04	1.16E+05	2.32E+07	1.34E+08	0.83	23.9	23.9	13.9	13.9	2.881	1.69			61.08		0.00	
M3	spring	4/19/2006	0	6.60E+05	4.01E+06	5.92E+07	3.60E+08	0.84	23.9	23.9	13.9	13.9	2.881	1.69			61.08		0.00	
M4	spring	4/19/2006	0	6.30E+05	3.74E+06	4.04E+07	2.40E+08	0.83	23.9	23.9	13.9	13.9	2.881	1.69			61.08		0.00	
M5	spring	4/19/2006	0	7.50E+05	4.08E+06	3.16E+07	1.72E+08	0.82	23.9	23.9	13.9	13.9	2.881	1.69			61.08		0.00	
M1	spring	4/21/2006	2	9.00E+05	5.08E+06	9.54E+07	5.38E+08	0.82	25.6	25.6	15.6	14.7	2.713	1.40	2.88	1.44	67.28	63.28	0.00	0.00
M2	spring	4/21/2006	2	6.20E+06	3.04E+07	1.86E+08	9.11E+08	0.80	25.6	25.6	15.6	14.7	2.713	1.40	2.88	1.44	67.28	63.28	0.00	0.00
M3	spring	4/21/2006	2	4.30E+06	2.34E+07	1.10E+08	5.97E+08	0.82	25.6	25.6	15.6	14.7	2.713	1.40	2.88	1.44	67.28	63.28	0.00	0.00
M4	spring	4/21/2006	2	5.60E+05	2.73E+06	1.43E+08	6.98E+08	0.79	25.6	25.6	15.6	14.7	2.713	1.40	2.88	1.44	67.28	63.28	0.00	0.00
M5	spring	4/21/2006	2	4.30E+05	2.52E+06	8.22E+07	4.82E+08	0.83	25.6	25.6	15.6	14.7	2.713	1.40	2.88	1.44	67.28	63.28	0.00	0.00
M1	spring	4/25/2006	6	3.50E+07	1.76E+08	3.30E+05	1.66E+06	0.80	23.3	23.3	17.2	15.6	2.973	1.09	2.97	0.86	62.25	76.00	0.00	3.28
M2	spring	4/25/2006	6	1.11E+06	5.83E+06	4.50E+05	2.36E+06	0.81	23.3	23.3	17.2	15.6	2.973	1.09	2.97	0.86	62.25	76.00	0.00	3.28
M3	spring	4/25/2006	6	1.65E+06	8.95E+06	3.60E+05	1.95E+06	0.82	23.3	23.3	17.2	15.6	2.973	1.09	2.97	0.86	62.25	76.00	0.00	3.28
M4	spring	4/25/2006	6	7.00E+06	4.03E+07	3.20E+05	1.84E+06	0.83	23.3	23.3	17.2	15.6	2.973	1.09	2.97	0.86	62.25	76.00	0.00	3.28
M5	spring	4/25/2006	6	2.10E+07	1.14E+08	1.90E+05	1.03E+06	0.82	23.3	23.3	17.2	15.6	2.973	1.09	2.97	0.86	62.25	76.00	0.00	3.28
M1	spring	4/27/2006	8	2.00E+07	1.01E+08	3.50E+05	1.77E+06	0.80	13.3	25.0	10.6	13.6	0.336	0.20	2.66	0.91	96.64	85.85	0.15	0.89
M2	spring	4/27/2006	8	1.02E+08	4.79E+08	1.30E+05	6.10E+05	0.79	13.3	25.0	10.6	13.6	0.336	0.20	2.66	0.91	96.64	85.85	0.15	0.89
M3	spring	4/27/2006	8	1.30E+07	5.87E+07	4.70E+05	2.12E+06	0.78	13.3	25.0	10.6	13.6	0.336	0.20	2.66	0.91	96.64	85.85	0.15	0.89
M4	spring	4/27/2006	8	2.70E+07	1.06E+08	8.60E+05	3.39E+06	0.75	13.3	25.0	10.6	13.6	0.336	0.20	2.66	0.91	96.64	85.85	0.15	0.89
M5	spring	4/27/2006	8	1.50E+07	5.65E+07	6.00E+05	2.26E+06	0.73	13.3	25.0	10.6	13.6	0.336	0.20	2.66	0.91	96.64	85.85	0.15	0.89
M1	spring	5/3/2006	14	9.60E+06	3.41E+07	1.06E+08	3.77E+08	0.72	23.3	23.3	13.3	12.4	2.764	1.20	3.07	1.26	67.78	57.63	0.13	0.15
M2	spring	5/3/2006	14	1.09E+07	3.43E+07	4.70E+08	1.48E+09	0.68	23.3	23.3	13.3	12.4	2.764	1.20	3.07	1.26	67.78	57.63	0.13	0.15
M3	spring	5/3/2006	14	4.10E+06	1.28E+07	8.00E+07	2.51E+08	0.68	23.3	23.3	13.3	12.4	2.764	1.20	3.07	1.26	67.78	57.63	0.13	0.15
M4	spring	5/3/2006	14	3.10E+06	1.05E+07	1.45E+08	4.91E+08	0.70	23.3	23.3	13.3	12.4	2.764	1.20	3.07	1.26	67.78	57.63	0.13	0.15
M5	spring	5/3/2006	14	1.17E+07	3.68E+07	3.10E+08	9.74E+08	0.68	23.3	23.3	13.3	12.4	2.764	1.20	3.07	1.26	67.78	57.63	0.13	0.15
M1	spring	5/10/2006	21	3.00E+06	1.28E+07	2.20E+07	9.37E+07	0.77	16.7	16.7	12.2	9.8	1.971	0.74	2.93	0.70	85.53	81.31	0.00	1.55
M2	spring	5/10/2006	21	3.80E+06	1.91E+07	4.40E+07	2.21E+08	0.80	16.7	16.7	12.2	9.8	1.971	0.74	2.93	0.70	85.53	81.31	0.00	1.55
M3	spring	5/10/2006	21	5.00E+06	2.66E+07	4.10E+07	2.18E+08	0.81	16.7	16.7	12.2	9.8	1.971	0.74	2.93	0.70	85.53	81.31	0.00	1.55
M4	spring	5/10/2006	21	1.90E+06	1.03E+07	4.50E+07	2.44E+08	0.82	16.7	16.7	12.2	9.8	1.971	0.74	2.93	0.70	85.53	81.31	0.00	1.55
M5	spring	5/10/2006	21	5.70E+06	2.88E+07	4.70E+07	2.38E+08	0.80	16.7	16.7	12.2	9.8	1.971	0.74	2.93	0.70	85.53	81.31	0.00	1.55
M1	spring	5/17/2006	28	2.30E+06	7.36E+06	4.30E+07	1.38E+08	0.69	16.7	23.9	10.6	11.8	3.237	1.08	3.24	0.94	72.80	77.97	0.00	2.08

Sample No.	Season	Sample date	Time (d)	*E. coli* (cfu g^{-1}) wet wt.	*E. coli* (cfu g^{-1}) dry wt.	ENT (cfu g^{-1}) wet wt.	ENT (cfu g^{-1}) dry wt.	MC (%)	Temp PDH (ºC)	Temp PWH (ºC)	Temp PDA (ºC)	Temp PWA (ºC)	SR PDH (MJ)	SR PDA (MJ)	SR PWH (MJ)	SR PWA (MJ)	RH PDA (%)	RH PWA (%)	rainfall PDT (cm)	Rainfall PWT (cm)
M2	spring	5/17/2006	28	1.24E+07	5.01E+07	5.70E+07	2.30E+08	0.75	16.7	23.9	10.6	11.8	3.237	1.08	3.24	0.94	72.80	77.97	0.00	2.08
M3	spring	5/17/2006	28	1.30E+06	5.64E+06	3.70E+07	1.61E+08	0.77	16.7	23.9	10.6	11.8	3.237	1.08	3.24	0.94	72.80	77.97	0.00	2.08
M4	spring	5/17/2006	28	1.00E+05	3.61E+05	1.10E+07	3.97E+07	0.72	16.7	23.9	10.6	11.8	3.237	1.08	3.24	0.94	72.80	77.97	0.00	2.08
M5	spring	5/17/2006	28	1.70E+07	7.24E+07	1.14E+08	4.85E+08	0.77	16.7	23.9	10.6	11.8	3.237	1.08	3.24	0.94	72.80	77.97	0.00	2.08
M1	spring	5/24/2006	35	8.60E+05	1.71E+06	4.90E+07	9.76E+07	0.50	20.6	25.0	11.7	12.8	3.152	1.77	3.15	1.35	49.04	59.85	0.00	0.00
M2	spring	5/24/2006	35	3.80E+06	1.10E+07	7.80E+07	2.26E+08	0.66	20.6	25.0	11.7	12.8	3.152	1.77	3.15	1.35	49.04	59.85	0.00	0.00
M3	spring	5/24/2006	35	6.90E+05	1.58E+06	1.20E+07	2.75E+07	0.56	20.6	25.0	11.7	12.8	3.152	1.77	3.15	1.35	49.04	59.85	0.00	0.00
M4	spring	5/24/2006	35	2.40E+05	3.62E+05	1.80E+07	2.72E+07	0.34	20.6	25.0	11.7	12.8	3.152	1.77	3.15	1.35	49.04	59.85	0.00	0.00
M5	spring	5/24/2006	35	8.00E+05	1.42E+06	1.10E+07	1.96E+07	0.44	20.6	25.0	11.7	12.8	3.152	1.77	3.15	1.35	49.04	59.85	0.00	0.00
M1	spring	5/31/2006	42	8.60E+06	1.26E+07	7.70E+07	1.13E+08	0.32	31.7	31.7	22.8	19.6	3.023	1.67	3.08	1.45	70.41	71.41	0.00	1.73
M2	spring	5/31/2006	42	5.60E+06	8.99E+06	3.50E+07	5.62E+07	0.38	31.7	31.7	22.8	19.6	3.023	1.67	3.08	1.45	70.41	71.41	0.00	1.73
M3	spring	5/31/2006	42	3.20E+06	5.04E+06	5.60E+07	8.81E+07	0.36	31.7	31.7	22.8	19.6	3.023	1.67	3.08	1.45	70.41	71.41	0.00	1.73
M4	spring	5/31/2006	42	1.70E+05	2.34E+05	2.90E+07	3.99E+07	0.27	31.7	31.7	22.8	19.6	3.023	1.67	3.08	1.45	70.41	71.41	0.00	1.73
M5	spring	5/31/2006	42	7.50E+06	9.03E+06	8.80E+06	1.06E+07	0.17	31.7	31.7	22.8	19.6	3.023	1.67	3.08	1.45	70.41	71.41	0.00	1.73
M1	spring	6/7/2006	49	6.00E+04	7.60E+04	3.20E+06	4.05E+06	0.21	22.8	30.0	16.1	18.7	3.081	1.36	3.08	1.18	68.41	76.24	0.00	4.24
M2	spring	6/7/2006	49	7.50E+05	9.06E+05	1.10E+06	1.33E+06	0.17	22.8	30.0	16.1	18.7	3.081	1.36	3.08	1.18	68.41	76.24	0.00	4.24
M3	spring	6/7/2006	49	4.50E+05	5.36E+05	2.40E+06	2.86E+06	0.16	22.8	30.0	16.1	18.7	3.081	1.36	3.08	1.18	68.41	76.24	0.00	4.24
M4	spring	6/7/2006	49	5.70E+05	5.84E+05	3.80E+06	3.89E+06	0.02	22.8	30.0	16.1	18.7	3.081	1.36	3.08	1.18	68.41	76.24	0.00	4.24
M5	spring	6/7/2006	49	1.07E+06	1.29E+06	2.70E+06	3.26E+06	0.17	22.8	30.0	16.1	18.7	3.081	1.36	3.08	1.18	68.41	76.24	0.00	4.24
M1	spring	6/15/2006	57	1.60E+05	3.23E+05	2.00E+05	4.04E+05	0.50	24.4	28.3	19.4	17.9	2.489	1.03	2.94	1.03	74.56	75.77	0.00	1.83
M2	spring	6/15/2006	57	1.91E+06	3.09E+06	1.63E+07	2.64E+07	0.38	24.4	28.3	19.4	17.9	2.489	1.03	2.94	1.03	74.56	75.77	0.00	1.83
M3	spring	6/15/2006	57	4.00E+04	6.37E+04	3.00E+05	4.78E+05	0.37	24.4	28.3	19.4	17.9	2.489	1.03	2.94	1.03	74.56	75.77	0.00	1.83
M4	spring	6/15/2006	57	1.00E+04	1.63E+04	2.00E+05	3.27E+05	0.39	24.4	28.3	19.4	17.9	2.489	1.03	2.94	1.03	74.56	75.77	0.00	1.83
M5	spring	6/15/2006	57	1.10E+05	1.58E+05	4.00E+06	5.75E+06	0.30	24.4	28.3	19.4	17.9	2.489	1.03	2.94	1.03	74.56	75.77	0.00	1.83
M1	spring	6/22/2006	64	9.40E+04	1.55E+05	8.00E+04	1.32E+05	0.39	28.9	30.0	21.7	19.9	2.288	1.28	3.03	1.39	76.54	70.84	0.00	0.00
M2	spring	6/22/2006	64	3.90E+04	4.96E+04	2.50E+05	3.18E+05	0.21	28.9	30.0	21.7	19.9	2.288	1.28	3.03	1.39	76.54	70.84	0.00	0.00
M3	spring	6/22/2006	64	4.60E+04	7.34E+04	5.00E+04	7.98E+04	0.37	28.9	30.0	21.7	19.9	2.288	1.28	3.03	1.39	76.54	70.84	0.00	0.00
M4	spring	6/22/2006	64	1.60E+04		5.10E+05	4.25E+05		28.9	30.0	21.7	19.9	2.288	1.28	3.03	1.39	76.54	70.84	0.00	0.00
M5	spring	6/22/2006	64	1.40E+04	1.63E+04	3.20E+05	3.72E+05	0.14	28.9	30.0	21.7	19.9	2.288	1.28	3.03	1.39	76.54	70.84	0.00	0.00
M1	spring	6/28/2006	70	1.00E+02	4.50E+02	3.00E+03	1.35E+04	0.78	24.4	32.2	21.1	22.9	1.564	0.54	2.90	1.03	97.06	86.66	5.41	23.93
M2	spring	6/28/2006	70	1.50E+04	6.45E+04	1.20E+04	5.16E+04	0.77	24.4	32.2	21.1	22.9	1.564	0.54	2.90	1.03	97.06	86.66	5.41	23.93
M3	spring	6/28/2006	70	2.91E+04	1.17E+05	1.30E+05	5.22E+05	0.75	24.4	32.2	21.1	22.9	1.564	0.54	2.90	1.03	97.06	86.66	5.41	23.93
M4	spring	6/28/2006	70	6.00E+03	2.51E+04	6.70E+04	2.80E+05	0.76	24.4	32.2	21.1	22.9	1.564	0.54	2.90	1.03	97.06	86.66	5.41	23.93
M5	spring	6/28/2006	70	2.00E+03	8.94E+03	1.90E+04	8.49E+04	0.78	24.4	32.2	21.1	22.9	1.564	0.54	2.90	1.03	97.06	86.66	5.41	23.93

Sample No.	Season	Sample date	Time (d)	*E. coli* (cfu g^{-1}) wet wt.	*E. coli* (cfu g^{-1}) dry wt.	ENT (cfu g^{-1}) wet wt.	ENT (cfu g^{-1}) dry wt.	MC (%)	Temp PDH (ºC)	Temp PWH (ºC)	Temp PDA (ºC)	Temp PWA (ºC)	SR PDH (MJ)	SR PDA (MJ)	SR PWH (MJ)	SR PWA (MJ)	RH PDA (%)	RH PWA (%)	rainfall PDT (cm)	Rainfall PWT (cm)
M1	spring	7/5/2006	77	1.35E+04	2.59E+04	5.50E+04	1.05E+05	0.48	30.6	30.6	23.9	21.6	2.734	1.28	3.11	1.47	84.47	77.65	1.14	1.14
M2	spring	7/5/2006	77	2.58E+04	4.98E+04	6.30E+04	1.22E+05	0.48	30.6	30.6	23.9	21.6	2.734	1.28	3.11	1.47	84.47	77.65	1.14	1.14
M3	spring	7/5/2006	77	2.20E+04	4.20E+04	3.47E+05	6.63E+05	0.48	30.6	30.6	23.9	21.6	2.734	1.28	3.11	1.47	84.47	77.65	1.14	1.14
M4	spring	7/5/2006	77	1.90E+03	3.44E+03	3.00E+04	5.42E+04	0.45	30.6	30.6	23.9	21.6	2.734	1.28	3.11	1.47	84.47	77.65	1.14	1.14
M5	spring	7/5/2006	77	6.00E+04	1.10E+05	7.80E+04	1.44E+05	0.46	30.6	30.6	23.9	21.6	2.734	1.28	3.11	1.47	84.47	77.65	1.14	1.14
M1	spring	7/12/2006	84	5.40E+03	8.56E+03	8.20E+04	1.30E+05	0.37	28.9	28.9	22.2	19.6	2.371	1.09	2.74	1.16	88.03	82.83	0.53	5.13
M2	spring	7/12/2006	84	3.03E+04	5.52E+04	3.10E+04	5.65E+04	0.45	28.9	28.9	22.2	19.6	2.371	1.09	2.74	1.16	88.03	82.83	0.53	5.13
M3	spring	7/12/2006	84	3.45E+04	5.67E+04	5.30E+04	8.70E+04	0.39	28.9	28.9	22.2	19.6	2.371	1.09	2.74	1.16	88.03	82.83	0.53	5.13
M4	spring	7/12/2006	84	2.62E+04	3.83E+04			0.32	28.9	28.9	22.2	19.6	2.371	1.09	2.74	1.16	88.03	82.83	0.53	5.13
M1	spring	7/19/2006	91	1.40E+05	1.58E+05	5.40E+04	6.11E+04	0.12	32.8	32.8	25.0	24.2	2.986	1.65	3.07	1.33	75.31	81.67	0.00	0.91
M2	spring	7/19/2006	91	8.70E+04	1.04E+05	6.00E+03	7.16E+03	0.16	32.8	32.8	25.0	24.2	2.986	1.65	3.07	1.33	75.31	81.67	0.00	0.91
M3	spring	7/19/2006	91	2.00E+04	2.26E+04	3.60E+04	4.07E+04	0.12	32.8	32.8	25.0	24.2	2.986	1.65	3.07	1.33	75.31	81.67	0.00	0.91
M4	spring	7/19/2006	91	5.00E+03	6.53E+03	5.70E+04	7.45E+04	0.23	32.8	32.8	25.0	24.2	2.986	1.65	3.07	1.33	75.31	81.67	0.00	0.91
M5	spring	7/19/2006	91	2.50E+03	2.94E+03	2.20E+04	2.59E+04	0.15	32.8	32.8	25.0	24.2	2.986	1.65	3.07	1.33	75.31	81.67	0.00	0.91
M1	spring	7/26/2006	98	4.00E+03	5.72E+03	7.60E+03	1.09E+04	0.30	25.6	31.7	21.7	22.3	1.704	0.61	2.93	1.25	89.66	82.21	0.00	1.42
M2	spring	7/26/2006	98	3.80E+03	5.21E+03	6.00E+04	8.23E+04	0.27	25.6	31.7	21.7	22.3	1.704	0.61	2.93	1.25	89.66	82.21	0.00	1.42
M3	spring	7/26/2006	98	9.00E+02	1.32E+03	7.12E+05	1.04E+06	0.32	25.6	31.7	21.7	22.3	1.704	0.61	2.93	1.25	89.66	82.21	0.00	1.42
M4	spring	7/26/2006	98	0.00E+00	0.00E+00	5.20E+04	7.73E+04	0.33	25.6	31.7	21.7	22.3	1.704	0.61	2.93	1.25	89.66	82.21	0.00	1.42
M5	spring	7/26/2006	98	7.00E+03	1.06E+04	1.15E+05	1.74E+05	0.34	25.6	31.7	21.7	22.3	1.704	0.61	2.93	1.25	89.66	82.21	0.00	1.42
M1	spring	8/2/2006	105	4.80E+04	8.31E+04	8.50E+05	1.47E+06	0.42	32.2	32.2	25.6	23.6	2.849	1.50	2.85	1.16	78.00	82.90	0.00	0.56
M2	spring	8/2/2006	105	2.00E+04	4.98E+04	3.20E+04	7.96E+04	0.60	32.2	32.2	25.6	23.6	2.849	1.50	2.85	1.16	78.00	82.90	0.00	0.56
M3	spring	8/2/2006	105	5.40E+05	1.10E+06			0.51	32.2	32.2	25.6	23.6	2.849	1.50	2.85	1.16	78.00	82.90	0.00	0.56
M4	spring	8/2/2006	105	3.30E+05	6.69E+05	5.20E+05	1.05E+06	0.51	32.2	32.2	25.6	23.6	2.849	1.50	2.85	1.16	78.00	82.90	0.00	0.56
M5	spring	8/2/2006	105	4.10E+04	8.30E+04	3.40E+05	6.88E+05	0.51	32.2	32.2	25.6	23.6	2.849	1.50	2.85	1.16	78.00	82.90	0.00	0.56
M1	spring	8/9/2006	112	1.40E+04	3.68E+04	1.30E+04	3.41E+04	0.62	28.9	32.2	23.9	24.9	1.936	0.90	2.77	1.06	90.07	81.21	0.08	0.33
M2	spring	8/9/2006	112	4.00E+03	9.39E+03	5.00E+03	1.17E+04	0.57	28.9	32.2	23.9	24.9	1.936	0.90	2.77	1.06	90.07	81.21	0.08	0.33
M3	spring	8/9/2006	112	1.40E+05	3.43E+05	3.70E+04	9.07E+04	0.59	28.9	32.2	23.9	24.9	1.936	0.90	2.77	1.06	90.07	81.21	0.08	0.33
M4	spring	8/9/2006	112	5.30E+04	1.08E+05	7.50E+04	1.53E+05	0.51	28.9	32.2	23.9	24.9	1.936	0.90	2.77	1.06	90.07	81.21	0.08	0.33
M5	spring	8/9/2006	112	1.10E+04	2.33E+04	4.60E+04	9.73E+04	0.53	28.9	32.2	23.9	24.9	1.936	0.90	2.77	1.06	90.07	81.21	0.08	0.33
M1	spring	8/16/2006	119	8.00E+02	1.31E+03	5.00E+02	8.19E+02	0.39	26.7	29.4	22.2	20.9	1.247	0.58	2.62	0.84	88.44	87.55	0.03	2.39
M2	spring	8/16/2006	119	1.83E+04	3.06E+04	2.79E+04	4.67E+04	0.40	26.7	29.4	22.2	20.9	1.247	0.58	2.62	0.84	88.44	87.55	0.03	2.39
M3	spring	8/16/2006	119	8.00E+02	1.54E+03	5.34E+04	1.03E+05	0.48	26.7	29.4	22.2	20.9	1.247	0.58	2.62	0.84	88.44	87.55	0.03	2.39
M4	spring	8/16/2006	119	2.00E+02	3.53E+02	2.20E+03	3.88E+03	0.43	26.7	29.4	22.2	20.9	1.247	0.58	2.62	0.84	88.44	87.55	0.03	2.39
M5	spring	8/16/2006	119	1.20E+03	2.04E+03	4.20E+03	7.15E+03	0.41	26.7	29.4	22.2	20.9	1.247	0.58	2.62	0.84	88.44	87.55	0.03	2.39

Sample No.	Season	Sample date	Time (d)	*E. coli* (cfu g^{-1}) wet wt.	*E. coli* (cfu g^{-1}) dry wt.	ENT (cfu g^{-1}) wet wt.	ENT (cfu g^{-1}) dry wt.	MC (%)	Temp PDH (ºC)	Temp PWH (ºC)	Temp PDA (ºC)	Temp PWA (ºC)	SR PDH (MJ)	SR PDA (MJ)	SR PWH (MJ)	SR PWA (MJ)	RH PDA (%)	RH PWA (%)	rainfall PDT (cm)	Rainfall PWT (cm)
M1	spring	8/24/2006	127	1.00E+02	1.37E+02	1.10E+03	1.51E+03	0.27	28.9	28.9	21.7	21.6	2.85	1.40	2.88	1.31	73.35	78.86	0.00	2.41
M2	spring	8/24/2006	127	1.40E+03	1.88E+03	1.40E+03	1.88E+03	0.26	28.9	28.9	21.7	21.6	2.85	1.40	2.88	1.31	73.35	78.86	0.00	2.41
M3	spring	8/24/2006	127	9.40E+03	1.10E+04	8.60E+03	1.00E+04	0.14	28.9	28.9	21.7	21.6	2.85	1.40	2.88	1.31	73.35	78.86	0.00	2.41
M4	spring	8/24/2006	127	1.10E+03	6.89E+03	1.85E+04	1.16E+05	0.84	28.9	28.9	21.7	21.6	2.85	1.40	2.88	1.31	73.35	78.86	0.00	2.41
M5	spring	8/24/2006	127	1.00E+03	1.28E+03	2.03E+04	2.59E+04	0.22	28.9	28.9	21.7	21.6	2.85	1.40	2.88	1.31	73.35	78.86	0.00	2.41
M1	spring	8/30/2006	133	7.00E+02	1.37E+03	6.50E+03	1.27E+04	0.49	28.9	31.1	24.4	23.1	2.256	1.00	2.68	1.22	86.37	79.41	0.08	1.19
M2	spring	8/30/2006	133	2.00E+02	4.21E+02	2.00E+03	4.21E+03	0.53	28.9	31.1	24.4	23.1	2.256	1.00	2.68	1.22	86.37	79.41	0.08	1.19
M3	spring	8/30/2006	133	1.30E+03	2.51E+03	1.29E+04	2.49E+04	0.48	28.9	31.1	24.4	23.1	2.256	1.00	2.68	1.22	86.37	79.41	0.08	1.19
M4	spring	8/30/2006	133	2.00E+03	4.09E+03	4.20E+03	8.59E+03	0.51	28.9	31.1	24.4	23.1	2.256	1.00	2.68	1.22	86.37	79.41	0.08	1.19
M5	spring	8/30/2006	133	2.10E+03	5.73E+03	8.50E+03	2.32E+04	0.63	28.9	31.1	24.4	23.1	2.256	1.00	2.68	1.22	86.37	79.41	0.08	1.19
M1	summer	6/28/2006	0	8.00E+05	3.11E+06	1.20E+08	4.66E+08	0.74	24.4		21.1		2.93	1.47			97.06		0.00	
M2	summer	6/28/2006	0	1.00E+06	9.36E+06	1.10E+08	1.03E+09	0.89	24.4		21.1		2.93	1.47			97.06		0.00	
M3	summer	6/28/2006	0	9.00E+05	4.78E+06	1.90E+08	1.01E+09	0.81	24.4		21.1		2.93	1.47			97.06		0.00	
M4	summer	6/28/2006	0	1.00E+06	5.71E+06	1.20E+08	6.86E+08	0.82	24.4		21.1		2.93	1.47			97.06		0.00	
M5	summer	6/28/2006	0	4.00E+05	2.10E+06	1.10E+08	5.78E+08	0.81	24.4		21.1		2.93	1.47			97.06		0.00	
M1	summer	6/30/2006	2	5.00E+05	2.25E+06	2.30E+08	1.03E+09	0.78	25.0	27.8	18.3	14.7	2.888	1.36	2.93	1.42	77.72	78.54	0.00	0.00
M2	summer	6/30/2006	2	1.00E+05	4.30E+05	2.10E+08	9.03E+08	0.77	25.0	27.8	18.3	14.7	2.888	1.36	2.93	1.42	77.72	78.54	0.00	0.00
M3	summer	6/30/2006	2	1.00E+05	4.02E+05	1.20E+08	4.82E+08	0.75	25.0	27.8	18.3	14.7	2.888	1.36	2.93	1.42	77.72	78.54	0.00	0.00
M4	summer	6/30/2006	2	4.00E+05	1.67E+06	1.90E+08	7.93E+08	0.76	25.0	27.8	18.3	14.7	2.888	1.36	2.93	1.42	77.72	78.54	0.00	0.00
M5	summer	6/30/2006	2	3.00E+05	1.34E+06	1.10E+08	4.92E+08	0.78	25.0	27.8	18.3	14.7	2.888	1.36	2.93	1.42	77.72	78.54	0.00	0.00
M1	summer	7/3/2006	5	4.40E+06	1.08E+07	1.70E+08	4.16E+08	0.59	30.6	30.6	23.9	20.4	2.999	1.61	3.11	1.58	72.52	75.03	0.00	0.00
M2	summer	7/3/2006	5	2.86E+07	7.46E+07	2.50E+08	6.52E+08	0.62	30.6	30.6	23.9	20.4	2.999	1.61	3.11	1.58	72.52	75.03	0.00	0.00
M3	summer	7/3/2006	5	1.68E+07	5.11E+07	2.00E+08	6.09E+08	0.67	30.6	30.6	23.9	20.4	2.999	1.61	3.11	1.58	72.52	75.03	0.00	0.00
M4	summer	7/3/2006	5	1.76E+07	5.00E+07	1.30E+08	3.69E+08	0.65	30.6	30.6	23.9	20.4	2.999	1.61	3.11	1.58	72.52	75.03	0.00	0.00
M5	summer	7/3/2006	5	6.90E+06	1.76E+07	1.80E+08	4.60E+08	0.61	30.6	30.6	23.9	20.4	2.999	1.61	3.11	1.58	72.52	75.03	0.00	0.00
M1	summer	7/5/2006	7	2.18E+07	7.24E+07	1.60E+08	5.32E+08	0.70	30.6	30.6	23.9	21.6	2.734	1.28	3.11	1.47	84.47	77.65	1.14	1.14
M2	summer	7/5/2006	7	1.92E+07	7.13E+07	1.20E+08	4.45E+08	0.73	30.6	30.6	23.9	21.6	2.734	1.28	3.11	1.47	84.47	77.65	1.14	1.14
M3	summer	7/5/2006	7	5.30E+07	1.76E+08	1.30E+08	4.31E+08	0.70	30.6	30.6	23.9	21.6	2.734	1.28	3.11	1.47	84.47	77.65	1.14	1.14
M4	summer	7/5/2006	7	3.90E+07	1.51E+08	2.10E+08	8.11E+08	0.74	30.6	30.6	23.9	21.6	2.734	1.28	3.11	1.47	84.47	77.65	1.14	1.14
M5	summer	7/5/2006	7	1.09E+08	3.43E+08	1.90E+08	5.99E+08	0.68	30.6	30.6	23.9	21.6	2.734	1.28	3.11	1.47	84.47	77.65	1.14	1.14
M1	summer	7/12/2006	14	2.04E+07	6.68E+07	2.70E+07	8.85E+07	0.69	28.9	28.9	22.2	19.6	2.371	1.09	2.74	1.16	88.03	82.83	0.53	5.13
M2	summer	7/12/2006	14	4.40E+06	1.46E+07	2.10E+07	6.96E+07	0.70	28.9	28.9	22.2	19.6	2.371	1.09	2.74	1.16	88.03	82.83	0.53	5.13
M3	summer	7/12/2006	14	5.20E+06	1.57E+07	1.00E+07	3.02E+07	0.67	28.9	28.9	22.2	19.6	2.371	1.09	2.74	1.16	88.03	82.83	0.53	5.13
M4	summer	7/12/2006	14	1.01E+07	3.21E+07	3.20E+07	1.02E+08	0.69	28.9	28.9	22.2	19.6	2.371	1.09	2.74	1.16	88.03	82.83	0.53	5.13

Sample No.	Season	Sample date	Time (d)	*E. coli* (cfu g^{-1}) wet wt.	*E. coli* (cfu g^{-1}) dry wt.	ENT (cfu g^{-1}) wet wt.	ENT (cfu g^{-1}) dry wt.	MC (%)	Temp PDH (ºC)	Temp PWH (ºC)	Temp PDA (ºC)	Temp PWA (ºC)	SR PDH (MJ)	SR PDA (MJ)	SR PWH (MJ)	SR PWA (MJ)	RH PDA (%)	RH PWA (%)	rainfall PDT (cm)	Rainfall PWT (cm)
M1	summer	7/19/2006	21	1.00E+06	1.49E+06	4.30E+06	6.39E+06	0.33	32.8	32.8	25.0	24.2	2.986	1.65	3.07	1.33	75.31	81.67	0.00	0.91
M2	summer	7/19/2006	21	1.20E+06	3.42E+06	3.20E+06	9.12E+06	0.65	32.8	32.8	25.0	24.2	2.986	1.65	3.07	1.33	75.31	81.67	0.00	0.91
M3	summer	7/19/2006	21	2.80E+06	4.70E+06	2.10E+06	3.52E+06	0.40	32.8	32.8	25.0	24.2	2.986	1.65	3.07	1.33	75.31	81.67	0.00	0.91
M4	summer	7/19/2006	21	2.40E+06	7.21E+06	4.50E+06	1.35E+07	0.67	32.8	32.8	25.0	24.2	2.986	1.65	3.07	1.33	75.31	81.67	0.00	0.91
M5	summer	7/19/2006	21	1.10E+06	2.54E+06	5.70E+06	1.32E+07	0.57	32.8	32.8	25.0	24.2	2.986	1.65	3.07	1.33	75.31	81.67	0.00	0.91
M1	summer	7/26/2006	28	2.20E+05	6.70E+05	9.40E+05	2.86E+06	0.67	25.6	31.7	21.7	22.3	1.704	0.61	2.93	1.25	89.66	82.21	0.00	1.42
M2	summer	7/26/2006	28	5.30E+05	1.38E+06	1.30E+06	3.40E+06	0.62	25.6	31.7	21.7	22.3	1.704	0.61	2.93	1.25	89.66	82.21	0.00	1.42
M3	summer	7/26/2006	28	2.00E+05	6.27E+05	4.00E+05	1.25E+06	0.68	25.6	31.7	21.7	22.3	1.704	0.61	2.93	1.25	89.66	82.21	0.00	1.42
M4	summer	7/26/2006	28	4.40E+06	9.39E+06	5.30E+06	1.13E+07	0.53	25.6	31.7	21.7	22.3	1.704	0.61	2.93	1.25	89.66	82.21	0.00	1.42
M5	summer	7/26/2006	28	3.90E+05	9.32E+05	2.40E+06	5.74E+06	0.58	25.6	31.7	21.7	22.3	1.704	0.61	2.93	1.25	89.66	82.21	0.00	1.42
M1	summer	8/2/2006	35	2.30E+05	3.77E+05	9.00E+04	1.48E+05	0.39	32.2	32.2	25.6	23.6	2.849	1.50	2.85	1.16	78.00	82.90	0.00	0.56
M2	summer	8/2/2006	35	5.20E+05	9.56E+05	3.40E+05	6.25E+05	0.46	32.2	32.2	25.6	23.6	2.849	1.50	2.85	1.16	78.00	82.90	0.00	0.56
M3	summer	8/2/2006	35	1.47E+06	2.35E+06	1.64E+06	2.62E+06	0.37	32.2	32.2	25.6	23.6	2.849	1.50	2.85	1.16	78.00	82.90	0.00	0.56
M4	summer	8/2/2006	35	1.40E+05	2.36E+05	1.60E+05	2.70E+05	0.41	32.2	32.2	25.6	23.6	2.849	1.50	2.85	1.16	78.00	82.90	0.00	0.56
M5	summer	8/2/2006	35	2.30E+05	5.07E+05	7.00E+04	1.54E+05	0.55	32.2	32.2	25.6	23.6	2.849	1.50	2.85	1.16	78.00	82.90	0.00	0.56
M1	summer	8/9/2006	42	1.45E+06	4.48E+06	1.92E+06	5.93E+06	0.68	28.9	32.2	23.9	24.9	1.936	0.90	2.77	1.06	90.07	81.21	0.08	0.33
M2	summer	8/9/2006	42	6.40E+05	1.68E+06	2.68E+06	7.05E+06	0.62	28.9	32.2	23.9	24.9	1.936	0.90	2.77	1.06	90.07	81.21	0.08	0.33
M3	summer	8/9/2006	42	5.20E+06	1.21E+07	4.16E+06	9.67E+06	0.57	28.9	32.2	23.9	24.9	1.936	0.90	2.77	1.06	90.07	81.21	0.08	0.33
M4	summer	8/9/2006	42	5.10E+05	1.10E+06	2.73E+06	5.91E+06	0.54	28.9	32.2	23.9	24.9	1.936	0.90	2.77	1.06	90.07	81.21	0.08	0.33
M5	summer	8/9/2006	42	2.60E+06	6.08E+06	6.00E+05	1.40E+06	0.57	28.9	32.2	23.9	24.9	1.936	0.90	2.77	1.06	90.07	81.21	0.08	0.33
M1	summer	8/16/2006	49	9.10E+05	1.70E+06	4.00E+04	7.48E+04	0.47	26.7	29.4	22.2	20.9	1.247	0.58	2.62	0.84	88.44	87.55	0.03	2.39
M2	summer	8/16/2006	49	1.80E+05	2.89E+05	2.20E+05	3.53E+05	0.38	26.7	29.4	22.2	20.9	1.247	0.58	2.62	0.84	88.44	87.55	0.03	2.39
M3	summer	8/16/2006	49	9.00E+04	1.39E+05	1.50E+05	2.32E+05	0.35	26.7	29.4	22.2	20.9	1.247	0.58	2.62	0.84	88.44	87.55	0.03	2.39
M4	summer	8/16/2006	49	1.25E+06	2.19E+06	6.80E+05	1.19E+06	0.43	26.7	29.4	22.2	20.9	1.247	0.58	2.62	0.84	88.44	87.55	0.03	2.39
M5	summer	8/16/2006	49	5.00E+04	7.63E+04	1.10E+05	1.68E+05	0.34	26.7	29.4	22.2	20.9	1.247	0.58	2.62	0.84	88.44	87.55	0.03	2.39
M1	summer	8/24/2006	56	4.90E+05	6.69E+05	3.50E+04	4.78E+04	0.27	28.9	28.9	21.7	21.6	2.85	1.40	2.88	1.31	73.35	78.86	0.00	2.41
M2	summer	8/24/2006	56	1.20E+04	1.55E+04	1.00E+04	1.29E+04	0.23	28.9	28.9	21.7	21.6	2.85	1.40	2.88	1.31	73.35	78.86	0.00	2.41
M3	summer	8/24/2006	56	1.30E+04	1.64E+04	4.00E+03	5.03E+03	0.20	28.9	28.9	21.7	21.6	2.85	1.40	2.88	1.31	73.35	78.86	0.00	2.41
M4	summer	8/24/2006	56	4.60E+04	5.70E+04	3.90E+04	4.84E+04	0.19	28.9	28.9	21.7	21.6	2.85	1.40	2.88	1.31	73.35	78.86	0.00	2.41
M5	summer	8/24/2006	56	3.50E+04	4.45E+04	6.40E+04	8.13E+04	0.21	28.9	28.9	21.7	21.6	2.85	1.40	2.88	1.31	73.35	78.86	0.00	2.41
M1	summer	8/30/2006	63	1.56E+05	2.72E+05	1.29E+05	2.25E+05	0.43	28.9	31.1	24.4	23.1	2.256	1.00	2.68	1.22	86.37	79.41	0.08	1.19
M2	summer	8/30/2006	63	1.70E+04	2.99E+04	7.90E+04	1.39E+05	0.43	28.9	31.1	24.4	23.1	2.256	1.00	2.68	1.22	86.37	79.41	0.08	1.19
M3	summer	8/30/2006	63	3.50E+04	6.40E+04	8.40E+04	1.54E+05	0.45	28.9	31.1	24.4	23.1	2.256	1.00	2.68	1.22	86.37	79.41	0.08	1.19
M4	summer	8/30/2006	63	4.50E+04	8.31E+04	1.52E+05	2.81E+05	0.46	28.9	31.1	24.4	23.1	2.256	1.00	2.68	1.22	86.37	79.41	0.08	1.19

Sample No.	Season	Sample date	Time (d)	*E. coli* (cfu g^{-1}) wet wt.	*E. coli* (cfu g^{-1}) dry wt.	ENT (cfu g^{-1}) wet wt.	ENT (cfu g^{-1}) dry wt.	MC (%)	Temp PDH (ºC)	Temp PWH (ºC)	Temp PDA (ºC)	Temp PWA (ºC)	SR PDH (MJ)	SR PDA (MJ)	SR PWH (MJ)	SR PWA (MJ)	RH PDA (%)	RH PWA (%)	rainfall PDT (cm)	Rainfall PWT (cm)
M5	summer	8/30/2006	63	2.10E+05	3.80E+05	5.30E+04	9.58E+04	0.45	28.9	31.1	24.4	23.1	2.256	1.00	2.68	1.22	86.37	79.41	0.08	1.19
M1	summer	9/6/2006	70	5.00E+03	1.41E+04	1.07E+05	3.02E+05	0.65	22.2	26.1	19.4	18.7	1.434	0.63	2.52	0.63	95.68	90.48	1.19	3.68
M2	summer	9/6/2006	70	4.00E+02	1.16E+03	2.40E+04	6.93E+04	0.65	22.2	26.1	19.4	18.7	1.434	0.63	2.52	0.63	95.68	90.48	1.19	3.68
M3	summer	9/6/2006	70	6.00E+04	1.54E+05	4.00E+03	1.03E+04	0.61	22.2	26.1	19.4	18.7	1.434	0.63	2.52	0.63	95.68	90.48	1.19	3.68
M4	summer	9/6/2006	70	2.80E+05	6.63E+05	1.60E+04	3.79E+04	0.58	22.2	26.1	19.4	18.7	1.434	0.63	2.52	0.63	95.68	90.48	1.19	3.68
M5	summer	9/6/2006	70	6.00E+03	1.64E+04	4.00E+03	1.10E+04	0.63	22.2	26.1	19.4	18.7	1.434	0.63	2.52	0.63	95.68	90.48	1.19	3.68
M1	summer	9/13/2006	77	3.90E+03	1.28E+04	9.00E+03	2.96E+04	0.70	17.2	25.6	16.1	18.2	0.437	0.18	2.39	0.87	91.95	88.41	0.00	0.74
M2	summer	9/13/2006	77	1.10E+03	2.87E+03	7.00E+03	1.83E+04	0.62	17.2	25.6	16.1	18.2	0.437	0.18	2.39	0.87	91.95	88.41	0.00	0.74
M3	summer	9/13/2006	77	2.50E+03	5.78E+03	8.00E+03	1.85E+04	0.57	17.2	25.6	16.1	18.2	0.437	0.18	2.39	0.87	91.95	88.41	0.00	0.74
M4	summer	9/13/2006	77	8.00E+02	2.25E+03	4.60E+03	1.29E+04	0.64	17.2	25.6	16.1	18.2	0.437	0.18	2.39	0.87	91.95	88.41	0.00	0.74
M5	summer	9/13/2006	77	1.80E+03	4.98E+03	6.00E+03	1.66E+04	0.64	17.2	25.6	16.1	18.2	0.437	0.18	2.39	0.87	91.95	88.41	0.00	0.74
M1	summer	9/20/2007	84	2.40E+03	2.93E+03	1.70E+03	2.07E+03	0.18	22.2	27.2	17.8	17.2							0.00	1.75
M2	summer	9/20/2007	84	1.70E+03	2.14E+03	9.90E+03	1.25E+04	0.21	22.2	27.2	17.8	17.2							0.00	1.75
M3	summer	9/20/2007	84	2.10E+03	2.85E+03	1.20E+03	1.63E+03	0.26	22.2	27.2	17.8	17.2							0.00	1.75
M4	summer	9/20/2007	84	3.40E+03	4.19E+03	3.60E+03	4.44E+03	0.19	22.2	27.2	17.8	17.2							0.00	1.75
M5	summer	9/20/2007	84	5.10E+03	7.29E+03	4.50E+03	6.43E+03	0.30	22.2	27.2	17.8	17.2							0.00	1.75
M1	summer	9/27/2006	91	3.70E+03	5.53E+03	1.00E+03	1.50E+03	0.33	20.0	25.6	12.2	14.4							0.00	0.56
M2	summer	9/27/2006	91	8.50E+03	1.26E+04	1.10E+03	1.64E+03	0.33	20.0	25.6	12.2	14.4							0.00	0.56
M3	summer	9/27/2006	91	8.00E+02	1.27E+03	9.00E+02	1.43E+03	0.37	20.0	25.6	12.2	14.4							0.00	0.56
M4	summer	9/27/2006	91	2.00E+03	3.17E+03	1.10E+03	1.74E+03	0.37	20.0	25.6	12.2	14.4							0.00	0.56
M5	summer	9/27/2006	91	9.40E+02	1.40E+03	1.20E+03	1.79E+03	0.33	20.0	25.6	12.2	14.4							0.00	0.56
M1	summer	10/4/2006	98	2.80E+02	4.04E+02	1.07E+04	1.54E+04	0.31	24.4	24.4	15.6	13.7							0.00	2.34
M2	summer	10/4/2006	98	4.70E+03	7.16E+03	3.60E+03	5.48E+03	0.34	24.4	24.4	15.6	13.7							0.00	2.34
M3	summer	10/4/2006	98	1.20E+03	1.66E+03	8.00E+02	1.11E+03	0.28	24.4	24.4	15.6	13.7							0.00	2.34
M4	summer	10/4/2006	98	1.10E+04	1.50E+04	1.20E+02	1.64E+02	0.27	24.4	24.4	15.6	13.7							0.00	2.34
M5	summer	10/4/2006	98	2.20E+03	3.16E+03	3.30E+03	4.74E+03	0.30	24.4	24.4	15.6	13.7							0.00	2.34
M1	summer	10/11/2007	105	1.89E+04	2.87E+04	6.00E+03	9.12E+03	0.34	25.0	26.1	15.6	14.9							0.00	3.23
M2	summer	10/11/2007	105	4.50E+03	6.16E+03	3.10E+03	4.25E+03	0.27	25.0	26.1	15.6	14.9							0.00	3.23
M3	summer	10/11/2007	105	2.80E+03	5.18E+03	5.00E+03	9.24E+03	0.46	25.0	26.1	15.6	14.9							0.00	3.23
M4	summer	10/11/2007	105	1.90E+03	3.14E+03	4.20E+03	6.95E+03	0.40	25.0	26.1	15.6	14.9							0.00	3.23
M5	summer	10/11/2007	105	2.80E+03		2.30E+03		1.92	25.0	26.1	15.6	14.9							0.00	3.23
M1	summer	10/18/2007	112	2.50E+02	3.86E+02	2.20E+03	3.40E+03	0.35	15.6	18.3	11.1	8.3	0.279	0.11	2.20	0.82	98.67	68.30	3.00	3.25
M2	summer	10/18/2007	112	2.00E+01	3.09E+01	6.10E+03	9.42E+03	0.35	15.6	18.3	11.1	8.3	0.279	0.11	2.20	0.82	98.67	68.30	3.00	3.25

Sample No.	Season	Sample date	Time (d)	*E. coli* (cfu g^{-1}) wet wt.	*E. coli* (cfu g^{-1}) dry wt.	ENT (cfu g^{-1}) wet wt.	ENT (cfu g^{-1}) dry wt.	MC (%)	Temp PDH (ºC)	Temp PWH (ºC)	Temp PDA (ºC)	Temp PWA (ºC)	SR PDH (MJ)	SR PDA (MJ)	SR PWH (MJ)	SR PWA (MJ)	RH PDA (%)	RH PWA (%)	rainfall PDT (cm)	Rainfall PWT (cm)
M3	summer	10/18/2007	112	2.20E+03	3.47E+03	7.00E+03	1.10E+04	0.37	15.6	18.3	11.1	8.3	0.279	0.11	2.20	0.82	98.67	68.30	3.00	3.25
M4	summer	10/18/2007	112	3.30E+03	6.35E+03	3.60E+03	6.93E+03	0.48	15.6	18.3	11.1	8.3	0.279	0.11	2.20	0.82	98.67	68.30	3.00	3.25
M5	summer	10/18/2007	112	1.20E+02	1.96E+02	8.90E+03	1.46E+04	0.39	15.6	18.3	11.1	8.3	0.279	0.11	2.20	0.82	98.67	68.30	3.00	3.25
M1	summer	10/25/2007	119	2.10E+02	2.89E+02	1.30E+03	1.79E+03	0.27	5.0	25.6	1.7	9.2	1.011	0.52	2.08	0.69	62.13	78.61	0.00	1.57
M2	summer	10/25/2007	119	7.30E+02	1.10E+03	9.00E+02	1.35E+03	0.33	5.0	25.6	1.7	9.2	1.011	0.52	2.08	0.69	62.13	78.61	0.00	1.57
M3	summer	10/25/2007	119	5.60E+02	8.88E+02	2.00E+02	3.17E+02	0.37	5.0	25.6	1.7	9.2	1.011	0.52	2.08	0.69	62.13	78.61	0.00	1.57
M4	summer	10/25/2007	119	1.40E+02	1.88E+02	2.20E+03	2.95E+03	0.25	5.0	25.6	1.7	9.2	1.011	0.52	2.08	0.69	62.13	78.61	0.00	1.57
M5	summer	10/25/2007	119	8.50E+02	1.57E+03	9.00E+02	1.66E+03	0.46	5.0	25.6	1.7	9.2	1.011	0.52	2.08	0.69	62.13	78.61	0.00	1.57
M1	summer	11/1/2006	126	1.31E+04	2.09E+04	6.70E+02	1.07E+03	0.37	21.1	21.1	12.2	8.1	1.952	0.97	2.00	0.69	67.23	68.21	0.00	3.48
M2	summer	11/1/2006	126	6.40E+02	8.97E+02	4.00E+02	5.61E+02	0.29	21.1	21.1	12.2	8.1	1.952	0.97	2.00	0.69	67.23	68.21	0.00	3.48
M3	summer	11/1/2006	126	4.00E+02	5.98E+02	3.10E+02	4.63E+02	0.33	21.1	21.1	12.2	8.1	1.952	0.97	2.00	0.69	67.23	68.21	0.00	3.48
M4	summer	11/1/2006	126	3.00E+02	3.80E+02	1.13E+03	1.43E+03	0.21	21.1	21.1	12.2	8.1	1.952	0.97	2.00	0.69	67.23	68.21	0.00	3.48
M5	summer	11/1/2006	126	1.30E+02	1.84E+02	3.40E+03	4.80E+03	0.29	21.1	21.1	12.2	8.1	1.952	0.97	2.00	0.69	67.23	68.21	0.00	3.48
M1	summer	11/8/2007	133	5.30E+02	8.35E+02	1.10E+02	1.73E+02	0.37	9.4	21.1	5.6	5.6	0.231	0.11	1.94	0.84	86.43	64.63	0.56	1.88
M2	summer	11/8/2007	133	1.13E+03	1.94E+03	8.00E+01	1.38E+02	0.42	9.4	21.1	5.6	5.6	0.231	0.11	1.94	0.84	86.43	64.63	0.56	1.88
M3	summer	11/8/2007	133	4.60E+02	9.83E+02	2.70E+02	5.77E+02	0.53	9.4	21.1	5.6	5.6	0.231	0.11	1.94	0.84	86.43	64.63	0.56	1.88
M4	summer	11/8/2007	133	3.00E+02	7.32E+02	3.00E+02	7.32E+02	0.59	9.4	21.1	5.6	5.6	0.231	0.11	1.94	0.84	86.43	64.63	0.56	1.88
M5	summer	11/8/2007	133	1.20E+01	2.29E+01	3.50E+02	6.69E+02	0.48	9.4	21.1	5.6	5.6	0.231	0.11	1.94	0.84	86.43	64.63	0.56	1.88
M1	summer	11/15/2007	140	5.60E+02	8.73E+02	9.00E+01	1.40E+02	0.36	14.4	25.0	7.8	11.3	1.799	0.76	1.99	0.69	71.44	78.94	0.00	1.47
M2	summer	11/15/2007	140	1.81E+03	2.86E+03	4.30E+02	6.79E+02	0.37	14.4	25.0	7.8	11.3	1.799	0.76	1.99	0.69	71.44	78.94	0.00	1.47
M3	summer	11/15/2007	140	1.10E+02	1.75E+02	2.00E+01	3.18E+01	0.37	14.4	25.0	7.8	11.3	1.799	0.76	1.99	0.69	71.44	78.94	0.00	1.47
M4	summer	11/15/2007	140	1.10E+02	1.76E+02	2.10E+02	3.37E+02	0.38	14.4	25.0	7.8	11.3	1.799	0.76	1.99	0.69	71.44	78.94	0.00	1.47
M5	summer	11/15/2007	140	1.24E+03	2.35E+03	6.00E+01	1.14E+02	0.47	14.4	25.0	7.8	11.3	1.799	0.76	1.99	0.69	71.44	78.94	0.00	1.47
M1	summer	11/29/2006	154	7.00E+01	1.18E+02	2.00E+01	3.38E+01	0.41	18.9	19.4	8.9	6.6	1.474	0.81	1.79	0.72	72.79	72.21	0.00	5.97
M2	summer	11/29/2006	154	1.10E+02	1.66E+02	1.60E+02	2.42E+02	0.34	18.9	19.4	8.9	6.6	1.474	0.81	1.79	0.72	72.79	72.21	0.00	5.97
M3	summer	11/29/2006	154	2.20E+02	3.46E+02	3.00E+01	4.72E+01	0.36	18.9	19.4	8.9	6.6	1.474	0.81	1.79	0.72	72.79	72.21	0.00	5.97
M4	summer	11/29/2006	154	5.70E+02	1.01E+03	7.00E+01	1.24E+02	0.44	18.9	19.4	8.9	6.6	1.474	0.81	1.79	0.72	72.79	72.21	0.00	5.97
M5	summer	11/29/2006	154	3.40E+02	5.72E+02	8.00E+01	1.35E+02	0.41	18.9	19.4	8.9	6.6	1.474	0.81	1.79	0.72	72.79	72.21	0.00	5.97
M1	summer	12/13/2007	168	4.00E+01	6.76E+01	5.00E+01	8.45E+01	0.41	11.7	22.2	3.3	3.1							0.00	0.38
M2	summer	12/13/2007	168	6.00E+01	9.06E+01	1.40E+02	2.11E+02	0.34	11.7	22.2	3.3	3.1							0.00	0.38
M3	summer	12/13/2007	168	3.00E+01	4.72E+01	1.40E+02	2.20E+02	0.36	11.7	22.2	3.3	3.1							0.00	0.38
M4	summer	12/13/2007	168	0.00E+00	0.00E+00	1.00E+01	1.78E+01	0.44	11.7	22.2	3.3	3.1							0.00	0.38
M5	summer	12/13/2007	168	1.20E+02	2.02E+02	1.30E+02	2.19E+02	0.41	11.7	22.2	3.3	3.1							0.00	0.38
M1	fall	9/21/2006	0	1.00E+06	6.89E+06	2.00E+05	1.38E+06	0.85	19.4		11.1								0.00	

Sample No.	Season	Sample date	Time (d)	*E. coli* (cfu g^{-1}) wet wt.	*E. coli* (cfu g^{-1}) dry wt.	ENT (cfu g^{-1}) wet wt.	ENT (cfu g^{-1}) dry wt.	MC (%)	Temp PDH (ºC)	Temp PWH (ºC)	Temp PDA (ºC)	Temp PWA (ºC)	SR PDH (MJ)	SR PDA (MJ)	SR PWH (MJ)	SR PWA (MJ)	RH PDA (%)	RH PWA (%)	rainfall PDT (cm)	Rainfall PWT (cm)
M2	fall	9/21/2006	0	2.60E+06	1.81E+07	1.70E+06	1.18E+07	0.86	19.4		11.1								0.00	
M3	fall	9/21/2006	0	1.35E+07	8.63E+07			0.84	19.4		11.1								0.00	
M4	fall	9/21/2006	0	1.04E+07	6.54E+07			0.84	19.4		11.1								0.00	
M5	fall	9/21/2006	0	1.18E+07	7.65E+07	2.00E+05	1.30E+06	0.85	19.4		11.1								0.00	
M1	fall	9/25/2006	4	1.10E+07	5.62E+07	7.70E+06	3.93E+07	0.80	24.4	25.6	20.0	16.3							0.56	0.56
M2	fall	9/25/2006	4	4.10E+06	2.24E+07	3.90E+06	2.13E+07	0.82	24.4	25.6	20.0	16.3							0.56	0.56
M3	fall	9/25/2006	4	5.10E+06	2.59E+07	5.40E+06	2.74E+07	0.80	24.4	25.6	20.0	16.3							0.56	0.56
M4	fall	9/25/2006	4	2.50E+07	1.40E+08	9.30E+06	5.21E+07	0.82	24.4	25.6	20.0	16.3							0.56	0.56
M5	fall	9/25/2006	4	2.70E+07	1.26E+08	6.30E+06	2.94E+07	0.79	24.4	25.6	20.0	16.3							0.56	0.56
M1	fall	9/27/2006	6	2.70E+07	1.11E+08	5.70E+06	2.35E+07	0.76	20.0	25.6	12.2	14.4							0.00	0.56
M2	fall	9/27/2006	6	4.90E+06	1.64E+07	2.00E+06	6.69E+06	0.70	20.0	25.6	12.2	14.4							0.00	0.56
M3	fall	9/27/2006	6	2.10E+07	8.21E+07	6.20E+06	2.42E+07	0.74	20.0	25.6	12.2	14.4							0.00	0.56
M4	fall	9/27/2006	6	1.80E+06	6.79E+06	1.60E+06	6.04E+06	0.73	20.0	25.6	12.2	14.4							0.00	0.56
M5	fall	9/27/2006	6	1.50E+06	5.17E+06	2.50E+06	8.62E+06	0.71	20.0	25.6	12.2	14.4							0.00	0.56
M1	fall	10/4/2007	13	2.00E+05	2.88E+05	1.20E+06	1.73E+06	0.31	24.4	24.4	15.6	13.7							0.00	2.34
M2	fall	10/4/2007	13	4.00E+06	6.09E+06	6.10E+06	9.29E+06	0.34	24.4	24.4	15.6	13.7							0.00	2.34
M3	fall	10/4/2007	13	5.00E+05	6.93E+05	3.90E+06	5.41E+06	0.28	24.4	24.4	15.6	13.7							0.00	2.34
M4	fall	10/4/2007	13	3.00E+05	4.09E+05	1.40E+06	1.91E+06	0.27	24.4	24.4	15.6	13.7							0.00	2.34
M5	fall	10/4/2007	13	1.50E+06	2.16E+06	3.40E+06	4.89E+06	0.30	24.4	24.4	15.6	13.7							0.00	2.34
M1	fall	10/11/2007	20	7.80E+06	2.68E+07	4.70E+06	1.61E+07	0.71	25.0	26.1	15.6	14.9							0.00	3.23
M2	fall	10/11/2007	20	8.30E+06	3.16E+07	3.80E+06	1.45E+07	0.74	25.0	26.1	15.6	14.9							0.00	3.23
M3	fall	10/11/2007	20	7.10E+06	3.10E+07	2.40E+06	1.05E+07	0.77	25.0	26.1	15.6	14.9							0.00	3.23
M4	fall	10/11/2007	20	2.60E+06	1.09E+07	1.10E+06	4.60E+06	0.76	25.0	26.1	15.6	14.9							0.00	3.23
M5	fall	10/11/2007	20	7.50E+05	3.70E+06	3.10E+06	1.53E+07	0.80	25.0	26.1	15.6	14.9							0.00	3.23
M1	fall	10/18/2007	27	6.20E+05	3.34E+06	9.80E+05	5.28E+06	0.81	15.6	18.3	11.1	8.3	0.279	0.11	2.20	0.82	98.67	68.30	3.00	3.25
M2	fall	10/18/2007	27	3.80E+05	1.93E+06	4.50E+05	2.28E+06	0.80	15.6	18.3	11.1	8.3	0.279	0.11	2.20	0.82	98.67	68.30	3.00	3.25
M3	fall	10/18/2007	27	4.40E+05	2.07E+06	7.90E+05	3.72E+06	0.79	15.6	18.3	11.1	8.3	0.279	0.11	2.20	0.82	98.67	68.30	3.00	3.25
M4	fall	10/18/2007	27	1.00E+05	4.33E+05	9.20E+05	3.98E+06	0.77	15.6	18.3	11.1	8.3	0.279	0.11	2.20	0.82	98.67	68.30	3.00	3.25
M5	fall	10/18/2007	27	1.10E+06	5.48E+06	9.20E+05	4.59E+06	0.80	15.6	18.3	11.1	8.3	0.279	0.11	2.20	0.82	98.67	68.30	3.00	3.25
M1	fall	10/25/2007	34	1.30E+05	3.32E+05	2.80E+05	7.16E+05	0.61	5.0	25.6	1.7	9.2	1.011	0.52	2.08	0.69	62.13	78.61	0.00	1.57
M2	fall	10/25/2007	34	2.50E+05	1.05E+06	5.00E+05	2.10E+06	0.76	5.0	25.6	1.7	9.2	1.011	0.52	2.08	0.69	62.13	78.61	0.00	1.57
M3	fall	10/25/2007	34	3.00E+04	1.19E+05	9.00E+04	3.56E+05	0.75	5.0	25.6	1.7	9.2	1.011	0.52	2.08	0.69	62.13	78.61	0.00	1.57
M4	fall	10/25/2007	34	3.00E+04	1.06E+05	4.20E+05	1.48E+06	0.72	5.0	25.6	1.7	9.2	1.011	0.52	2.08	0.69	62.13	78.61	0.00	1.57
M5	fall	10/25/2007	34	2.00E+04	5.65E+04	7.00E+04	1.98E+05	0.65	5.0	25.6	1.7	9.2	1.011	0.52	2.08	0.69	62.13	78.61	0.00	1.57

Sample No.	Season	Sample date	Time (d)	*E. coli* (cfu g^{-1}) wet wt.	*E. coli* (cfu g^{-1}) dry wt.	ENT (cfu g^{-1}) wet wt.	ENT (cfu g^{-1}) dry wt.	MC (%)	Temp PDH (ºC)	Temp PWH (ºC)	Temp PDA (ºC)	Temp PWA (ºC)	SR PDH (MJ)	SR PDA (MJ)	SR PWH (MJ)	SR PWA (MJ)	RH PDA (%)	RH PWA (%)	rainfall PDT (cm)	Rainfall PWT (cm)
M1	fall	11/1/2006	41	1.50E+05	2.93E+05	4.40E+05	8.58E+05	0.49	21.1	21.1	12.2	8.1	1.952	0.97	2.00	0.69	67.23	68.21	0.00	3.48
M2	fall	11/1/2006	41	1.80E+04	2.86E+04	4.70E+05	7.46E+05	0.37	21.1	21.1	12.2	8.1	1.952	0.97	2.00	0.69	67.23	68.21	0.00	3.48
M3	fall	11/1/2006	41	3.10E+04	1.10E+05	5.80E+05	2.05E+06	0.72	21.1	21.1	12.2	8.1	1.952	0.97	2.00	0.69	67.23	68.21	0.00	3.48
M4	fall	11/1/2006	41	2.40E+05	8.22E+05	8.50E+05	2.91E+06	0.71	21.1	21.1	12.2	8.1	1.952	0.97	2.00	0.69	67.23	68.21	0.00	3.48
M5	fall	11/1/2006	41	1.00E+04	2.31E+04	6.10E+04	1.41E+05	0.57	21.1	21.1	12.2	8.1	1.952	0.97	2.00	0.69	67.23	68.21	0.00	3.48
M1	fall	11/8/2007	48	2.20E+04	6.45E+04	1.80E+04	5.28E+04	0.66	9.4	21.1	5.6	5.6	0.231	0.11	1.94	0.84	86.43	64.63	0.56	1.88
M2	fall	11/8/2007	48	4.00E+03	1.70E+04	4.60E+04	1.96E+05	0.77	9.4	21.1	5.6	5.6	0.231	0.11	1.94	0.84	86.43	64.63	0.56	1.88
M3	fall	11/8/2007	48	1.40E+04	3.89E+04	2.10E+04	5.83E+04	0.64	9.4	21.1	5.6	5.6	0.231	0.11	1.94	0.84	86.43	64.63	0.56	1.88
M4	fall	11/8/2007	48	5.00E+03	1.39E+04	2.10E+04	5.83E+04	0.64	9.4	21.1	5.6	5.6	0.231	0.11	1.94	0.84	86.43	64.63	0.56	1.88
M5	fall	11/8/2007	48	6.00E+03	1.56E+04	3.70E+04	9.62E+04	0.62	9.4	21.1	5.6	5.6	0.231	0.11	1.94	0.84	86.43	64.63	0.56	1.88
M1	fall	11/15/2007	55	1.50E+04	3.22E+04	2.10E+04	4.50E+04	0.53	14.4	25.0	7.8	11.3	1.799	0.76	1.99	0.69	71.44	78.94	0.00	1.47
M2	fall	11/15/2007	55	5.50E+03	1.06E+04	4.20E+03	8.13E+03	0.48	14.4	25.0	7.8	11.3	1.799	0.76	1.99	0.69	71.44	78.94	0.00	1.47
M3	fall	11/15/2007	55	1.50E+03	3.65E+03	5.00E+03	1.22E+04	0.59	14.4	25.0	7.8	11.3	1.799	0.76	1.99	0.69	71.44	78.94	0.00	1.47
M4	fall	11/15/2007	55	4.00E+03	9.85E+03	3.60E+03	8.86E+03	0.59	14.4	25.0	7.8	11.3	1.799	0.76	1.99	0.69	71.44	78.94	0.00	1.47
M5	fall	11/15/2007	55	3.60E+03	8.15E+03	1.20E+04	2.72E+04	0.56	14.4	25.0	7.8	11.3	1.799	0.76	1.99	0.69	71.44	78.94	0.00	1.47
M1	fall	11/29/2006	69	8.10E+03	1.39E+04	4.20E+04	7.22E+04	0.42	18.9	19.4	8.9	6.6	1.474	0.81	1.79	0.72	72.79	72.21	0.00	5.97
M2	fall	11/29/2006	69	8.60E+03	2.04E+04	3.30E+04	7.82E+04	0.58	18.9	19.4	8.9	6.6	1.474	0.81	1.79	0.72	72.79	72.21	0.00	5.97
M3	fall	11/29/2006	69	6.50E+03	1.21E+04	3.90E+03	7.24E+03	0.46	18.9	19.4	8.9	6.6	1.474	0.81	1.79	0.72	72.79	72.21	0.00	5.97
M4	fall	11/29/2006	69	5.00E+02	1.07E+03	5.90E+03	1.27E+04	0.53	18.9	19.4	8.9	6.6	1.474	0.81	1.79	0.72	72.79	72.21	0.00	5.97
M5	fall	11/29/2006	69	6.90E+03	1.31E+04	6.60E+03	1.25E+04	0.47	18.9	19.4	8.9	6.6	1.474	0.81	1.79	0.72	72.79	72.21	0.00	5.97
M1	fall	12/6/2007	76	5.70E+03	1.27E+04	3.50E+03	7.81E+03	0.55	5.6	22.2	0.0	5.7							0.00	0.00
M2	fall	12/6/2007	76	2.90E+03	4.59E+03	1.40E+03	2.22E+03	0.37	5.6	22.2	0.0	5.7							0.00	0.00
M3	fall	12/6/2007	76	1.00E+03	1.66E+03	1.60E+03	2.65E+03	0.40	5.6	22.2	0.0	5.7							0.00	0.00
M4	fall	12/6/2007	76	5.00E+02	7.10E+02	1.40E+03	1.99E+03	0.30	5.6	22.2	0.0	5.7							0.00	0.00
M5	fall	12/6/2007	76	2.10E+03	3.19E+03	6.80E+03	1.03E+04	0.34	5.6	22.2	0.0	5.7							0.00	0.00
M1	fall	12/13/2006	83	1.40E+03	2.73E+03	4.00E+03	7.80E+03	0.49	11.7	16.1	3.3	0.6							0.00	0.25
M2	fall	12/13/2006	83	1.50E+03	2.98E+03	3.10E+03	6.15E+03	0.50	11.7	16.1	3.3	0.6							0.00	0.25
M3	fall	12/13/2006	83	6.00E+02	1.29E+03	3.00E+04	6.45E+04	0.53	11.7	16.1	3.3	0.6							0.00	0.25
M4	fall	12/13/2006	83	5.00E+02	9.33E+02	3.20E+03	5.97E+03	0.46	11.7	16.1	3.3	0.6							0.00	0.25
M5	fall	12/13/2006	83	2.00E+02	3.97E+02	2.50E+03	4.96E+03	0.50	11.7	16.1	3.3	0.6							0.00	0.25
M1	fall	12/19/2006	89	4.40E+03	8.80E+03	1.04E+04	2.08E+04	0.50	18.9	18.9	4.4	7.8							0.00	0.28
M2	fall	12/19/2006	89	4.40E+03	8.77E+03	1.27E+04	2.53E+04	0.50	18.9	18.9	4.4	7.8							0.00	0.28
M3	fall	12/19/2006	89	1.30E+03	1.85E+03	5.50E+03	7.84E+03	0.30	18.9	18.9	4.4	7.8							0.00	0.28
M4	fall	12/19/2006	89	3.40E+03	5.77E+03	8.10E+03	1.37E+04	0.41	18.9	18.9	4.4	7.8							0.00	0.28

Sample No.	Season	Sample date	Time (d)	*E. coli* (cfu g^{-1}) wet wt.	*E. coli* (cfu g^{-1}) dry wt.	ENT (cfu g^{-1}) wet wt.	ENT (cfu g^{-1}) dry wt.	MC (%)	Temp PDH (ºC)	Temp PWH (ºC)	Temp PDA (ºC)	Temp PWA (ºC)	SR PDH (MJ)	SR PDA (MJ)	SR PWH (MJ)	SR PWA (MJ)	RH PDA (%)	RH PWA (%)	rainfall PDT (cm)	Rainfall PWT (cm)
M5	fall	12/19/2006	89	1.40E+03	2.28E+03	4.00E+03	6.51E+03	0.39	18.9	18.9	4.4	7.8							0.00	0.28
M1	fall	1/3/2007	104	4.10E+03	7.71E+03	3.20E+03	6.02E+03	0.47	7.8	14.4	2.2	5.1	1.741	0.87	3.24	0.71	75.15	76.06	0.00	5.84
M2	fall	1/3/2007	104	7.00E+02	1.45E+03	1.50E+03	3.11E+03	0.52	7.8	14.4	2.2	5.1	1.741	0.87	3.24	0.71	75.15	76.06	0.00	5.84
M3	fall	1/3/2007	104	7.00E+02	1.28E+03	4.70E+03	8.60E+03	0.45	7.8	14.4	2.2	5.1	1.741	0.87	3.24	0.71	75.15	76.06	0.00	5.84
M4	fall	1/3/2007	104	9.00E+02	1.89E+03	2.90E+03	6.09E+03	0.52	7.8	14.4	2.2	5.1	1.741	0.87	3.24	0.71	75.15	76.06	0.00	5.84
M5	fall	1/3/2007	104	4.10E+03	7.40E+03	2.80E+03	5.06E+03	0.45	7.8	14.4	2.2	5.1	1.741	0.87	3.24	0.71	75.15	76.06	0.00	5.84
M1	fall	1/11/2007	112	1.57E+03	3.98E+03	3.80E+03	9.62E+03	0.61	1.1	18.3	-2.8	5.8	1.585	0.83	1.80	0.65	60.12	73.30	0.00	3.45
M2	fall	1/11/2007	112					0.64	1.1	18.3	-2.8	5.8	1.585	0.83	1.80	0.65	60.12	73.30	0.00	3.45
M3	fall	1/11/2007	112	2.10E+02	5.22E+02	1.60E+03	3.98E+03	0.60	1.1	18.3	-2.8	5.8	1.585	0.83	1.80	0.65	60.12	73.30	0.00	3.45
M4	fall	1/11/2007	112	5.20E+02	1.00E+03	1.00E+02	1.92E+02	0.48	1.1	18.3	-2.8	5.8	1.585	0.83	1.80	0.65	60.12	73.30	0.00	3.45
M5	fall	1/11/2007	112	7.30E+02	1.70E+03	1.40E+03	3.27E+03	0.57	1.1	18.3	-2.8	5.8	1.585	0.83	1.80	0.65	60.12	73.30	0.00	3.45
M1	fall	1/24/2007	125	4.80E+02	9.73E+02	1.45E+03	2.94E+03	0.51	2.8	19.4	0.0	2.7	0.895	0.38	1.86	0.47	87.93	74.73	0.00	1.91
M2	fall	1/24/2007	125	1.30E+03	3.05E+03	1.10E+03	2.58E+03	0.57	2.8	19.4	0.0	2.7	0.895	0.38	1.86	0.47	87.93	74.73	0.00	1.91
M3	fall	1/24/2007	125	9.30E+02	1.67E+03	6.50E+02	1.17E+03	0.44	2.8	19.4	0.0	2.7	0.895	0.38	1.86	0.47	87.93	74.73	0.00	1.91
M4	fall	1/24/2007	125	1.74E+03	3.64E+03	2.90E+03	6.06E+03	0.52	2.8	19.4	0.0	2.7	0.895	0.38	1.86	0.47	87.93	74.73	0.00	1.91
M5	fall	1/24/2007	125	2.18E+03	4.56E+03	9.40E+03	1.97E+04	0.52	2.8	19.4	0.0	2.7	0.895	0.38	1.86	0.47	87.93	74.73	0.00	1.91
M1	fall	2/1/2007	133	2.70E+02	4.60E+02	2.90E+02	4.94E+02	0.41	-1.1	12.2	-6.1	-1.8	2.06	1.07	2.06	0.81	52.32	58.16	0.00	0.08
M2	fall	2/1/2007	133	3.90E+02	6.50E+02	2.20E+02	3.67E+02	0.40	-1.1	12.2	-6.1	-1.8	2.06	1.07	2.06	0.81	52.32	58.16	0.00	0.08
M3	fall	2/1/2007	133	7.60E+02	1.47E+03	4.40E+03	8.49E+03	0.48	-1.1	12.2	-6.1	-1.8	2.06	1.07	2.06	0.81	52.32	58.16	0.00	0.08
M4	fall	2/1/2007	133	3.00E+01	5.62E+01	1.10E+02	2.06E+02	0.47	-1.1	12.2	-6.1	-1.8	2.06	1.07	2.06	0.81	52.32	58.16	0.00	0.08
M5	fall	2/1/2007	133	3.80E+02	7.98E+02	7.40E+03	1.55E+04	0.52	-1.1	12.2	-6.1	-1.8	2.06	1.07	2.06	0.81	52.32	58.16	0.00	0.08
M1	fall	2/13/2007	145	9.00E+01	2.46E+02	1.40E+03	3.82E+03	0.63	7.2	7.2	-0.6	-4.1	1.16	0.58	2.43	0.87	63.66	54.59	0.03	0.89
M2	fall	2/13/2007	145	2.00E+02	4.85E+02			0.59	7.2	7.2	-0.6	-4.1	1.16	0.58	2.43	0.87	63.66	54.59	0.03	0.89
M3	fall	2/13/2007	145	6.60E+02	1.99E+03	7.00E+02	2.11E+03	0.67	7.2	7.2	-0.6	-4.1	1.16	0.58	2.43	0.87	63.66	54.59	0.03	0.89
M4	fall	2/13/2007	145	9.00E+01	2.20E+02	4.00E+02	9.78E+02	0.59	7.2	7.2	-0.6	-4.1	1.16	0.58	2.43	0.87	63.66	54.59	0.03	0.89
M5	fall	2/13/2007	145	2.10E+02	6.45E+02	4.60E+03	1.41E+04	0.67	7.2	7.2	-0.6	-4.1	1.16	0.58	2.43	0.87	63.66	54.59	0.03	0.89
M1	fall	3/2/2007	162	1.50E+02	3.00E+02	5.00E+01	9.99E+01	0.50	9.4	14.4	4.4	1.1	0.534	0.21	2.61	0.81	81.11	66.14	1.57	3.81
M2	fall	3/2/2007	162	7.70E+02	1.64E+03	8.70E+02	1.85E+03	0.53	9.4	14.4	4.4	1.1	0.534	0.21	2.61	0.81	81.11	66.14	1.57	3.81
M3	fall	3/2/2007	162	1.10E+02	2.08E+02	3.00E+01	5.66E+01	0.47	9.4	14.4	4.4	1.1	0.534	0.21	2.61	0.81	81.11	66.14	1.57	3.81
M4	fall	3/2/2007	162	7.90E+02	1.49E+03	7.20E+02	1.35E+03	0.47	9.4	14.4	4.4	1.1	0.534	0.21	2.61	0.81	81.11	66.14	1.57	3.81
M5	fall	3/2/2007	162	1.21E+03	2.74E+03	5.70E+02	1.29E+03	0.56	9.4	14.4	4.4	1.1	0.534	0.21	2.61	0.81	81.11	66.14	1.57	3.81
M1	fall	3/8/2007	168	4.20E+02	7.06E+02	2.90E+02	4.87E+02	0.40	16.7	16.7	7.8	3.3	2.743	1.27	2.76	1.32	37.72	47.41	0.00	0.43
M2	fall	3/8/2007	168	3.50E+02	6.35E+02	5.90E+02	1.07E+03	0.45	16.7	16.7	7.8	3.3	2.743	1.27	2.76	1.32	37.72	47.41	0.00	0.43
M3	fall	3/8/2007	168	2.20E+02	3.82E+02	1.70E+02	2.95E+02	0.42	16.7	16.7	7.8	3.3	2.743	1.27	2.76	1.32	37.72	47.41	0.00	0.43

Sample No.	Season	Sample date	Time (d)	*E. coli* (cfu g^{-1}) wet wt.	*E. coli* (cfu g^{-1}) dry wt.	ENT (cfu g^{-1}) wet wt.	ENT (cfu g^{-1}) dry wt.	MC (%)	Temp PDH (ºC)	Temp PWH (ºC)	Temp PDA (ºC)	Temp PWA (ºC)	SR PDH (MJ)	SR PDA (MJ)	SR PWH (MJ)	SR PWA (MJ)	RH PDA (%)	RH PWA (%)	rainfall PDT (cm)	Rainfall PWT (cm)
M4	fall	3/8/2007	168	3.40E+02	6.12E+02	6.20E+02	1.12E+03	0.44	16.7	16.7	7.8	3.3	2.743	1.27	2.76	1.32	37.72	47.41	0.00	0.43
M5	fall	3/8/2007	168	7.00E+01	1.24E+02	2.10E+02	3.71E+02	0.43	16.7	16.7	7.8	3.3	2.743	1.27	2.76	1.32	37.72	47.41	0.00	0.43
M1	fall	3/19/2007	179	3.00E+02	4.13E+02	1.00E+03	1.38E+03	0.27	3.9	25.0	-0.6	7.2	2.36	1.41	2.83	1.11	51.67	59.67	0.00	7.01
M2	fall	3/19/2007	179	1.30E+03	1.84E+03	5.00E+02	7.08E+02	0.29	3.9	25.0	-0.6	7.2	2.36	1.41	2.83	1.11	51.67	59.67	0.00	7.01
M3	fall	3/19/2007	179	3.40E+03	4.78E+03	2.50E+03	3.51E+03	0.29	3.9	25.0	-0.6	7.2	2.36	1.41	2.83	1.11	51.67	59.67	0.00	7.01
M4	fall	3/19/2007	179	1.00E+03	1.44E+03	3.10E+03	4.46E+03	0.30	3.9	25.0	-0.6	7.2	2.36	1.41	2.83	1.11	51.67	59.67	0.00	7.01
M5	fall	3/19/2007	179	7.00E+02	9.21E+02	1.60E+03	2.10E+03	0.24	3.9	25.0	-0.6	7.2	2.36	1.41	2.83	1.11	51.67	59.67	0.00	7.01
M1	fall	3/29/2007	189	4.00E+01	7.48E+01	3.00E+01	5.61E+01	0.47	27.2	27.2	18.3	14.2	2.858	1.05	2.90	1.17	81.36	65.83	7.44	7.77
M2	fall	3/29/2007	189	9.00E+01	1.75E+02	1.20E+02	2.33E+02	0.49	27.2	27.2	18.3	14.2	2.858	1.05	2.90	1.17	81.36	65.83	7.44	7.77
M3	fall	3/29/2007	189	2.40E+02	5.35E+02	1.14E+03	2.54E+03	0.55	27.2	27.2	18.3	14.2	2.858	1.05	2.90	1.17	81.36	65.83	7.44	7.77
M4	fall	3/29/2007	189	4.10E+02	8.18E+02	3.20E+02	6.38E+02	0.50	27.2	27.2	18.3	14.2	2.858	1.05	2.90	1.17	81.36	65.83	7.44	7.77
M5	fall	3/29/2007	189	4.00E+01	7.74E+01	3.00E+01	5.80E+01	0.48	27.2	27.2	18.3	14.2	2.858	1.05	2.90	1.17	81.36	65.83	7.44	7.77
M1	fall	4/5/2007	196	2.40E+02	2.95E+02	3.50E+02	4.31E+02	0.19	20.0	25.0	12.2	12.6	2.924	1.07	2.98	1.08	56.65	72.40	0.00	0.36
M2	fall	4/5/2007	196	6.00E+01	7.92E+01	6.70E+02	8.85E+02	0.24	20.0	25.0	12.2	12.6	2.924	1.07	2.98	1.07	56.65	72.40	0.00	0.36
M3	fall	4/5/2007	196	4.00E+01	5.54E+01	5.00E+01	6.92E+01	0.28	20.0	25.0	12.2	12.6	2.924	1.07	2.98	1.07	56.65	72.40	0.00	0.36
M4	fall	4/5/2007	196	7.00E+01	8.60E+01	5.00E+01	6.14E+01	0.19	20.0	25.0	12.2	12.6	2.924	1.07	2.98	1.07	56.65	72.40	0.00	0.36
M5	fall	4/5/2007	196	1.10E+02	1.41E+02	7.00E+01	9.00E+01	0.22	20.0	25.0	12.2	12.6	2.924	1.07	2.98	1.07	56.65	72.40	0.00	0.36
M1	winter	2/13/2007	0	3.50E+06	2.33E+07	9.00E+06	6.00E+07	0.85	7.2	7.2	-0.6	-4.1	1.16	0.58			63.66	54.59	0.03	0.89
M2	winter	2/13/2007	0			5.00E+06	3.29E+07	0.85	7.2	7.2	-0.6	-4.1	1.16	0.58			63.66	54.59	0.03	0.89
M3	winter	2/13/2007	0	1.40E+06	9.34E+06	1.90E+07	1.27E+08	0.85	7.2	7.2	-0.6	-4.1	1.16	0.58			63.66	54.59	0.03	0.89
M4	winter	2/13/2007	0	6.00E+05	4.03E+06	4.00E+06	2.69E+07	0.85	7.2	7.2	-0.6	-4.1	1.16	0.58			63.66	54.59	0.03	0.89
M5	winter	2/13/2007	0	4.00E+05	2.70E+06	7.00E+06	4.73E+07	0.85	7.2	7.2	-0.6	-4.1	1.16	0.58			63.66	54.59	0.03	0.89
M1	winter	2/16/2007	3	4.00E+05	2.08E+06	7.00E+06	3.64E+07	0.81	-3.9	2.8	-7.2	-3.3	1.745	0.88	1.75	0.43	64.46	78.68	0.00	2.01
M2	winter	2/16/2007	3	2.00E+05	1.13E+06	1.00E+07	5.64E+07	0.82	-3.9	2.8	-7.2	-3.3	1.745	0.88	1.75	0.43	64.46	78.68	0.00	2.01
M3	winter	2/16/2007	3	1.00E+05	5.68E+05	8.00E+06	4.55E+07	0.82	-3.9	2.8	-7.2	-3.3	1.745	0.88	1.75	0.43	64.46	78.68	0.00	2.01
M4	winter	2/16/2007	3	9.00E+05	4.61E+06	4.00E+06	2.05E+07	0.80	-3.9	2.8	-7.2	-3.3	1.745	0.88	1.75	0.43	64.46	78.68	0.00	2.01
M5	winter	2/16/2007	3	1.00E+05	5.39E+05	3.00E+06	1.62E+07	0.81	-3.9	2.8	-7.2	-3.3	1.745	0.88	1.75	0.43	64.46	78.68	0.00	2.01
M1	winter	2/20/2007	7	2.30E+04	1.12E+05	7.00E+05	3.42E+06	0.80	7.8	7.8	-0.6	-4.4	2.262	1.17	2.29	0.94	56.85	63.27	0.00	0.03
M2	winter	2/20/2007	7	1.90E+04	9.08E+04	2.20E+06	1.05E+07	0.79	7.8	7.8	-0.6	-4.4	2.262	1.17	2.29	0.94	56.85	63.27	0.00	0.03
M3	winter	2/20/2007	7	1.50E+04	7.86E+04	1.10E+06	5.76E+06	0.81	7.8	7.8	-0.6	-4.4	2.262	1.17	2.29	0.94	56.85	63.27	0.00	0.03
M4	winter	2/20/2007	7	5.00E+03	2.35E+04	7.00E+06	3.29E+07	0.79	7.8	7.8	-0.6	-4.4	2.262	1.17	2.29	0.94	56.85	63.27	0.00	0.03
M5	winter	2/20/2007	7	2.00E+04	9.85E+04	2.40E+06	1.18E+07	0.80	7.8	7.8	-0.6	-4.4	2.262	1.17	2.29	0.94	56.85	63.27	0.00	0.03
M1	winter	2/25/2007	12	7.40E+03	3.84E+04	3.10E+06	1.61E+07	0.81	11.1	14.4	3.9	5.3	2.384	1.26	2.49	1.00	29.31	57.71	0.00	0.66
M2	winter	2/25/2007	12	2.52E+04	1.26E+05	7.00E+05	3.49E+06	0.80	11.1	14.4	3.9	5.3	2.384	1.26	2.49	1.00	29.31	57.71	0.00	0.66

Sample No.	Season	Sample date	Time (d)	*E. coli* (cfu g^{-1}) wet wt.	*E. coli* (cfu g^{-1}) dry wt.	ENT (cfu g^{-1}) wet wt.	ENT (cfu g^{-1}) dry wt.	MC (%)	Temp PDH (ºC)	Temp PWH (ºC)	Temp PDA (ºC)	Temp PWA (ºC)	SR PDH (MJ)	SR PDA (MJ)	SR PWH (MJ)	SR PWA (MJ)	RH PDA (%)	RH PWA (%)	rainfall PDT (cm)	Rainfall PWT (cm)
M3	winter	2/25/2007	12	1.01E+04	4.78E+04	1.56E+07	7.39E+07	0.79	11.1	14.4	3.9	5.3	2.384	1.26	2.49	1.00	29.31	57.71	0.00	0.66
M4	winter	2/25/2007	12	3.20E+03	1.57E+04	5.10E+06	2.51E+07	0.80	11.1	14.4	3.9	5.3	2.384	1.26	2.49	1.00	29.31	57.71	0.00	0.66
M5	winter	2/25/2007	12	1.50E+03	7.72E+03	1.70E+06	8.75E+06	0.81	11.1	14.4	3.9	5.3	2.384	1.26	2.49	1.00	29.31	57.71	0.00	0.66
M1	winter	3/2/2007	17	1.50E+05	6.92E+05	1.99E+07	9.18E+07	0.78	9.4	14.4	4.4	4.1	0.534	0.21	2.61	0.81	81.11	66.14	1.57	2.69
M2	winter	3/2/2007	17	7.40E+04	3.29E+05	4.90E+06	2.18E+07	0.78	9.4	14.4	4.4	4.1	0.534	0.21	2.61	0.81	81.11	66.14	1.57	2.69
M3	winter	3/2/2007	17	4.90E+05	2.28E+06	2.10E+06	9.77E+06	0.79	9.4	14.4	4.4	4.1	0.534	0.21	2.61	0.81	81.11	66.14	1.57	2.69
M4	winter	3/2/2007	17	5.80E+05	2.70E+06	2.80E+06	1.30E+07	0.78	9.4	14.4	4.4	4.1	0.534	0.21	2.61	0.81	81.11	66.14	1.57	2.69
M5	winter	3/2/2007	17	2.09E+04	1.04E+05	1.80E+06	8.97E+06	0.80	9.4	14.4	4.4	4.1	0.534	0.21	2.61	0.81	81.11	66.14	1.57	2.69
M1	winter	3/8/2007	23	5.90E+04	1.49E+05	1.00E+07	2.53E+07	0.60	16.7	16.7	7.8	3.3	2.743	1.27	2.76	1.32	37.72	47.41	0.00	0.43
M2	winter	3/8/2007	23	1.50E+04	4.33E+04	5.30E+06	1.53E+07	0.65	16.7	16.7	7.8	3.3	2.743	1.27	2.76	1.32	37.72	47.41	0.00	0.43
M3	winter	3/8/2007	23	1.24E+05	3.56E+05	7.90E+05	2.27E+06	0.65	16.7	16.7	7.8	3.3	2.743	1.27	2.76	1.32	37.72	47.41	0.00	0.43
M4	winter	3/8/2007	23	2.34E+05	6.57E+05	1.20E+06	3.37E+06	0.64	16.7	16.7	7.8	3.3	2.743	1.27	2.76	1.32	37.72	47.41	0.00	0.43
M5	winter	3/8/2007	23	5.40E+05	1.70E+06	8.60E+06	2.70E+07	0.68	16.7	16.7	7.8	3.3	2.743	1.27	2.76	1.32	37.72	47.41	0.00	0.43
M1	winter	3/19/2007	34	3.80E+04	8.79E+04	1.70E+05	3.93E+05	0.57	3.9	25.0	-0.6	7.2	2.36	1.41	2.83	1.11	51.67	59.67	0.00	7.01
M2	winter	3/19/2007	34	2.41E+05	4.84E+05	8.30E+05	1.67E+06	0.50	3.9	25.0	-0.6	7.2	2.36	1.41	2.83	1.11	51.67	59.67	0.00	7.01
M3	winter	3/19/2007	34	1.06E+08	2.74E+08	1.42E+07	3.68E+07	0.61	3.9	25.0	-0.6	7.2	2.36	1.41	2.83	1.11	51.67	59.67	0.00	7.01
M4	winter	3/19/2007	34	6.10E+06	1.17E+07	7.50E+05	1.43E+06	0.48	3.9	25.0	-0.6	7.2	2.36	1.41	2.83	1.11	51.67	59.67	0.00	7.01
M5	winter	3/19/2007	34	3.60E+05	6.18E+05	5.10E+06	8.75E+06	0.42	3.9	25.0	-0.6	7.2	2.36	1.41	2.83	1.11	51.67	59.67	0.00	7.01
M1	winter	3/29/2007	44	1.50E+05	4.92E+05	1.40E+05	4.59E+05	0.69	27.2	27.2	18.3	14.2	2.858	1.05	2.90	1.17	81.36	65.83	7.44	7.77
M2	winter	3/29/2007	44	9.00E+04	2.55E+05	1.07E+06	3.03E+06	0.65	27.2	27.2	18.3	14.2	2.858	1.05	2.90	1.17	81.36	65.83	7.44	7.77
M3	winter	3/29/2007	44	1.90E+05	5.52E+05	5.90E+05	1.72E+06	0.66	27.2	27.2	18.3	14.2	2.858	1.05	2.90	1.17	81.36	65.83	7.44	7.77
M4	winter	3/29/2007	44	1.00E+04	4.16E+04	5.00E+05	2.08E+06	0.76	27.2	27.2	18.3	14.2	2.858	1.05	2.90	1.17	81.36	65.83	7.44	7.77
M5	winter	3/29/2007	44	4.00E+05	1.14E+06	4.90E+06	1.39E+07	0.65	27.2	27.2	18.3	14.2	2.858	1.05	2.90	1.17	81.36	65.83	7.44	7.77
M1	winter	4/5/2007	51	1.00E+03	1.97E+03	4.30E+05	8.45E+05	0.49	20.0	25.0	12.2	12.6	2.924	1.07	2.98	1.07	56.65	72.40	0.00	0.36
M2	winter	4/5/2007	51	1.00E+03	3.09E+03	1.40E+05	4.32E+05	0.68	20.0	25.0	12.2	12.6	2.924	1.07	2.98	1.07	56.65	72.40	0.00	0.36
M3	winter	4/5/2007	51	1.10E+05	3.63E+05	1.10E+05	3.63E+05	0.70	20.0	25.0	12.2	12.6	2.924	1.07	2.98	1.07	56.65	72.40	0.00	0.36
M4	winter	4/5/2007	51	1.00E+03	2.86E+03	6.10E+05	1.75E+06	0.65	20.0	25.0	12.2	12.6	2.924	1.07	2.98	1.07	56.65	72.40	0.00	0.36
M5	winter	4/5/2007	51	6.30E+05	2.26E+06	2.02E+06	7.26E+06	0.72	20.0	25.0	12.2	12.6	2.924	1.07	2.98	1.07	56.65	72.40	0.00	0.36
M1	winter	4/12/2007	58	2.40E+04	1.06E+05	5.30E+04	2.35E+05	0.77	8.3	13.3	6.1	2.0	0.464	0.17	3.31	1.27	80.68	58.47	1.80	1.98
M2	winter	4/12/2007	58	5.00E+06	2.17E+07	4.60E+05	2.00E+06	0.77	8.3	13.3	6.1	2.0	0.464	0.17	3.31	1.27	80.68	58.47	1.80	1.98
M3	winter	4/12/2007	58	1.90E+04	8.32E+04	2.90E+04	1.27E+05	0.77	8.3	13.3	6.1	2.0	0.464	0.17	3.31	1.27	80.68	58.47	1.80	1.98
M4	winter	4/12/2007	58	2.70E+04	1.05E+05	5.50E+04	2.14E+05	0.74	8.3	13.3	6.1	2.0	0.464	0.17	3.31	1.27	80.68	58.47	1.80	1.98
M5	winter	4/12/2007	58	2.20E+04	1.05E+05	6.70E+04	3.19E+05	0.79	8.3	13.3	6.1	2.0	0.464	0.17	3.31	1.27	80.68	58.47	1.80	1.98
M1	winter	4/26/2007	68	3.90E+03	6.62E+03	5.80E+03	9.85E+03	0.41	28.3	28.3	18.9	11.6	3.037	1.43	3.34	1.36	59.90	61.82	0.00	4.75

Sample No.	Season	Sample date	Time (d)	*E. coli* (cfu g^{-1}) wet wt.	*E. coli* (cfu g^{-1}) dry wt.	ENT (cfu g^{-1}) wet wt.	ENT (cfu g^{-1}) dry wt.	MC (%)	Temp PDH (ºC)	Temp PWH (ºC)	Temp PDA (ºC)	Temp PWA (ºC)	SR PDH (MJ)	SR PDA (MJ)	SR PWH (MJ)	SR PWA (MJ)	RH PDA (%)	RH PWA (%)	rainfall PDT (cm)	Rainfall PWT (cm)
M2	winter	4/26/2007	68	1.04E+06	1.72E+06	3.60E+05	5.96E+05	0.40	28.3	28.3	18.9	11.6	3.037	1.43	3.34	1.36	59.90	61.82	0.00	4.75
M3	winter	4/26/2007	68	4.30E+03	6.02E+03	6.80E+03	9.52E+03	0.29	28.3	28.3	18.9	11.6	3.037	1.43	3.34	1.36	59.90	61.82	0.00	4.75
M4	winter	4/26/2007	68	8.00E+02	1.69E+03	9.60E+04	2.03E+05	0.53	28.3	28.3	18.9	11.6	3.037	1.43	3.34	1.36	59.90	61.82	0.00	4.75
M5	winter	4/26/2007	68	8.20E+04	2.06E+05	2.30E+05	5.77E+05	0.60	28.3	28.3	18.9	11.6	3.037	1.43	3.34	1.36	59.90	61.82	0.00	4.75
M1	winter	5/3/2007	75	5.40E+02	2.12E+03	2.00E+04	7.85E+04	0.75	30.0	30.0	20.0	16.7	2.492	1.19	3.31	1.31	60.79	60.22	0.00	2.08
M2	winter	5/3/2007	75	1.10E+03	4.53E+03	2.90E+04	1.19E+05	0.76	30.0	30.0	20.0	16.7	2.492	1.19	3.31	1.31	60.79	60.22	0.00	2.08
M3	winter	5/3/2007	75	1.03E+04	3.33E+04	6.10E+04	1.97E+05	0.69	30.0	30.0	20.0	16.7	2.492	1.19	3.31	1.31	60.79	60.22	0.00	2.08
M4	winter	5/3/2007	75	1.00E+03	2.22E+03	4.30E+04	9.56E+04	0.55	30.0	30.0	20.0	16.7	2.492	1.19	3.31	1.31	60.79	60.22	0.00	2.08
M5	winter	5/3/2007	75	2.40E+02	5.36E+02	1.00E+04	2.23E+04	0.55	30.0	30.0	20.0	16.7	2.492	1.19	3.31	1.31	60.79	60.22	0.00	2.08
M1	winter	5/10/2007	82	6.80E+02	1.16E+03	1.40E+04	2.40E+04	0.42	24.4	24.4	17.8	12.6	2.862	1.08	3.30	1.02	80.56	80.29	0.00	2.90
M2	winter	5/10/2007	82	6.00E+01	1.17E+02	1.30E+05	2.54E+05	0.49	24.4	24.4	17.8	12.6	2.862	1.08	3.30	1.02	80.56	80.29	0.00	2.90
M3	winter	5/10/2007	82	3.00E+01	8.01E+01	3.70E+04	9.87E+04	0.63	24.4	24.4	17.8	12.6	2.862	1.08	3.30	1.02	80.56	80.29	0.00	2.90
M4	winter	5/10/2007	82	2.40E+03	5.22E+03	1.00E+04	2.17E+04	0.54	24.4	24.4	17.8	12.6	2.862	1.08	3.30	1.02	80.56	80.29	0.00	2.90
M5	winter	5/10/2007	82	1.40E+03	2.86E+03	3.00E+03	6.13E+03	0.51	24.4	24.4	17.8	12.6	2.862	1.08	3.30	1.02	80.56	80.29	0.00	2.90
M1	winter	5/16/2007	88	1.60E+02	3.43E+02	1.80E+03	3.86E+03	0.53	26.7	28.9	17.2	17.6	2.91	1.62	3.29	1.54	64.85	69.08	0.00	0.08
M2	winter	5/16/2007	88	1.00E+02	1.31E+02	2.70E+03	3.55E+03	0.24	26.7	28.9	17.2	17.6	2.91	1.62	3.29	1.54	64.85	69.08	0.00	0.08
M3	winter	5/16/2007	88	1.20E+02	2.51E+02	2.60E+04	5.43E+04	0.52	26.7	28.9	17.2	17.6	2.91	1.62	3.29	1.54	64.85	69.08	0.00	0.08
M4	winter	5/16/2007	88	1.00E+03	1.32E+03	2.80E+03	3.69E+03	0.24	26.7	28.9	17.2	17.6	2.91	1.62	3.29	1.54	64.85	69.08	0.00	0.08
M5	winter	5/16/2007	88	1.00E+01	1.48E+01	3.90E+03	5.77E+03	0.32	26.7	28.9	17.2	17.6	2.91	1.62	3.29	1.54	64.85	69.08	0.00	0.08
M1	winter	5/23/2007	95	0.00E+00	0.00E+00	3.50E+03	4.09E+03	0.14	25.0	26.1	18.9	15.2	3.131	1.35	3.36	1.37	78.76	64.82	0.00	0.61
M2	winter	5/23/2007	95	4.30E+02	4.90E+02	4.00E+02	4.56E+02	0.12	25.0	26.1	18.9	15.2	3.131	1.35	3.36	1.37	78.76	64.82	0.00	0.61
M3	winter	5/23/2007	95	0.00E+00	0.00E+00	7.00E+02	8.33E+02	0.16	25.0	26.1	18.9	15.2	3.131	1.35	3.36	1.37	78.76	64.82	0.00	0.61
M4	winter	5/23/2007	95	2.00E+01	2.29E+01	1.40E+02	1.60E+02	0.13	25.0	26.1	18.9	15.2	3.131	1.35	3.36	1.37	78.76	64.82	0.00	0.61
M5	winter	5/23/2007	95	1.80E+03	2.17E+03	1.60E+03	1.93E+03	0.17	25.0	26.1	18.9	15.2	3.131	1.35	3.36	1.37	78.76	64.82	0.00	0.61
M1	winter	5/29/2007	101	1.00E+01	1.13E+01	8.60E+02	9.73E+02	0.12	28.3	28.3	20.6	19.9	2.885	1.09	3.20	1.39	69.50	73.27	0.00	1.40
M2	winter	5/29/2007	101	5.10E+02	5.93E+02	4.00E+03	4.65E+03	0.14	28.3	28.3	20.6	19.9	2.885	1.09	3.20	1.39	69.50	73.27	0.00	1.40
M3	winter	5/29/2007	101	4.00E+02	4.55E+02	6.50E+02	7.40E+02	0.12	28.3	28.3	20.6	19.9	2.885	1.09	3.20	1.39	69.50	73.27	0.00	1.40
M4	winter	5/29/2007	101	2.00E+01	2.32E+01	5.80E+02	6.74E+02	0.14	28.3	28.3	20.6	19.9	2.885	1.09	3.20	1.39	69.50	73.27	0.00	1.40
M5	winter	5/29/2007	101	2.00E+01	2.26E+01	6.00E+02	6.79E+02	0.12	28.3	28.3	20.6	19.9	2.885	1.09	3.20	1.39	69.50	73.27	0.00	1.40
M1	winter	6/6/2007	109	1.20E+02	3.58E+02	9.00E+03	2.68E+04	0.66	23.3	28.9	18.3	19.7							0.69	6.60
M2	winter	6/6/2007	109	1.00E+01	2.37E+01	2.80E+03	6.62E+03	0.58	23.3	28.9	18.3	19.7							0.69	6.60
M3	winter	6/6/2007	109	3.50E+02	8.74E+02	1.30E+03	3.24E+03	0.60	23.3	28.9	18.3	19.7							0.69	6.60
M4	winter	6/6/2007	109	0.00E+00	0.00E+00	1.50E+03	3.92E+03	0.62	23.3	28.9	18.3	19.7							0.69	6.60
M5	winter	6/6/2007	109	1.00E+01	3.06E+01	1.52E+03	4.65E+03	0.67	23.3	28.9	18.3	19.7							0.69	6.60

Sample No.	Season	Sample date	Time (d)	*E. coli* (cfu g^{-1}) wet wt.	*E. coli* (cfu g^{-1}) dry wt.	ENT (cfu g^{-1}) wet wt.	ENT (cfu g^{-1}) dry wt.	MC (%)	Temp PDH (ºC)	Temp PWH (ºC)	Temp PDA (ºC)	Temp PWA (ºC)	SR PDH (MJ)	SR PDA (MJ)	SR PWH (MJ)	SR PWA (MJ)	RH PDA (%)	RH PWA (%)	rainfall PDT (cm)	Rainfall PWT (cm)
M1	winter	6/13/2007	116	0.00E+00	0.00E+00	4.80E+03	7.41E+03	0.35	27.2	30.6	20.0	20.6	3.03	1.62	3.19	1.57	72.03	70.87	0.00	0.03
M2	winter	6/13/2007	116	0.00E+00	0.00E+00	1.60E+03	2.15E+03	0.26	27.2	30.6	20.0	20.6	3.03	1.62	3.19	1.57	72.03	70.87	0.00	0.03
M3	winter	6/13/2007	116	0.00E+00	0.00E+00	3.30E+02	5.94E+02	0.44	27.2	30.6	20.0	20.6	3.03	1.62	3.19	1.57	72.03	70.87	0.00	0.03
M4	winter	6/13/2007	116	1.00E+01	1.55E+01	2.00E+02	3.10E+02	0.36	27.2	30.6	20.0	20.6	3.03	1.62	3.19	1.57	72.03	70.87	0.00	0.03
M5	winter	6/13/2007	116	1.00E+01	1.54E+01	2.30E+03	3.54E+03	0.35	27.2	30.6	20.0	20.6	3.03	1.62	3.19	1.57	72.03	70.87	0.00	0.03
M1	winter	6/20/2007	123	1.00E+01	1.33E+01	2.10E+02	2.79E+02	0.25	28.9	31.1	22.8	19.5	3.06	1.29	3.17	1.19	74.02	78.81	0.18	0.28
M2	winter	6/20/2007	123	2.00E+01	2.60E+01	3.70E+02	4.82E+02	0.23	28.9	31.1	22.8	19.5	3.06	1.29	3.17	1.19	74.02	78.81	0.18	0.28
M3	winter	6/20/2007	123	0.00E+00	0.00E+00	3.00E+01	3.81E+01	0.21	28.9	31.1	22.8	19.5	3.06	1.29	3.17	1.19	74.02	78.81	0.18	0.28
M4	winter	6/20/2007	123	5.50E+02	7.90E+02	1.30E+03	1.87E+03	0.30	28.9	31.1	22.8	19.5	3.06	1.29	3.17	1.19	74.02	78.81	0.18	0.28
M5	winter	6/20/2007	123	3.00E+01	4.09E+01	1.10E+03	1.50E+03	0.27	28.9	31.1	22.8	19.5	3.06	1.29	3.17	1.19	74.02	78.81	0.18	0.28
M1	winter	6/27/2007	130	0.00E+00	0.00E+00	1.00E+01	1.25E+01	0.20	30.6	30.6	24.4	21.1	3.16	1.70	3.29	1.51	75.90	71.26	0.00	2.90
M2	winter	6/27/2007	130	0.00E+00	0.00E+00	2.00E+02	2.38E+02	0.16	30.6	30.6	24.4	21.1	3.16	1.70	3.29	1.51	75.90	71.26	0.00	2.90
M3	winter	6/27/2007	130	0.00E+00	0.00E+00	1.10E+02	1.29E+02	0.15	30.6	30.6	24.4	21.1	3.16	1.70	3.29	1.51	75.90	71.26	0.00	2.90
M4	winter	6/27/2007	130	3.00E+01	3.37E+01	2.20E+03	2.47E+03	0.11	30.6	30.6	24.4	21.1	3.16	1.70	3.29	1.51	75.90	71.26	0.00	2.90
M5	winter	6/27/2007	130	1.00E+01	1.30E+01	4.00E+01	5.21E+01	0.23	30.6	30.6	24.4	21.1	3.16	1.70	3.29	1.51	75.90	71.26	0.00	2.90
M1	winter	7/2/2007	135	7.40E+02	8.30E+02	8.00E+02	8.97E+02	0.11	26.1	30.0	18.9	21.9	3.24	1.65	3.24	1.32	65.29	78.69	0.00	0.33
M2	winter	7/2/2007	135	2.00E+01	2.22E+01	2.00E+01	2.22E+01	0.10	26.1	30.0	18.9	21.9	3.24	1.65	3.24	1.32	65.29	78.69	0.00	0.33
M3	winter	7/2/2007	135	1.00E+01	1.14E+01	4.60E+02	5.22E+02	0.12	26.1	30.0	18.9	21.9	3.24	1.65	3.24	1.32	65.29	78.69	0.00	0.33
M4	winter	7/2/2007	135	0.00E+00	0.00E+00	9.00E+02	1.10E+03	0.19	26.1	30.0	18.9	21.9	3.24	1.65	3.24	1.32	65.29	78.69	0.00	0.33
M5	winter	7/2/2007	135	0.00E+00	0.00E+00	2.60E+02	2.75E+02	0.06	26.1	30.0	18.9	21.9	3.24	1.65	3.24	1.32	65.29	78.69	0.00	0.33

Appendix C. Supplemental Statistical Data for Die-off Analysis

C.1. First-order decay models

C.1.1. Predictions of ln E. coli decay during the fall season:

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	1571.43283	1571.43283	374.31	<.0001
Error	117	491.18637	4.19817		
Corrected Total	118	2062.61919			

Root MSE	2.04894	R-Square	0.7619
Dependent Mean	9.94902	Adj R-Sq	0.7598
Coeff Var	20.59443		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	Intercept	1	14.96569	0.32018	46.74	<.0001
Time	Time	1	-0.05808	0.00300	-19.35	<.0001

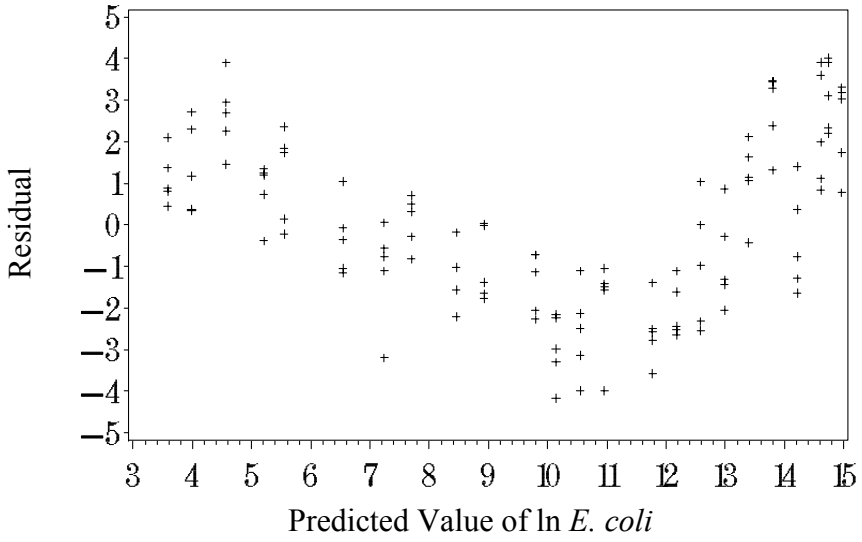


Figure C.1. Residual plots predicted ln *E. coli* during the fall season for first-order decay model.

C.1.2. Predictions of ln E. coli decay during the spring season:

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	1107.29889	1107.29889	250.08	<.0001
Error	106	469.33877	4.42772		
Corrected Total	107	1576.63766			

Root MSE	2.10422	R-Square	0.7023
Dependent Mean	12.51236	Adj R-Sq	0.6995
Coeff Var	16.81709		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	Intercept	1	17.05568	0.35148	48.53	<.0001
Time	Time	1	-0.07478	0.00473	-15.81	<.0001

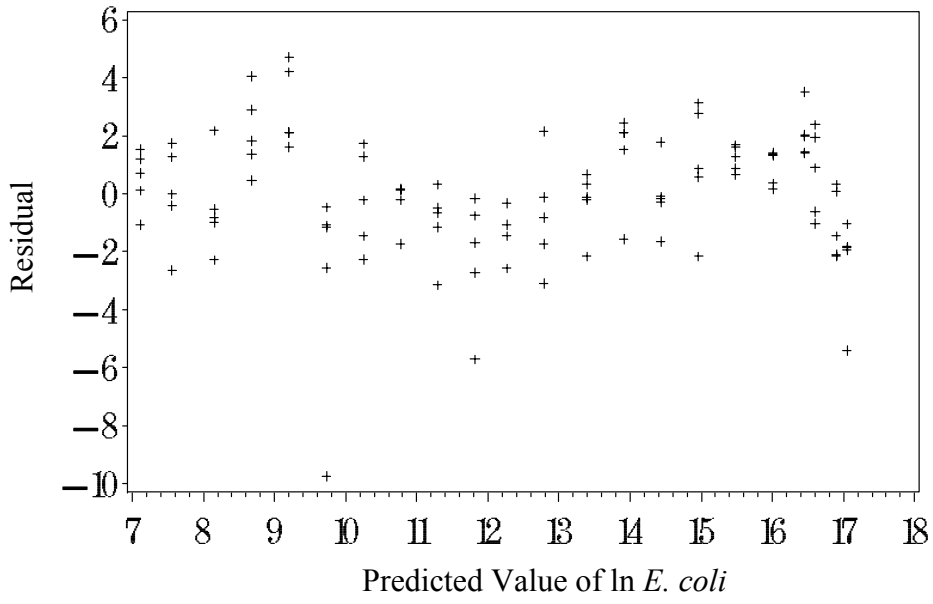


Figure C.2. Residual plots predicted ln *E. coli* during the spring season for first-order decay model.

C.1.3. Predictions of ln E. coli decay during the summer season:

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	1932.28485	1932.28485	645.75	<.0001
Error	121	362.06991	2.99231		
Corrected Total	122	2294.35476			

Root MSE	1.72983	R-Square	0.8422
Dependent Mean	10.72214	Adj R-Sq	0.8409
Coeff Var	16.13326		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	Intercept	1	16.40788	0.27275	60.16	<.0001
Time	Time	1	-0.07879	0.00310	-25.41	<.0001

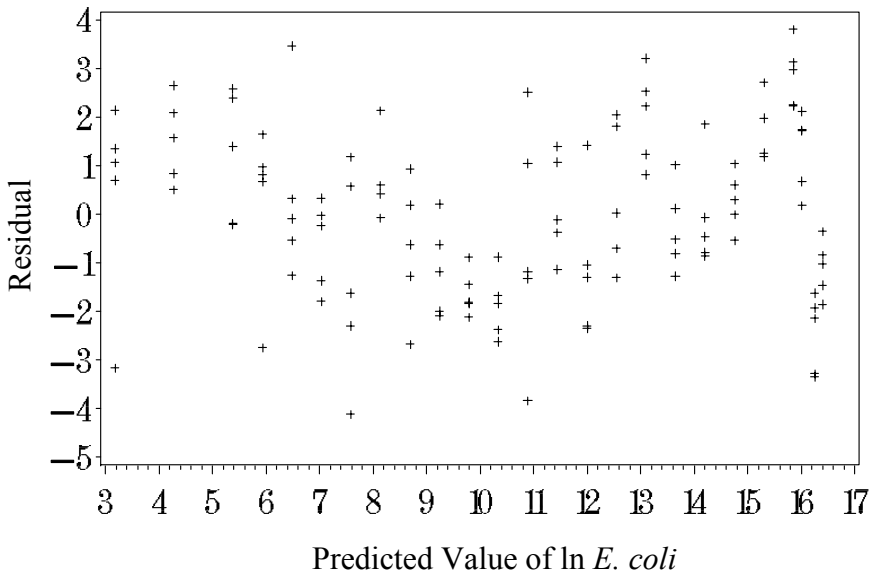


Figure C.3. Residual plots predicted ln *E. coli* during the summer season for first-order decay model.

C.1.4. Predictions of ln E. coli decay during the winter season:

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	1940.87664	1940.87664	282.18	<.0001
Error	102	701.58225	6.87826		
Corrected Total	103	2642.45889			

Root MSE	2.62264	R-Square	0.7345
Dependent Mean	8.37674	Adj R-Sq	0.7319
Coeff Var	31.30862		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	Intercept	1	14.93221	0.46737	31.95	<.0001
Time	Time	1	-0.09946	0.00592	-16.80	<.0001

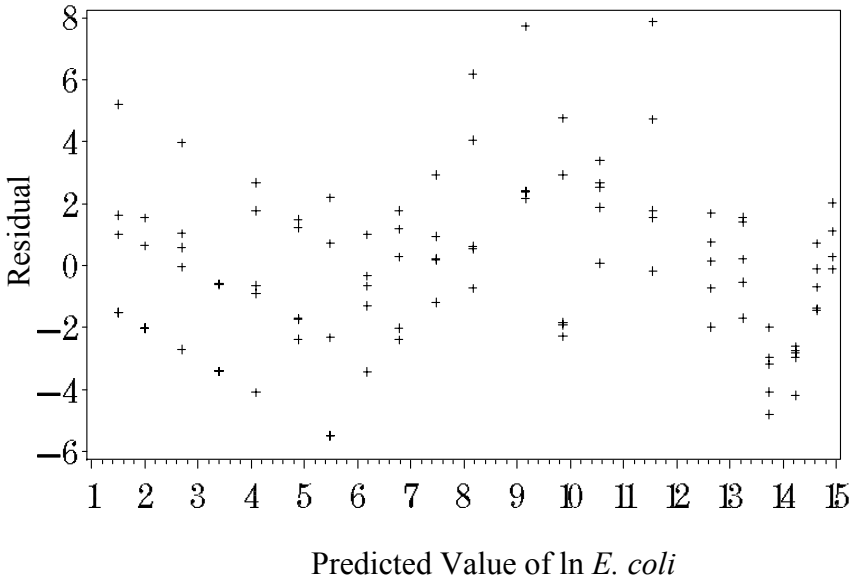


Figure C.4. Residual plots predicted ln *E. coli* during the winter season for first-order decay model.

C.1.5. Predictions of ln enterococci decay during the fall season:

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	1388.94000	1388.94000	493.43	<.0001
Error	114	320.89250	2.81485		
Corrected Total	115	1709.83250			

Root MSE	1.67775	R-Square	0.8123
Dependent Mean	10.41652	Adj R-Sq	0.8107
Coeff Var	16.10663		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	Intercept	1	15.28411	0.26886	56.85	<.0001
Time	Time	1	-0.05572	0.00251	-22.21	<.0001

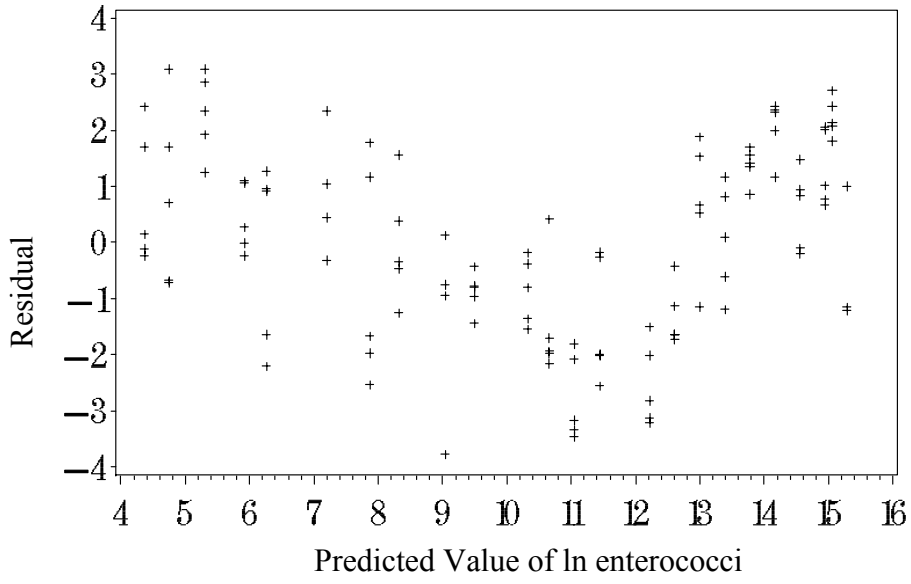


Figure C.5. Residual plots predicted ln enterococci during the fall season for first-order decay model.

C.1.6. Predictions of ln enterococci decay during the spring season:

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	1125.77378	1125.77378	259.20	<.0001
Error	105	456.04113	4.34325		
Corrected Total	106	1581.81491			

Root MSE	2.08405	R-Square	0.7117
Dependent Mean	14.23798	Adj R-Sq	0.7090
Coeff Var	14.63723		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	Intercept	1	18.80298	0.34783	54.06	<.0001
Time	Time	1	-0.07588	0.00471	-16.10	<.0001

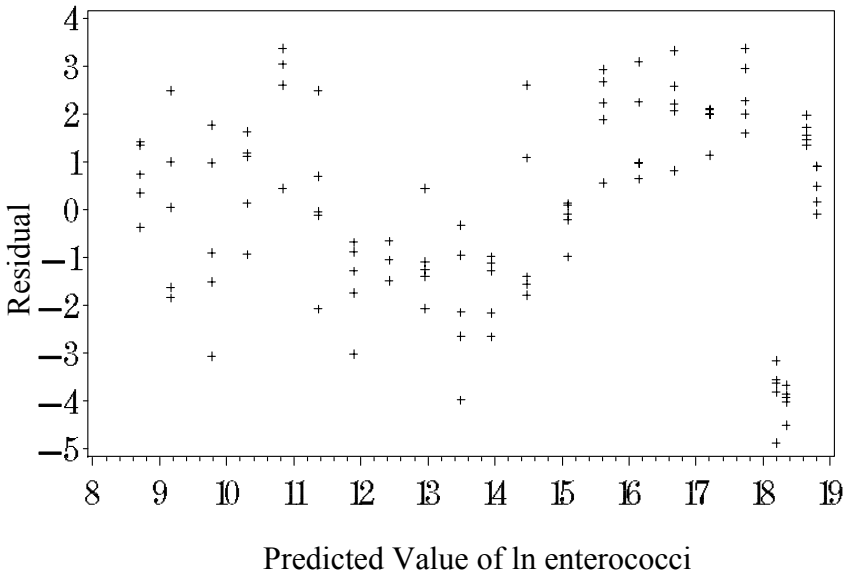


Figure C.6. Residual plots predicted ln enterococci during the spring season for first-order decay model.

C.1.7. Predictions of ln enterococci decay during the summer season:

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	2977.43399	2977.43399	997.52	<.0001
Error	121	361.16584	2.98484		
Corrected Total	122	3338.59982			

Root MSE	1.72767	R-Square	0.8918
Dependent Mean	11.53693	Adj R-Sq	0.8909
Coeff Var	14.97512		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	Intercept	1	18.59478	0.27240	68.26	<.0001
Time	Time	1	-0.09780	0.00310	-31.58	<.0001

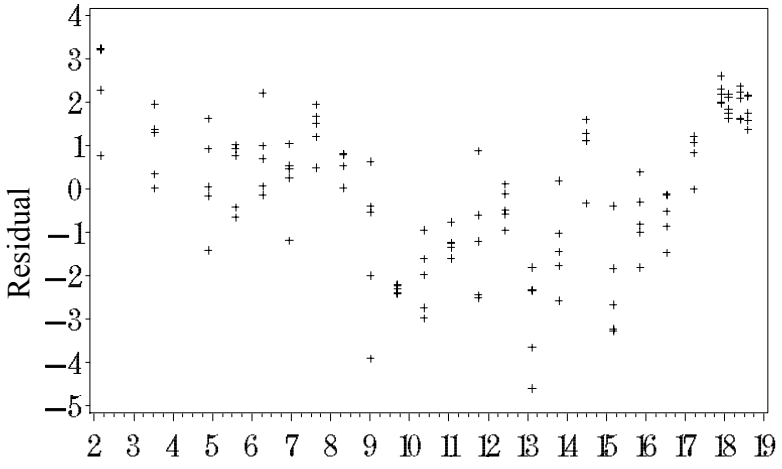


Figure C.7. Residual plots predicted ln enterococci during the summer season for first-order decay model.

C.1.8. Predictions of ln enterococci decay during the winter season:

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	1813.35179	1813.35179	920.66	<.0001
Error	103	202.87105	1.96962		
Corrected Total	104	2016.22284			

Root MSE	1.40343	R-Square	0.8994
Dependent Mean	11.69151	Adj R-Sq	0.8984
Coeff Var	12.00385		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	Intercept	1	17.89990	0.24622	72.70	<.0001
Time	Time	1	-0.09510	0.00313	-30.34	<.0001

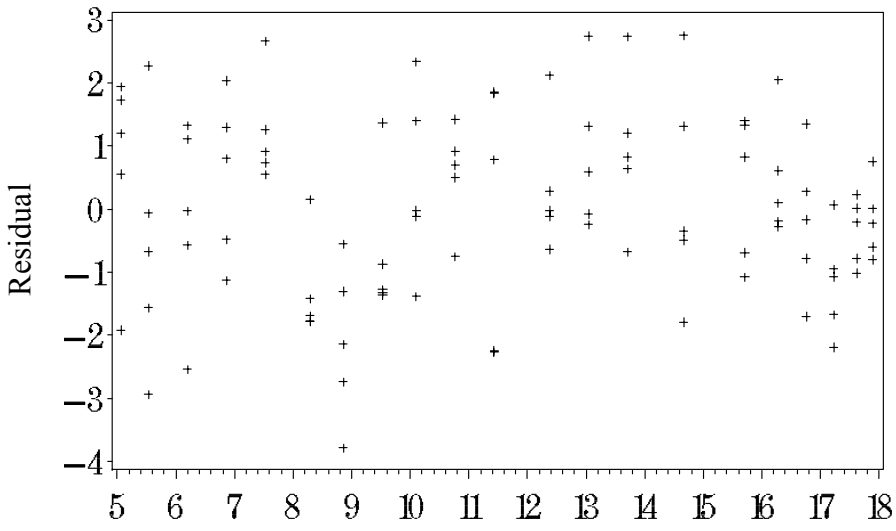


Figure C.8. Residual plots predicted ln enterococci during the winter season for first-order decay model.

C.2. Two-staged decay models

C.2.1. Prediction of ln E. coli decay during the summer spring and fall season – first stage:

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	46.53837	15.51279	7.64	0.0004
Error	41	83.24771	2.03043		
Corrected Total	44	129.78608			

Root MSE	1.42493	R-Square	0.3586
Dependent Mean	16.25941	Adj R-Sq	0.3116
Coeff Var	8.76374		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	Intercept	1	15.76182	0.36779	42.85	<.0001
Time	Time	1	-2.23430	0.79175	-2.82	0.0073
Sq_time		1	1.15042	0.36040	3.19	0.0027
Cu_time		1	-0.12381	0.04055	-3.05	0.0040

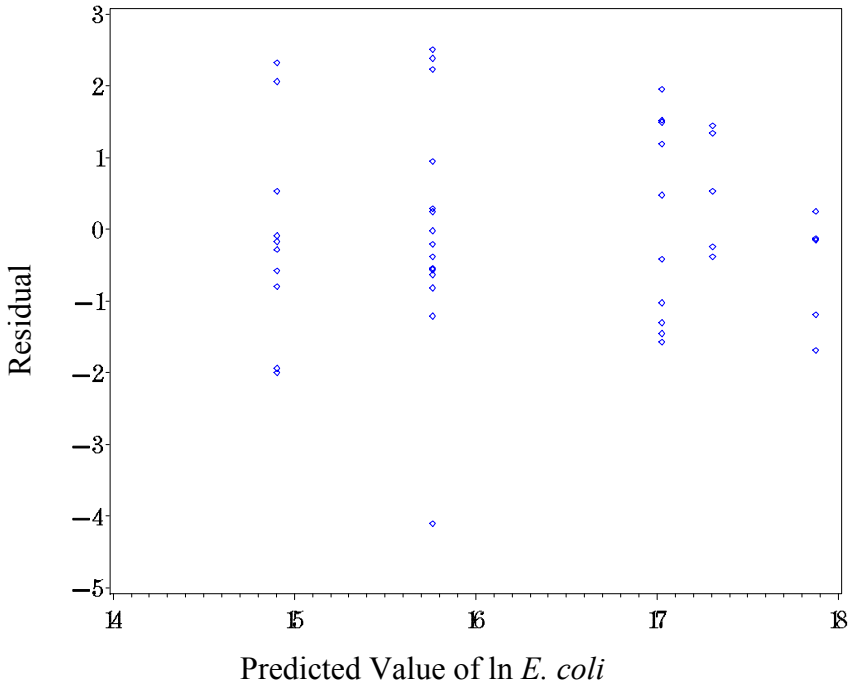


Figure C.9. Residual plots predicted ln *E. coli* during the fall, spring and summer seasons for first stage, time range 0 to 6.5 days.

C.2.1. Prediction of ln E. coli decay during the summer spring and fall season – second stage:

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	3826.05810	1275.35270	406.82	<.0001
Error	301	943.60798	3.13491		
Corrected Total	304	4769.66607			

Root MSE	1.77057	R-Square	0.8022
Dependent Mean	10.23744	Adj R-Sq	0.8002
Coeff Var	17.29503		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	Intercept	1	19.37631	0.45594	42.50	<.0001
Time	Time	1	-0.19978	0.02001	-9.99	<.0001
Sq_time		1	0.00116	0.00023568	4.93	<.0001
Cu_time		1	-0.00000267	7.907467E-7	-3.38	0.0008

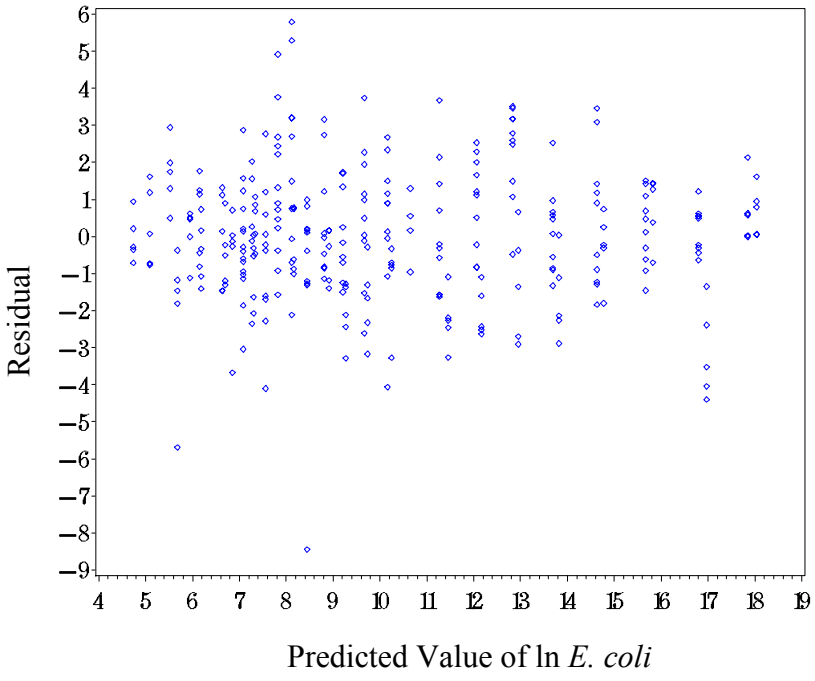


Figure C.10. Residual plots predicted ln E. coli during the fall, spring and summer seasons for first stage, beginning at 6.5 days.

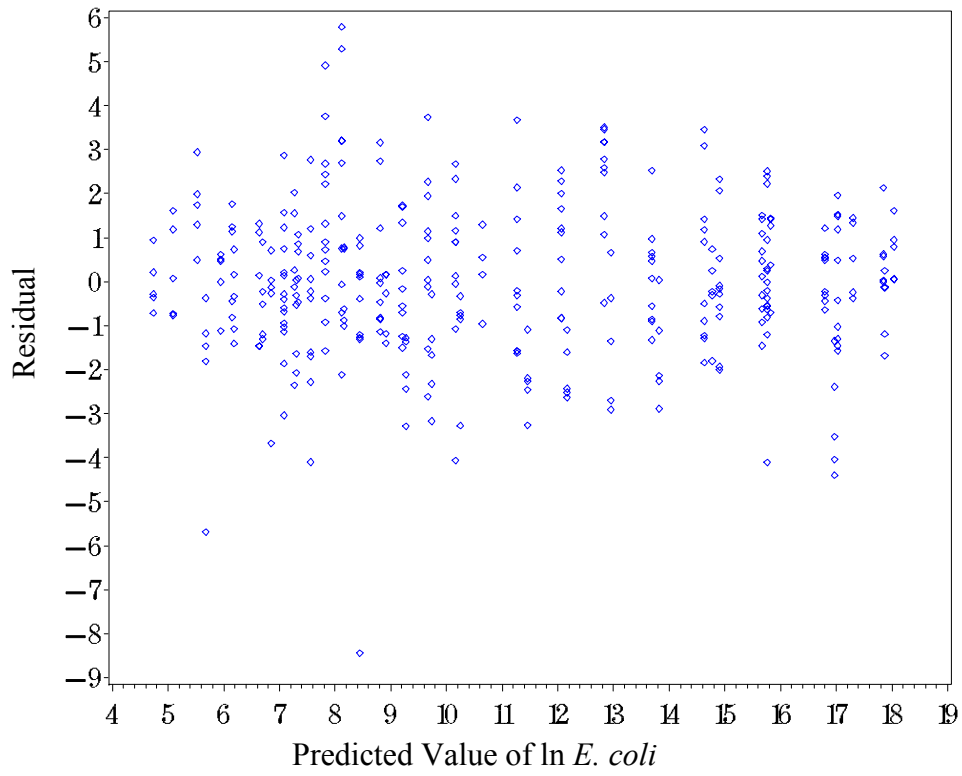


Figure C.11. Residual plots predicted ln *E. coli* during the fall, spring and summer seasons combined first and second stage.

C.3. Higher-order approximations

C.3.1. Predictions of ln E. coli decay during the fall season:

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	1882.22882	627.40961	399.98	<.0001
Error	115	180.39038	1.56861		
Corrected Total	118	2062.61919			

Root MSE	1.25244	R-Square	0.9125
Dependent Mean	9.94902	Adj R-Sq	0.9103
Coeff Var	12.58859		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	Intercept	1	18.22374	0.31923	57.09	<.0001
Time	Time	1	-0.22262	0.01622	-13.72	<.0001
Sq_time		1	0.00153	0.00020591	7.42	<.0001
Cu_time		1	-0.00000372	7.071029E-7	-5.26	<.0001

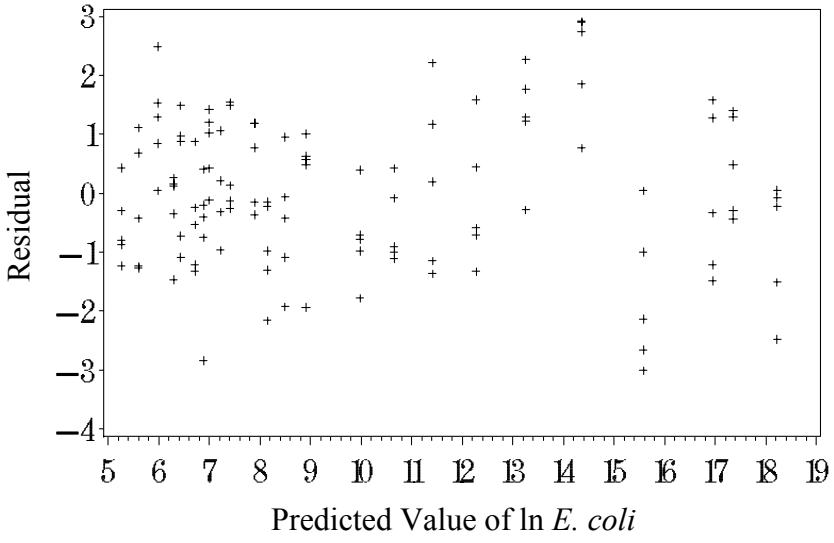


Figure C.12. Residual plots predicted ln *E. coli* during the fall season for higher order approximation.

C.3.2. Predictions of ln E. coli decay during the spring season:

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1212.31957	303.07989	85.69	<.0001
Error	103	364.31809	3.53707		
Corrected Total	107	1576.63766			

Root MSE	1.88071	R-Square	0.7689
Dependent Mean	12.51236	Adj R-Sq	0.7600
Coeff Var	15.03081		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	Intercept	1	15.51578	0.55742	27.84	<.0001
Time	Time	1	0.27554	0.07221	3.82	0.0002
Sq_time		1	-0.01227	0.00240	-5.12	<.0001
Cu_time		1	0.00013858	0.00002784	4.98	<.0001
Qu_time		1	-4.93188E-7	1.045539E-7	-4.72	<.0001

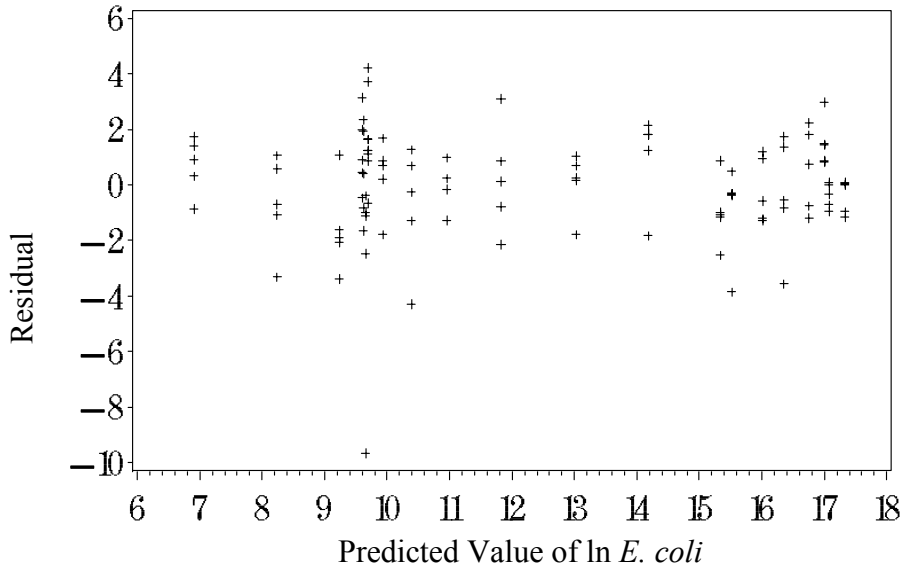


Figure C.13. Residual plots predicted ln *E. coli* during the spring season for higher order approximation.

C.3.3. Predictions of ln E. coli decay during the summer season:

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	2011.48715	502.87179	209.78	<.0001
Error	118	282.86761	2.39718		
Corrected Total	122	2294.35476			

Root MSE	1.54828	R-Square	0.8767
Dependent Mean	10.72214	Adj R-Sq	0.8725
Coeff Var	14.44006		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	Intercept	1	15.80443	0.41979	37.65	<.0001
Time	Time	1	0.09486	0.04324	2.19	0.0302
Sq_time		1	-0.00534	0.00115	-4.64	<.0001
Cu_time		1	0.00004994	0.00001069	4.67	<.0001
Qu_time		1	-1.43308E-7	3.199232E-8	-4.48	<.0001

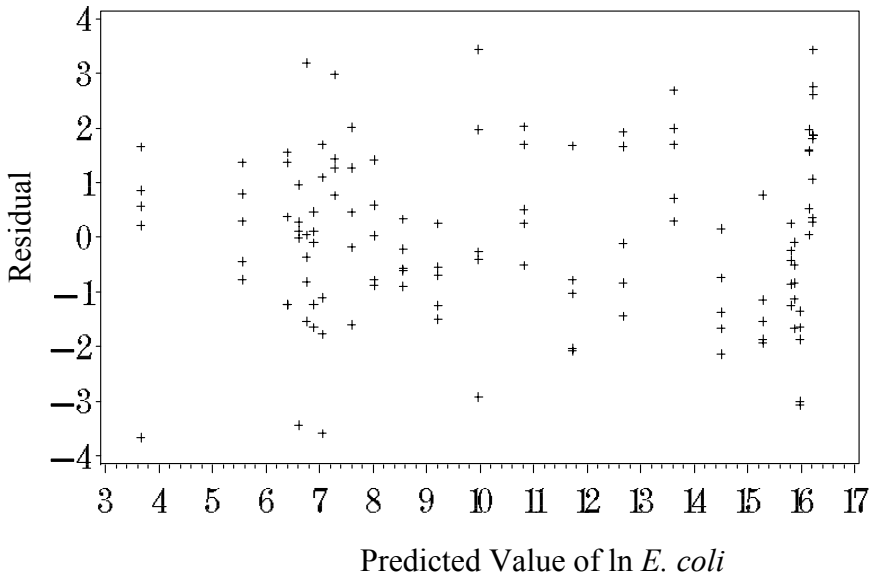


Figure C.14. Residual plots predicted ln *E. coli* during the summer season for higher order approximation.

C.3.4. Predictions of ln E. coli decay during the winter season:

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	1891.80700	630.60233	116.15	<.0001
Error	96	521.20488	5.42922		
Corrected Total	99	2413.01188			

Root MSE	2.33007	R-Square	0.7840
Dependent Mean	8.08146	Adj R-Sq	0.7773
Coeff Var	28.83227		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	Intercept	1	10.88696	0.82403	13.21	<.0001
Time	Time	1	0.21670	0.05731	3.78	0.0003
Sq_time		1	-0.00503	0.00098526	-5.11	<.0001
Cu_time		1	0.00002182	0.00000469	4.65	<.0001

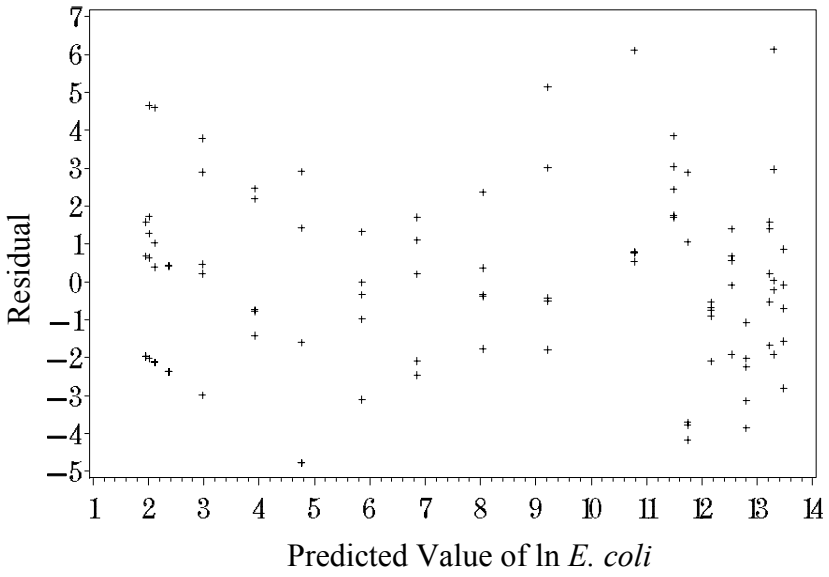


Figure C.15. Residual plots predicted ln *E. coli* during the winter season for higher order approximation.

C.3.5. Predictions of ln enterococci decay during the fall season:

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	1557.75452	259.62575	186.08	<.0001
Error	109	152.07798	1.39521		
Corrected Total	115	1709.83250			

Root MSE	1.18119	R-Square	0.9111
Dependent Mean	10.41652	Adj R-Sq	0.9062
Coeff Var	11.33959		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	Intercept	1	15.37542	0.49110	31.31	<.0001
Time	Time	1	0.26625	0.08434	3.16	0.0021
Sq_time		1	-0.01745	0.00410	-4.26	<.0001
Cu_time		1	0.00031971	0.00008200	3.90	0.0002
Qu_time		1	-0.00000271	7.764822E-7	-3.49	0.0007
Qi_time		1	1.098878E-8	3.465963E-9	3.17	0.0020
Si_time		1	-1.7194E-11	5.87152E-12	-2.93	0.0041

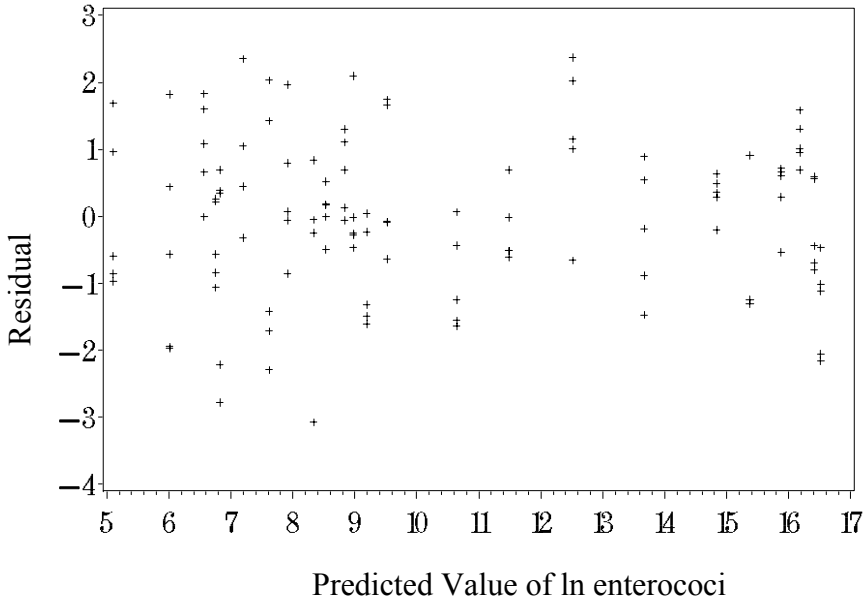


Figure C.16. Residual plots predicted ln enterococci during the fall season for higher order approximation.

C.3.6. Predictions of ln enterococci decay during the spring season:

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1192.53752	298.13438	78.12	<.0001
Error	102	389.27739	3.81645		
Corrected Total	106	1581.81491			

Root MSE	1.95357	R-Square	0.7539
Dependent Mean	14.23798	Adj R-Sq	0.7443
Coeff Var	13.72085		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	Intercept	1	17.27294	0.57925	29.82	<.0001
Time	Time	1	0.20863	0.07507	2.78	0.0065
Sq_time		1	-0.00893	0.00249	-3.59	0.0005
Cu_time		1	0.00009306	0.00002893	3.22	0.0017
Qu_time		1	-3.10098E-7	1.08703E-7	-2.85	0.0052

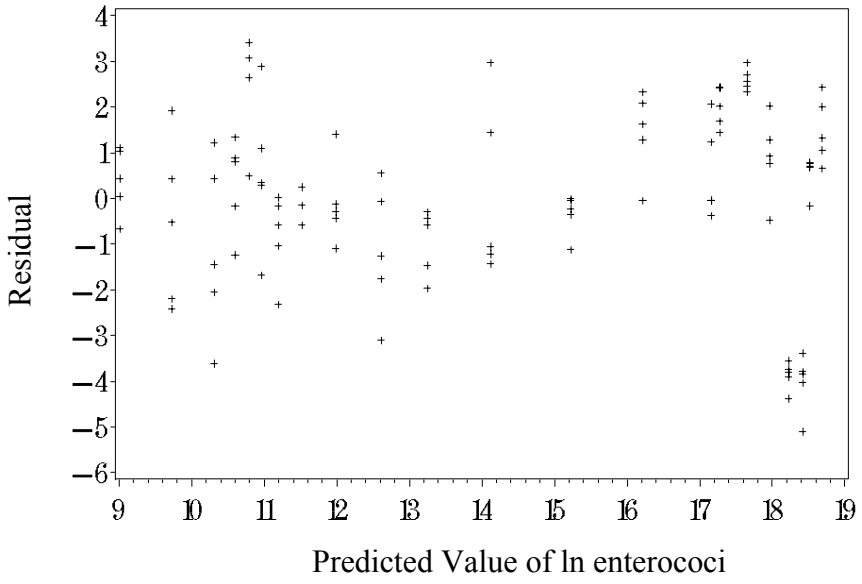


Figure C.17. Residual plots predicted ln enterococci during the spring season for higher order approximation.

C.3.7. Predictions of ln enterococci decay during the summer season:

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	3173.13993	1057.71331	760.72	<.0001
Error	119	165.45989	1.39042		
Corrected Total	122	3338.59982			

Root MSE	1.17916	R-Square	0.9504
Dependent Mean	11.53693	Adj R-Sq	0.9492
Coeff Var	10.22075		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	Intercept	1	20.88073	0.28274	73.85	<.0001
Time	Time	1	-0.23507	0.01693	-13.88	<.0001
Sq_time		1	0.00146	0.00025288	5.78	<.0001
Cu_time		1	-0.00000390	0.00000103	-3.80	0.0002

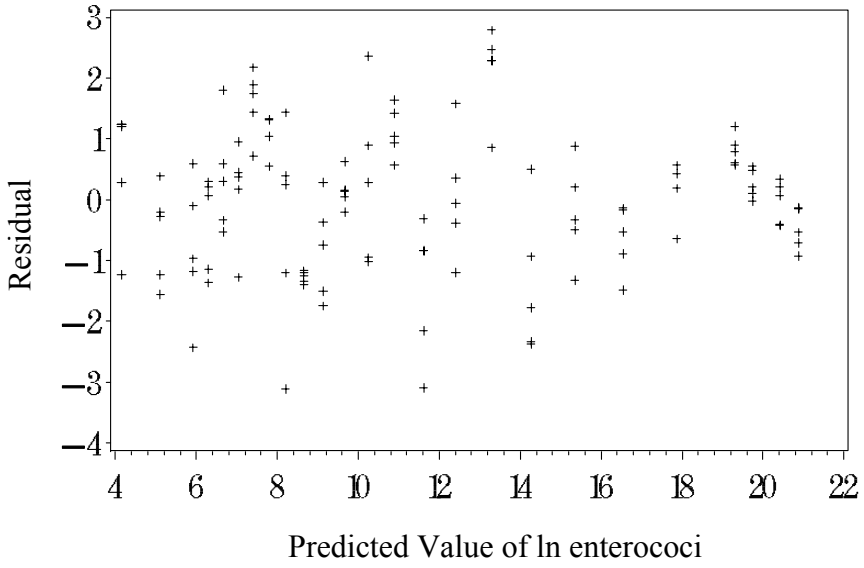


Figure C.18. Residual plots predicted ln enterococci during the summer season for higher order approximation.

C.3.8. Predictions of ln enterococci decay during the winter season:

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	1813.35179	1813.35179	920.66	<.0001
Error	103	202.87105	1.96962		
Corrected Total	104	2016.22284			

Root MSE	1.40343	R-Square	0.8994
Dependent Mean	11.69151	Adj R-Sq	0.8984
Coeff Var	12.00385		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	Intercept	1	17.89990	0.24622	72.70	<.0001
Time	Time	1	-0.09510	0.00313	-30.34	<.0001

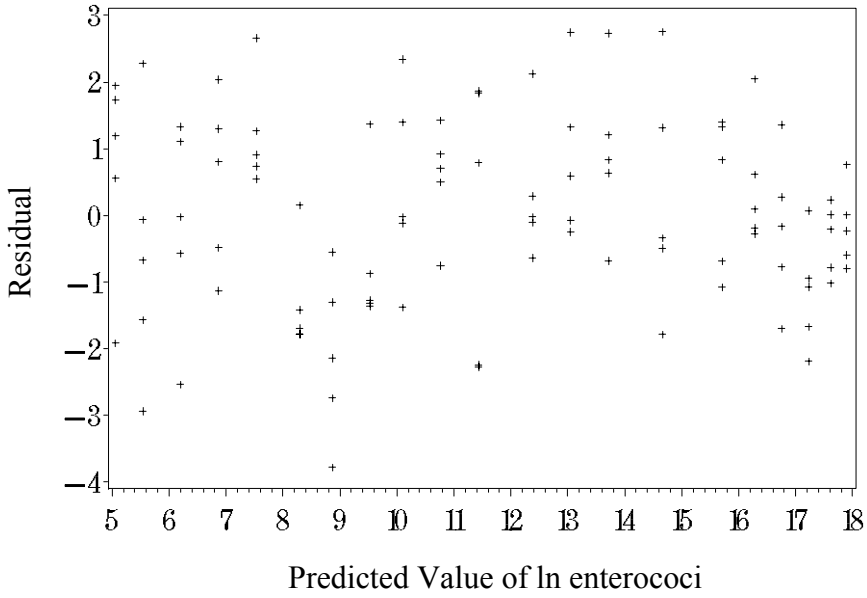


Figure C.19. Residual plots predicted ln enterococci during the winter season for higher order approximation.

C.4. Higher-order approximation best models including weather variables

C.4.1. Predictions of ln E. coli decay during the spring season:

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	1279.65549	159.95694	53.32	<.0001
Error	99	296.98217	2.99982		
Corrected Total	107	1576.63766			

Root MSE	1.73200	R-Square	0.8116
Dependent Mean	12.51236	Adj R-Sq	0.7964
Coeff Var	13.84230		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Variance Inflation
Intercept	Intercept	1	4.45897	3.51602	1.27	0.2077	0
Time	Time	1	0.39328	0.07635	5.15	<.0001	384.85388
Sq_time		1	-0.01793	0.00264	-6.80	<.0001	7965.66400
Cu_time		1	0.00020163	0.00002982	6.76	<.0001	16416
Qu_time		1	-7.07486E-7	1.088354E-7	-6.50	<.0001	3527.21448
Temp_high	Temp_high	1	-0.49165	0.20691	-2.38	0.0194	40.35464
Temp_avg	Temp_avg	1	0.66522	0.22215	2.99	0.0035	44.21906
Solar_rad_avg	Solar_rad_avg	1	4.54629	1.37589	3.30	0.0013	11.61557
humidity_avg	humidity_avg	1	0.09619	0.03622	2.66	0.0092	6.89171

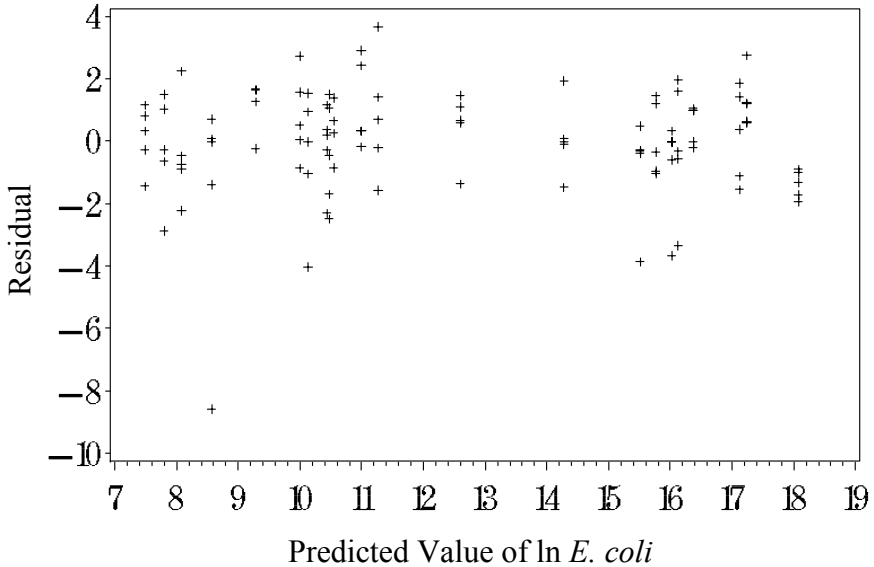


Figure C.20. Residual plots predicted ln *E. coli* during the spring season for higher order approximation with weather variables.

C.4.2. Predictions of ln E. coli decay during the summer season:

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	1587.55883	226.79412	122.85	<.0001
Error	86	158.75873	1.84603		
Corrected Total	93	1746.31755			

Root MSE	1.35869	R-Square	0.9091
Dependent Mean	11.35599	Adj R-Sq	0.9017
Coeff Var	11.96450		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Variance Inflation
Intercept	Intercept	1	28.85322	5.14619	5.61	<.0001	0
Time	Time	1	-0.32324	0.05365	-6.02	<.0001	352.67108
Sq_time		1	0.00313	0.00088853	3.53	0.0007	2328.40337
Cu_time		1	-0.00000978	0.00000359	-2.72	0.0078	808.57607
Temp_weekly_avg	Temp_weekly_avg	1	0.64946	0.09855	6.59	<.0001	20.85459
Solar_rad_weekly_avg	Solar_rad_weekly_avg	1	-5.91786	1.62069	-3.65	0.0004	11.87387
humidity_weekly_avg	humidity_weekly_avg	1	-0.18879	0.04615	-4.09	<.0001	4.86466
rainfall_weekly_total	rainfall_weekly_total	1	0.29990	0.12946	2.32	0.0229	2.19124

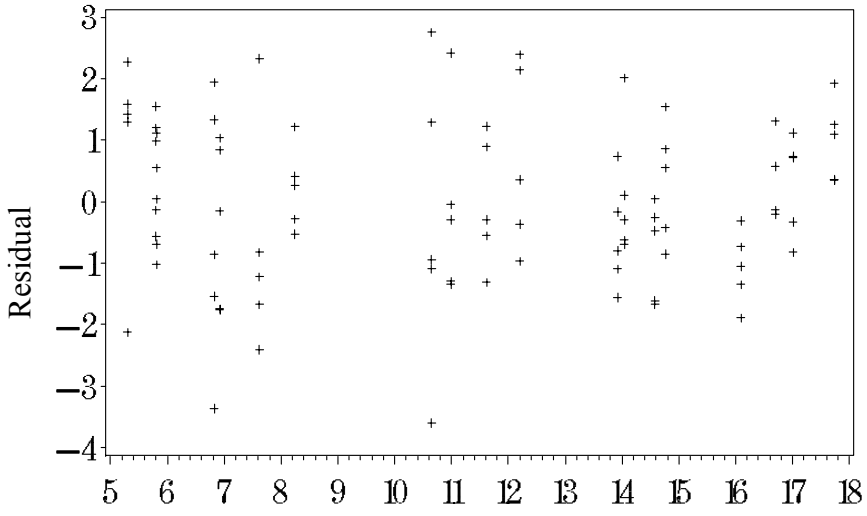


Figure C.21. Residual plots predicted ln *E. coli* during the summer season for higher order approximation with weather variables.

C.4.3. Predictions of ln E. coli decay during the winter season:

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	1893.89920	315.64987	69.90	<.0001
Error	88	397.36346	4.51549		
Corrected Total	94	2291.26266			

Root MSE	2.12497	R-Square	0.8266
Dependent Mean	8.30350	Adj R-Sq	0.8147
Coeff Var	25.59125		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Variance Inflation
Intercept	Intercept	1	19.48140	4.12337	4.72	<.0001	0
Time	Time	1	0.47350	0.13325	3.55	0.0006	666.60080
Sq_time		1	-0.00708	0.00163	-4.35	<.0001	1945.94587
Cu_time		1	0.00002796	0.00000649	4.31	<.0001	540.07458
Temp_weekly_avg	Temp_weekly_avg	1	-0.24117	0.07259	-3.32	0.0013	7.27337
Solar_rad_weekly_high	Solar_rad_weekly_high	1	-4.99053	2.20895	-2.26	0.0263	18.56346
rainfall_weekly_total	rainfall_weekly_total	1	0.28478	0.12613	2.26	0.0264	1.66605

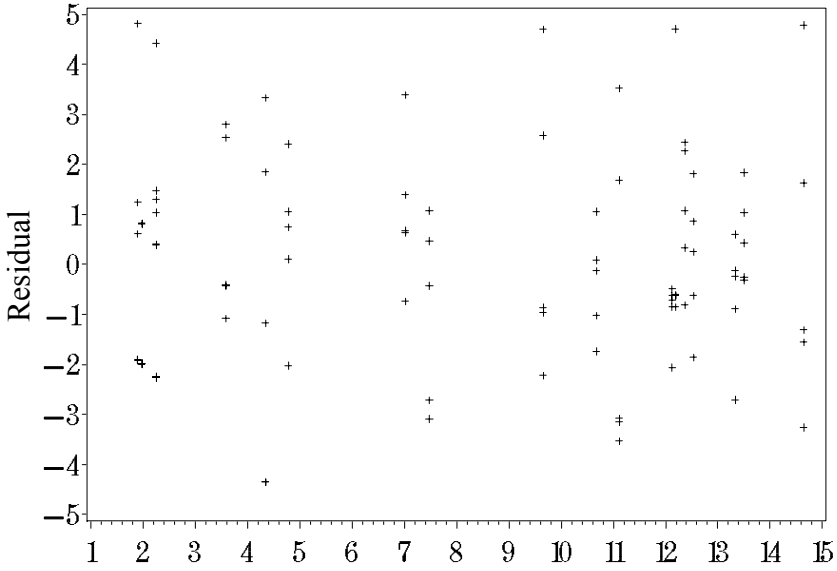


Figure C.22. Residual plots predicted ln *E. coli* during the winter season for higher order approximation with weather variables.

C.4.4. Predictions of ln enterococci decay during the spring season:

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	1332.61255	166.57657	65.51	<.0001
Error	98	249.20236	2.54288		
Corrected Total	106	1581.81491			

Root MSE	1.59464	R-Square	0.8425
Dependent Mean	14.23798	Adj R-Sq	0.8296
Coeff Var	11.19991		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Variance Inflation
Intercept	Intercept	1	3.40476	3.33361	1.02	0.3096	0
Time	Time	1	0.24266	0.07216	3.36	0.0011	400.37456
Sq_time		1	-0.00955	0.00244	-3.91	0.0002	7985.80539
Cu_time		1	0.00010057	0.00002736	3.68	0.0004	16225
Qu_time		1	-3.39331E-7	9.951037E-8	-3.41	0.0009	3472.08301
Temp_high	Temp_high	1	0.70917	0.14226	4.99	<.0001	22.12359
Temp_avg	Temp_avg	1	-0.85997	0.17536	-4.90	<.0001	31.92042
Solar_rad_high	Solar_rad_high	1	1.12009	0.46687	2.40	0.0183	4.68354
humidity_avg	humidity_avg	1	0.10777	0.03154	3.42	0.0009	6.12057

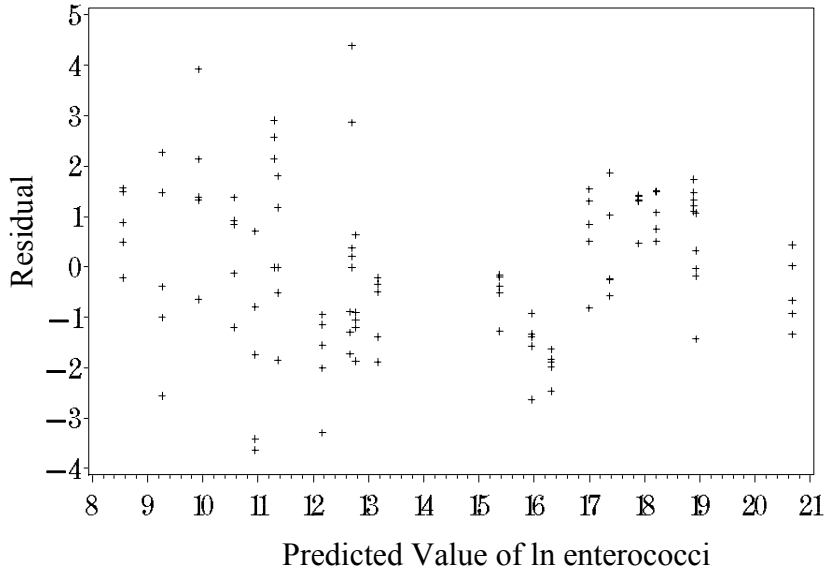


Figure C.23. Residual plots predicted ln enterococci during the spring season for higher order approximation with weather variables.

C.4.5. Predictions of ln enterococci decay during the summer season:

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	2651.22117	530.24423	521.91	<.0001
Error	93	94.48435	1.01596		
Corrected Total	98	2745.70552			

Root MSE	1.00795	R-Square	0.9656
Dependent Mean	12.55219	Adj R-Sq	0.9637
Coeff Var	8.03006		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Variance Inflation
Intercept	Intercept	1	20.07105	0.79332	25.30	<.0001	0
Time	Time	1	-0.32012	0.02325	-13.77	<.0001	131.52905
Sq_time		1	0.00299	0.00041597	7.19	<.0001	964.94758
Cu_time		1	-0.00001056	0.00000182	-5.79	<.0001	389.37715
Temp_high	Temp_high	1	0.13912	0.03765	3.69	0.0004	7.96574
Solar_rad_avg	Solar_rad_avg	1	-1.89212	0.41689	-4.54	<.0001	3.91667

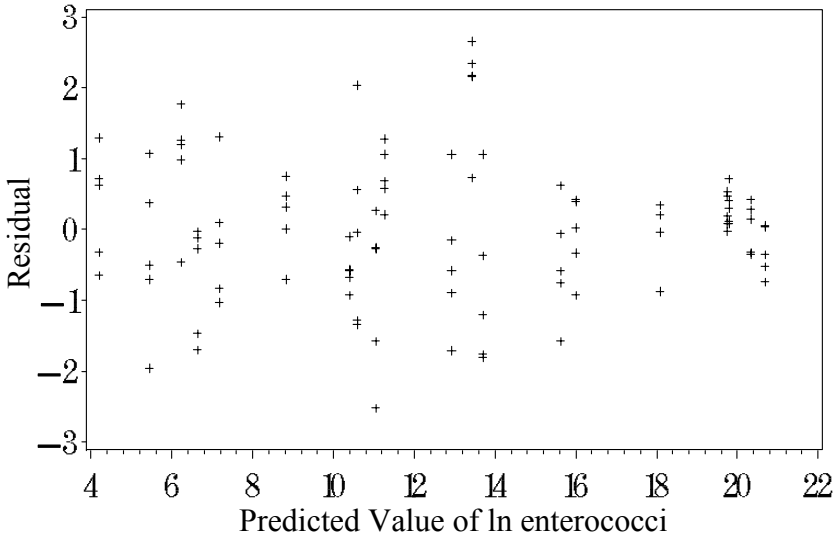


Figure C.24. Residual plots predicted ln enterococci during the summer season for higher order approximation with weather variables.

C.4.5. Predictions of ln enterococci decay during the winter season:

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	1783.55083	891.77541	468.36	<.0001
Error	97	184.69078	1.90403		
Corrected Total	99	1968.24161			

Root MSE	1.37987	R-Square	0.9062
Dependent Mean	11.83810	Adj R-Sq	0.9042
Coeff Var	11.65614		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Variance Inflation
Intercept	Intercept	1	17.78529	0.25427	69.95	<.0001	0
Time	Time	1	-0.09566	0.00320	-29.92	<.0001	1.02250
rainfall_prev_total	rainfall_prev_total	1	0.16150	0.08408	1.92	0.0577	1.02250

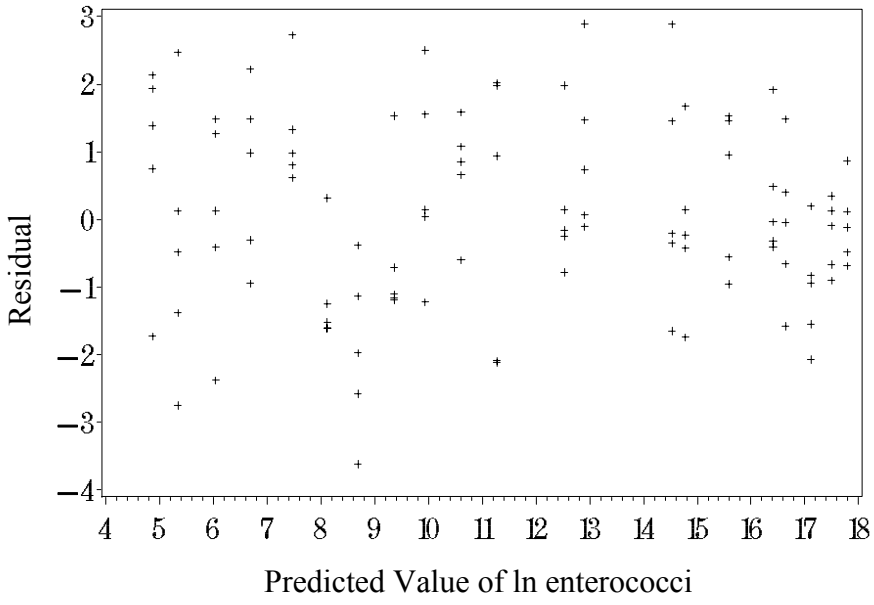


Figure C.25. Residual plots predicted ln enterococci during the winter season for higher order approximation with weather variables.

C.5. Predicted and observed plots

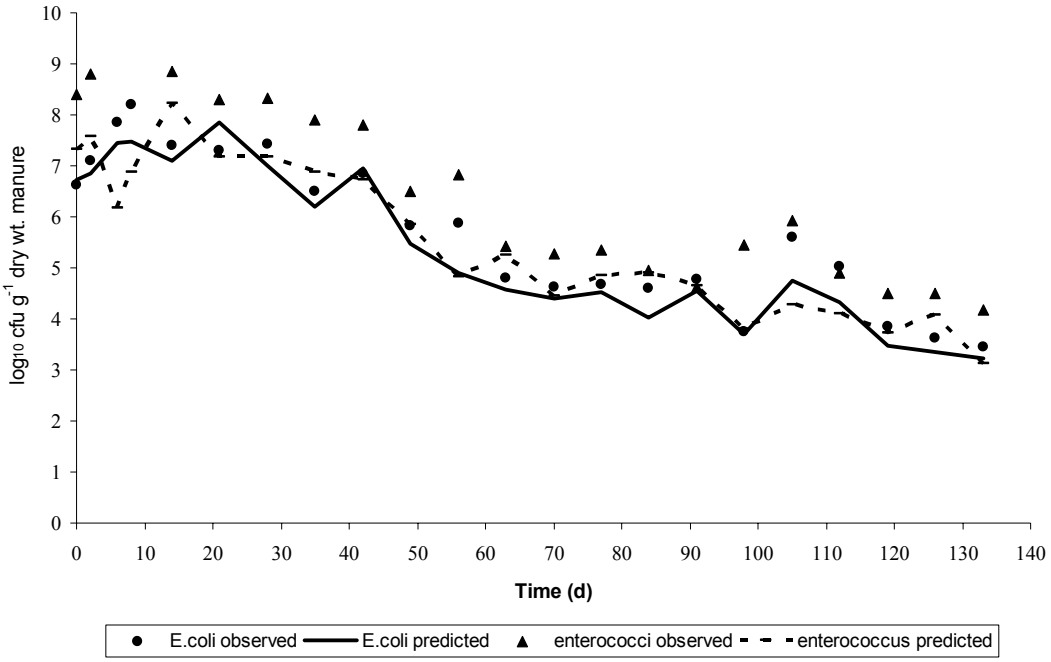


Figure C.26. Predicted and observed *E. coli* and enterococci decay. Predicted values are calculated using the equations presented in Table 4.4 for *E. coli* and enterococci decay beginning during the spring monitoring period.

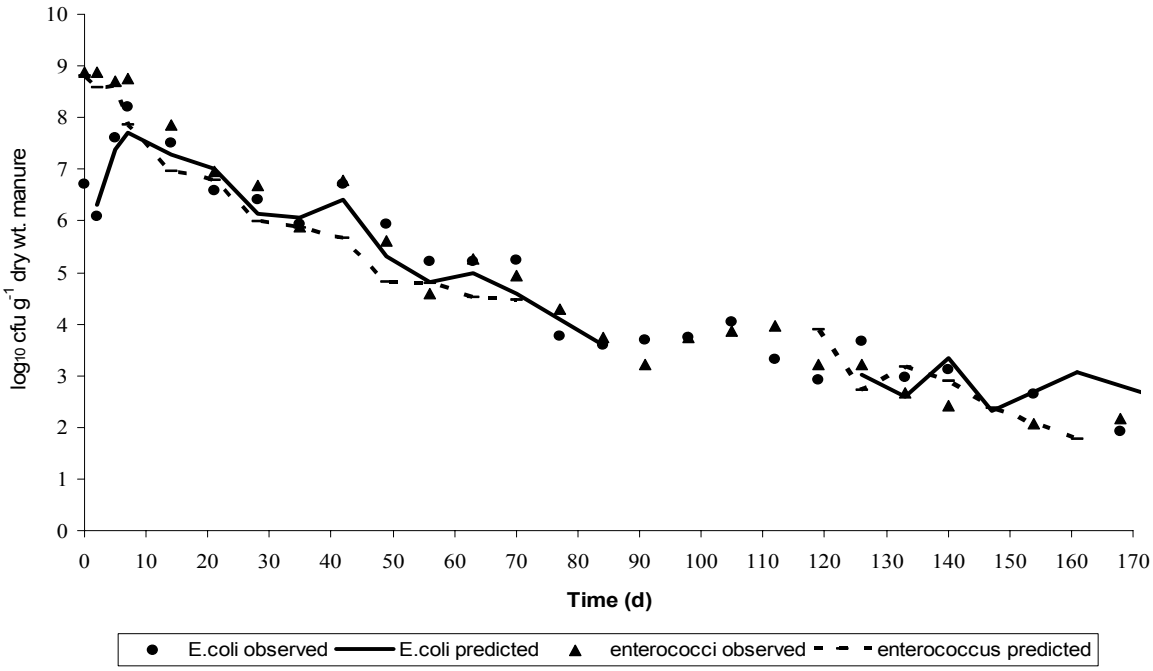


Figure C.27. Predicted and observed *E. coli* and enterococci decay. Predicted values are calculated using the equations presented in Table 4.4 for *E. coli* and enterococci decay beginning during the summer monitoring period.

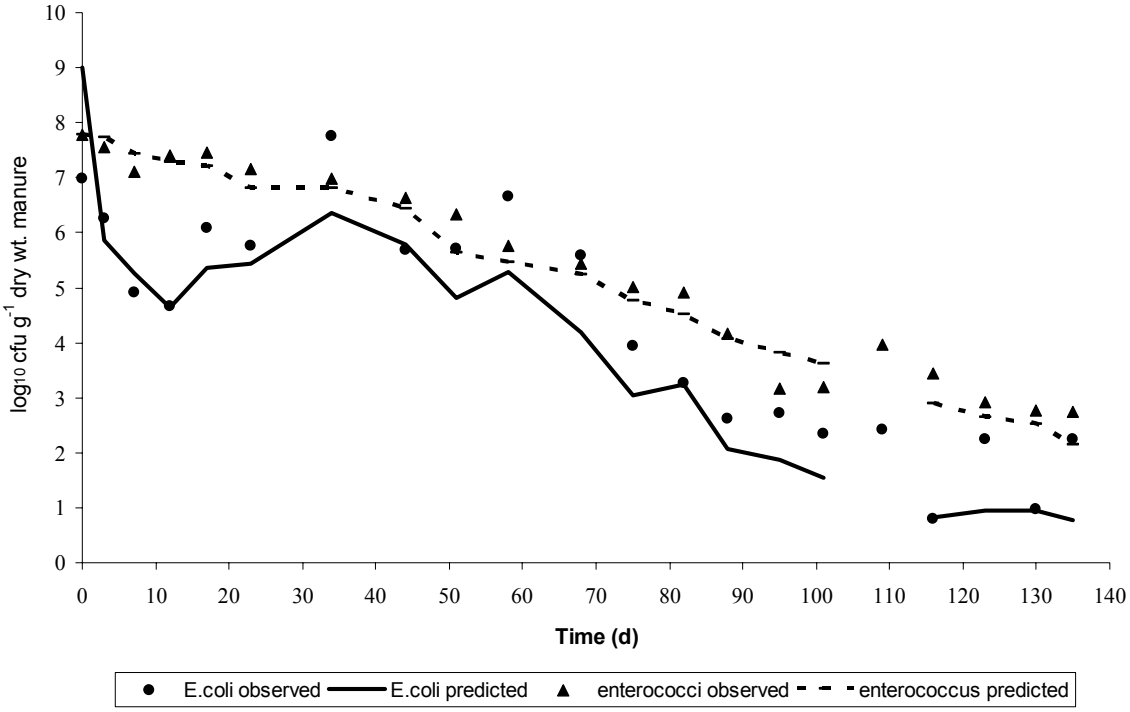


Figure C.28. Predicted and observed *E. coli* and enterococci decay. Predicted values are calculated using the equations presented in Table 4.4 for *E. coli* and enterococci decay beginning during the winter monitoring period.

Appendix D. Box Plot Study Data

Table D.1. Box Plot Study Bacteria, TSS, and Nutrient Results

Sample No.	Simu-lation	Date	*E. coli* TC	*E. coli* PC	*E. coli* PAF	ENT TC	ENT PC	ENT PAF	TSS	TDP	DOP	TP	TOP	SOP	Nitrate	Ammonia	TKN	TOC	DOC
PF1-1	1	6/6/06	7.00E+08			4.40E+06	0.54	1.17	2990	4.20	1.75	12.47	5.94	4.193	0.903	0.340	2.805	14.96	13.40
PF1-2	1	6/6/06	1.10E+09	0.22	0.28	4.20E+06	0.42	0.74	2916	4.90	2.77	8.15	4.08	1.305	1.820	0.422	5.200	16.00	14.00
PF1-3	1	6/6/06	2.00E+08	0.61	1.56	2.50E+06	0.76	3.18	1588	3.20	1.54	6.75	3.02	1.477	1.130	0.325	3.704	15.15	12.80
PF2-1	1	6/6/06	4.50E+03	0.41	0.70	3.30E+03	0.90	9.52	1268	3.40	1.67	7.55	4.27	2.606	1.353	0.449	4.660	17.63	13.90
PF2-2	1	6/6/06	2.00E+03			3.20E+03	0.63	1.70	4386	3.30	1.65	9.47	4.74	3.085	1.282	0.260	3.824	15.91	13.70
PF2-3	1	6/6/06	1.00E+03	0.51	1.06	2.00E+03	0.80	4.03	1912	3.50	1.75	9.20	5.19	3.443	1.199	0.207	3.817	15.69	12.80
PF3-1	1	6/6/06	1.20E+03	0.00	0.00	4.30E+03	0.14	0.17	6636	4.20	2.20	11.25	5.72	3.525	1.865	0.776	4.536	19.45	14.70
PF3-2	1	6/6/06	1.00E+03	0.35	0.55	6.50E+03	0.42	0.73	3806	4.80	3.12	7.85	4.70	1.586	2.704	0.890	6.604	20.67	15.10
PF3-3	1	6/6/06	1.20E+03	0.27	0.38	5.10E+03	0.37	0.60	5464	4.00	1.99	8.23	3.72	1.724	1.857	0.693	4.230	19.28	14.60
PF4-1	1	6/6/06	5.00E+02	0.42	0.72	3.50E+03	0.41	0.70	1134	3.30	2.24	9.20	5.17	2.939	0.913	0.124	3.711	14.24	13.80
PF4-2	1	6/6/06	1.10E+03	0.23	0.30	2.00E+03	0.10	0.11	4478	3.70	1.85	10.05	4.82	2.971	1.006	0.250	4.433	14.85	14.50
PF4-3	1	6/6/06	9.00E+02	0.21	0.26	3.40E+03	0.44	0.77	4932	4.70	2.17	11.16	5.35	3.175	0.844	0.177	3.358	13.90	13.12
PF5-1	1	6/6/06	1.00E+03	0.04	0.04	2.60E+03	0.42	0.72	2858	3.70	1.85	13.40	6.69	4.842	0.951	0.155	2.100	13.80	12.80
PF5-2	1	6/6/06	5.00E+02			3.80E+03			2600	3.51	1.79	11.24	5.61	3.823	3.672	0.430	3.878	19.61	19.13
PF5-3	1	6/6/06	1.20E+03	0.37	0.59	3.90E+03	0.47	0.88	13952	3.60	1.70	15.08	7.49	5.787	1.322	0.179	3.000	14.78	14.30
PF6-1	1	6/6/06							6546	0.430	0.229	0.931	0.455	0.226	0.011	0.006	0.021	5.09	3.57
PF6-2	1	6/6/06							7704	0.367	0.185	0.732	0.378	0.193	0.010	0.004	0.020	4.59	3.57
PF6-3	1	6/6/06							9076	0.280	0.136	0.412	0.212	0.076	0.003	0.001	0.007	3.31	3.12
S1-1	1	6/12/06							5734	0.450	0.229	0.932	0.476	0.247	0.021	0.013	0.055	2.96	2.28
S1-2	1	6/12/06							5648	0.400	0.203	0.787	0.388	0.185	0.039	0.014	0.061	3.35	2.13
S1-3	1	6/12/06							5644	0.357	0.187	0.761	0.390	0.203	0.036	0.012	0.046	2.33	2.06
S2-1	1	6/12/06	5.00E+08	0.25	0.34	5.50E+06	0.07	0.08	2678	6.55	3.28	13.25	6.60	3.318	4.655	1.220	11.260	26.28	16.10
S2-2	1	6/12/06	4.00E+08	0.62	1.02	1.60E+06	0.49	0.98	3816	7.40	3.69	15.74	7.87	4.178	3.201	0.450	10.490	25.61	16.00
S2-3	1	6/12/06	1.00E+08	0.52	0.40	6.00E+05	0.27	0.38	4326	7.95	4.19	26.69	13.03	8.843	2.100	0.400	6.331	24.07	15.20
S3-1	1	6/12/06	2.00E+08	0.35	0.15	5.20E+06	0.30	0.43	1874	7.31	3.66	14.55	7.16	3.504	8.422	0.462	13.800	28.16	15.80
S3-2	1	6/12/06	1.00E+08			3.60E+06	0.35	0.54	5312	9.00	4.47	17.56	8.68	4.213	7.944	0.435	11.419	23.32	15.30
S3-3	1	6/12/06	2.00E+08	0.23	0.23	1.40E+06	0.21	0.27	6246	9.10	4.52	20.82	10.30	5.778	2.116	0.400	7.822	21.01	16.10
S4-1	1	6/12/06	3.00E+08	0.50	0.55	6.20E+03	0.35	0.53	2832	6.85	3.43	22.00	10.48	7.050	2.015	0.660	10.550	22.47	16.90
S4-2	1	6/12/06	1.00E+08	0.63	0.74	2.80E+03	0.33	0.50	4364	6.60	3.27	15.15	7.39	4.126	2.008	0.651	10.541	22.30	16.70
S4-3	1	6/12/06	2.47E+08	0.53	0.55	1.00E+06	0.37	0.58	5038	5.65	2.83	12.36	6.18	3.352	2.000	0.640	10.500	22.09	16.20
S5-1	1	6/12/06	4.00E+08	0.16	0.11	1.11E+07	0.27	0.36	4392	7.50	3.75	18.55	9.16	5.413	8.507	7.910	15.103	29.36	16.60
S5-2	1	6/12/06	2.00E+08	0.14	0.10	5.60E+06	0.26	0.35	3774	6.91	3.44	16.18	7.99	4.545	8.432	4.430	12.712	24.04	16.20
S5-3	1	6/12/06	2.00E+08	0.39	0.27	1.40E+06	0.36	0.56	3698	6.34	3.17	12.27	6.03	2.855	8.401	1.210	11.604	22.61	16.00

Sample No.	Simu-lation	Date	*E. coli* TC	*E. coli* PC	*E. coli* PAF	ENT TC	ENT PC	ENT PAF	TSS	TDP	DOP	TP	TOP	SOP	Nitrate	Ammonia	TKN	TOC	DOC
S6-1	1	6/12/06	2.00E+08	0.73	1.51	7.70E+06	0.11	0.13	2732	9.21	4.51	11.65	5.83	1.325	5.839	3.614	15.100	24.72	16.10
S6-2	1	6/12/06	5.00E+08			5.10E+06			4128	9.44	4.71	15.45	7.75	3.037	5.800	1.710	11.414	21.96	15.20
S6-3	1	6/12/06	2.04E+08	0.49	0.57	3.70E+06	0.22	0.28	4550	9.87	4.92	17.90	9.39	4.474	1.911	1.011	10.802	21.55	15.20
C1-1	1	6/13/06	7.20E+07	0.08	0.08	2.81E+07	0.18	0.22	1354	2.58	1.37	8.25	4.03	2.654	1.300	0.220	6.650	17.93	16.20
C1-2	1	6/13/06	3.40E+07	0.43	0.24	1.71E+07	0.45	0.82	1588	2.79	1.25	8.95	4.36	3.113	7.706	0.655	12.516	26.86	16.70
C1-3	1	6/13/06	5.70E+07	0.53	0.24	1.52E+07	0.39	0.64	1780	3.90	1.93	15.01	7.39	5.463	5.124	0.219	6.822	19.03	16.40
C2-1	1	6/13/06	5.10E+07	0.33	0.24	3.63E+07	0.20	0.25	2314	2.47	1.22	10.44	5.11	3.892	2.788	0.221	7.166	18.42	16.30
C2-2	1	6/13/06	4.60E+07	0.64	0.61	1.62E+07	0.41	0.70	2340	2.91	1.37	15.35	7.68	6.315	3.542	0.225	8.120	19.73	16.70
C2-3	1	6/13/06	6.10E+07	0.04	0.03	1.80E+07	0.25	0.33	2280	2.88	1.44	14.85	7.43	5.985	2.762	0.221	7.068	18.17	16.10
C3-1	1	6/13/06	6.00E+07	0.61	0.51	1.70E+07	0.57	1.31	2866	2.52	0.25	16.22	8.00	7.750	2.522	0.222	7.331	18.91	16.90
C3-2	1	6/13/06	7.30E+07	0.57	0.45	7.00E+06	0.59	1.43	2614	2.24	1.11	9.75	4.76	3.654	2.500	0.200	6.809	18.40	16.90
C3-3	1	6/13/06	5.40E+07	0.71	0.76	1.70E+07	0.56	1.27	2786	2.33	1.16	10.96	5.37	4.214	2.501	0.220	6.809	18.07	16.70
C4-1	1	6/13/06	5.60E+07	0.44	0.39	1.10E+07	0.68	2.10	3092	3.14	1.57	14.12	7.05	5.484	2.566	0.657	8.600	23.08	16.90
C4-2	1	6/13/06	4.60E+07	0.31	0.33	4.00E+06	0.52	1.08	2806	3.12	1.56	11.26	5.63	4.072	3.100	0.659	10.944	23.93	17.20
C4-3	1	6/13/06	4.60E+05	0.92	1.29	5.00E+06	0.44	0.79	2348	2.22	1.11	11.09	5.43	4.326	1.339	0.401	8.109	19.18	17.00
C5-1	1	6/13/06							1920	4.65	2.32	11.12	5.45	3.134	1.806	3.218	8.422	20.25	17.30
C5-2	1	6/13/06	2.00E+08	0.01	0.01	3.44E+07	0.30	0.43	2218	6.75	3.37	16.35	8.16	4.798	4.000	3.790	11.100	39.58	17.90
C5-3	1	6/13/06	1.00E+08	0.19	0.18	3.01E+07	0.42	0.71	2124	6.58	3.29	13.38	6.59	3.301	1.223	0.714	0.851	18.30	16.40
C6-1	1	6/13/06							2902	0.042	0.020	0.093	0.045	0.025	0.060	0.022	0.111	4.64	3.24
C6-2	1	6/13/06							2614	0.040	0.019	0.086	0.042	0.023	0.065	0.029	0.128	5.11	3.48
C6-3	1	6/13/06							2254	0.029	0.013	0.079	0.039	0.026	0.039	0.021	0.112	4.89	3.37
PF1-1	2	8/24/06	0			0			1636	2.93	1.46	7.20	3.64	2.181	2.440	0.161	4.344	4.86	4.74
PF1-2	2	8/24/06	1000			0			2676	3.65	1.82	12.05	6.04	4.220	2.208	0.130	3.911	4.80	4.60
PF1-3	2	8/24/06	0			0			2148	3.42	1.70	8.13	4.08	2.378	2.115	0.124	3.155	4.66	4.54
PF2-1	2	8/24/06	0			0			2805	4.26	2.12	8.29	4.26	2.135	2.500	0.170	3.419	4.18	4.04
PF2-2	2	8/24/06	0			0			2629	3.58	1.78	8.00	4.00	2.220	2.127	0.142	3.120	3.66	3.14
PF2-3	2	8/24/06	0			0			1946	3.22	1.60	7.23	3.73	2.122	1.950	0.113	2.956	2.61	2.42
PF3-1	2	8/24/06	0			0			1968	3.80	1.89	11.46	5.84	3.942	2.102	0.145	3.188	3.26	2.98
PF3-2	2	8/24/06	0			0			1632	2.90	1.45	7.10	3.65	2.206	1.933	0.126	2.117	2.87	2.78
PF3-3	2	8/24/06	0			0			1918	2.96	1.47	7.35	3.79	2.316	1.623	0.120	1.904	2.67	2.60
PF4-1	2	8/24/06	0			0			1370	2.69	1.34	7.67	3.95	2.604	2.106	0.152	2.65	2.82	2.62
PF4-2	2	8/24/06	0			0			1316	2.46	1.22	6.38	3.30	2.076	1.902	0.144	2.212	2.36	2.12
PF4-3	2	8/24/06	0			0			1197	2.40	1.20	6.04	3.03	1.830	1.774	0.140	2.009	2.03	1.92
PF5-1	2	8/24/06	0			0			1660	2.33	1.16	8.15	4.09	2.923	1.855	0.182	3.534	3.57	3.40

Sample No.	Simu-lation	Date	*E. coli* TC	*E. coli* PC	*E. coli* PAF	ENT TC	ENT PC	ENT PAF	TSS	TDP	DOP	TP	TOP	SOP	Nitrate	Ammonia	TKN	TOC	DOC
PF5-2	2	8/24/06	0			0			1829	2.87	1.43	10.60	5.30	3.871	1.795	0.119	2.922	3.01	2.95
PF5-3	2	8/24/06	0			0			1652	2.24	1.12	7.10	3.62	2.503	1.500	0.110	2.651	2.72	2.64
PF6-1	2	8/29/06	0			0			5411	0.422	0.207	0.841	0.521	0.314	0.007	0.002	0.012	2.66	2.62
PF6-2	2	8/29/06	0			0			5174	0.345	0.165	0.510	0.342	0.177	0.005	0.002	0.007	2.19	2.09
PF6-3	2	8/29/06	0			0			4697	0.216	0.084	0.331	0.244	0.160	0.002	0.001	0.002	1.91	1.84
S1-1	2	8/29/06	0			400			1325	0.300	0.145	0.755	0.477	0.332	0.014	0.008	0.046	1.76	1.66
S1-2	2	8/29/06	0			700			1357	0.341	0.168	0.776	0.488	0.320	0.035	0.011	0.051	1.90	1.78
S1-3	2	8/29/06	0			400			1446	0.352	0.172	0.861	0.541	0.369	0.030	0.005	0.036	1.31	1.30
S2-1	2	8/29/06	200			300			621	3.36	1.68	6.51	3.36	1.679	1.100	0.065	2.251	4.60	4.51
S2-2	2	8/29/06	400			700			542	3.20	1.60	6.42	3.31	1.711	1.002	0.041	1.325	4.37	4.22
S2-3	2	8/29/06	100			400			588	3.12	1.55	6.28	3.24	1.686	0.640	0.032	0.977	3.95	3.69
S3-1	2	8/29/06	1500			600			1176	6.94	3.46	8.53	4.37	0.905	0.712	0.105	2.466	4.49	4.37
S3-2	2	8/29/06	2100			600			856	3.15	1.57	7.00	3.60	2.025	0.700	0.059	2.107	3.81	3.75
S3-3	2	8/29/06	2300			400			818	2.98	1.48	6.57	3.39	1.908	0.508	0.040	0.602	3.09	2.91
S4-1	2	8/29/06	0			300			659	3.83	1.91	6.76	3.49	1.581	0.633	0.079	1.344	3.70	3.45
S4-2	2	8/29/06	0			0			518	3.14	1.57	6.15	3.09	1.516	0.415	0.070	0.755	3.06	2.52
S4-3	2	8/29/06	0			100			523	3.01	1.50	5.10	2.66	1.155	0.367	0.042	0.511	2.55	2.28
S5-1	2	8/29/06	0			0			799	3.59	1.79	6.67	3.45	1.656	1.443	0.215	2.635	5.75	4.69
S5-2	2	8/29/06	700			200			577	3.34	1.67	5.92	3.06	1.393	1.660	0.047	1.817	4.57	3.95
S5-3	2	8/29/06	0			500			594	2.57	1.28	5.79	3.01	1.723	0.200	0.013	1.684	3.32	2.95
S6-1	2	8/29/06	0			500			844	5.94	2.97	6.26	3.23	0.261	0.749	0.065	1.705	3.53	2.96
S6-2	2	8/29/06	0			1900			578	3.80	1.89	5.84	3.03	1.141	0.634	0.032	1.536	3.23	2.69
S6-3	2	8/29/06	0			1800			577	3.61	1.81	5.70	2.95	1.147	0.600	0.020	1.872	2.59	2.16
C1-1	2	8/29/06	0			5100			332	2.65	1.30	3.78	2.00	0.702	1.224	0.132	3.614	12.35	11.00
C1-2	2	8/29/06	0			8800			279	2.37	1.13	2.57	1.40	0.264	1.103	0.115	3.566	10.62	9.89
C1-3	2	8/29/06	4500			3500			261	2.00	1.00	2.33	1.28	0.281	1.100	0.080	2.778	9.00	8.06
C2-1	2	8/29/06	100			300			343	3.21	1.60	3.68	1.94	0.346	2.421	0.158	4.355	8.50	8.44
C2-2	2	8/29/06	0			700			284	3.00	1.48	3.60	1.90	0.420	0.855	0.110	3.398	7.46	7.40
C2-3	2	8/29/06	0			1200			230	2.54	1.26	2.76	1.48	0.218	0.300	0.067	3.300	6.18	5.10
C3-1	2	8/29/06	0			200			273	2.80	1.40	3.53	1.88	0.476	1.008	0.284	2.366	5.38	4.18
C3-2	2	8/29/06	100			400			283	2.69	1.30	2.84	1.53	0.232	0.712	0.129	2.025	4.76	3.94
C3-3	2	8/29/06	0			0			286	2.54	1.22	2.75	1.49	0.271	0.700	0.054	1.611	3.83	3.35
C4-1	2	8/29/06	0			400			349	2.88	1.44	5.26	3.74	2.303	1.122	0.158	2.872	5.27	4.78
C4-2	2	8/29/06	0			700			282	2.26	1.08	2.48	1.36	0.278	1.009	0.140	2.655	4.82	4.32
C4-3	2	8/29/06	0			100			277	2.11	1.04	2.20	1.10	0.056	1.000	0.077	2.008	4.53	3.66

Sample No.	Simu-lation	Date	*E. coli* TC	*E. coli* PC	*E. coli* PAF	ENT TC	ENT PC	ENT PAF	TSS	TDP	DOP	TP	TOP	SOP	Nitrate	Ammonia	TKN	TOC	DOC
C5-1	2	8/29/06	100			900			503	2.90	1.45	4.10	2.08	0.630	1.335	0.128	4.533	9.81	7.96
C5-2	2	8/29/06	0			1100			456	2.73	1.36	3.80	1.91	0.550	1.277	0.120	4.225	8.81	7.35
C5-3	2	8/29/06	0			900			412	2.70	1.35	3.73	1.98	0.628	1.200	0.072	3.921	7.78	6.72
C6-1	2	8/29/06	0			100			767	0.032	0.009	0.052	0.033	0.024	0.039	0.014	0.027	5.69	4.65
C6-2	2	8/29/06	0			0			651	0.026	0.011	0.032	0.020	0.009	0.022	0.004	0.025	4.17	2.90
C6-3	2	8/29/06	0			0			333	0.016	0.006	0.023	0.016	0.010	0.007	0.003	0.010	2.82	2.72

Table D.2. Box Plot study preferential attachment to particulates

Sample no.	Soil	Screen Size	*E.coli* cfu/mg	Enterococci cfu/mg	Sample no.	Soil	Screen Size	*E.coli* cfu/mg	Enterococci cfu/mg	Sample no.	Soil	Screen Size	*E.coli* cfu/mg	Enterococci cfu/mg
PF1-1	Grosclose	500	2505	8	C1-1	Levy	500			S2-1	Eunola	500	21687	65
	Grosclose	63	86050	245		Levy	63	5600434			Eunola	63	866523	2124
	Grosclose	8	137695	233		Levy	8	701587	1187		Eunola	8	425000	719
PF1-2	Grosclose	500	12852	27	C1-2	Levy	500	192990	401	S2-2	Eunola	500		
	Grosclose	63	24779			Levy	63	585774			Eunola	63	52790	1367
	Grosclose	8	327174	538		Levy	8	2543662	4179		Eunola	8	832258	
PF1-3	Grosclose	500	7735	22	C1-3	Levy	500	15124	43	S2-3	Eunola	500	6196	17
	Grosclose	63		261		Levy	63		6390		Eunola	63		549
	Grosclose	8	1756731	1301		Levy	8	7612500	5639		Eunola	8	4567500	3383
PF2-1	Grosclose	500		11	C2-1	Levy	500		145	S3-1	Eunola	500		14
	Grosclose	63		431		Levy	63		6359		Eunola	63		791
	Grosclose	8	242888	2619		Levy	8	2176471	23470		Eunola	8	1681818	18136
PF2-2	Grosclose	500		16	C2-2	Levy	500		126	S3-2	Eunola	500		
	Grosclose	63		178		Levy	63		4595		Eunola	63		210
	Grosclose	8	97107	816		Levy	8	510870	4291		Eunola	8	252688	2123
PF2-3	Grosclose	500		30	C2-3	Levy	500		443	S3-3	Eunola	500		16
	Grosclose	63	67523	408		Levy	63	625316	3777		Eunola	63	87858	531
	Grosclose	8	615811	2421		Levy	8	4481325	17614		Eunola	8	1660491	6527
PF3-1	Grosclose	500			C3-1	Levy	500			S4-1	Eunola	500		
	Grosclose	63				Levy	63				Eunola	63		
	Grosclose	8		521		Levy	8		840		Eunola	8		1067
PF3-2	Grosclose	500		15	C3-2	Levy	500		189	S4-2	Eunola	500		48
	Grosclose	63	80402	232		Levy	63	1656363	4789		Eunola	63	187961	543
	Grosclose	8	573504	479		Levy	8	912925	763		Eunola	8	912925	763
PF3-3	Grosclose	500		74	C3-3	Levy	500		571	S4-3	Eunola	500		116
	Grosclose	63	32051	83		Levy	63	483425	1257		Eunola	63	154867	403
	Grosclose	8	295359	554		Levy	8	979021	1836		Eunola	8	654206	1227
PF4-1	Grosclose	500	28704	50	C4-1	Levy	500	81207	141	S5-1	Eunola	500	7112	12
	Grosclose	63	79476	159		Levy	63	139144	278		Eunola	63	8844	18
	Grosclose	8	540323	470		Levy	8	1736280	1512		Eunola	8	2093750	1823

Sample no.	Soil	Screen Size	*E.coli* cfu/mg	Enterococci cfu/mg	Sample no.	Soil	Screen Size	*E.coli* cfu/mg	Enterococci cfu/mg	Sample no.	Soil	Screen Size	*E.coli* cfu/mg	Enterococci cfu/mg
PF4-2	Grosclose	500	8527	28	C4-2	Levy	500	85140	275	S5-2	Eunola	500	9117	29
	Grosclose	63	19952	65		Levy	63	308320	1005		Eunola	63	39421	129
	Grosclose	8	225377			Levy	8	891060			Eunola	8	672750	
PF4-3	Grosclose	500		44	C4-3	Levy	500		157	S5-3	Eunola	500		36
	Grosclose	63	24222	91		Levy	63	373180	1408		Eunola	63	38053	144
	Grosclose	8	171429	433		Levy	8	752239	1901		Eunola	8	586047	1481
PF5-1	Grosclose	500	1685	20	C5-2	Levy	500	15847	185	S6-1	Eunola	500	3133	37
	Grosclose	63	29149	142		Levy	63	206422	1008		Eunola	63	19550	95
	Grosclose	8		266		Levy	8		2159		Eunola	8		1378
PF5-2	Grosclose	500	1256	27	C5-3	Levy	500	13617	295	S6-2	Eunola	500	1929	42
	Grosclose	63	72370	206		Levy	63	1534462	4376		Eunola	63	278840	795
	Grosclose	8	417783	349		Levy	8	2319231	1938		Eunola	8	1330147	1112
PF5-3	Grosclose	500		15						S6-3	Eunola	500		26
	Grosclose	63	96077	210							Eunola	63	120336	263
	Grosclose	8	262500	199							Eunola	8	644554	489

Appendix E. Supplemental Statistical Data for Box-plot Study

E.1. Multiple Regression Analysis Details for Box Plot Study

E.1.1. E. coli partitioning coefficient: full model

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	3.89986	0.35453	5.11	0.0003
Error	27	1.87353	0.06939		
Corrected Total	38	5.77339			

Root MSE	0.26342	R-Square	0.6755
Dependent Mean	0.47057	Adj R-Sq	0.5433
Coeff Var	55.97944		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Variance Inflation
Intercept	Intercept	1	-11.76438	3.26301	-3.61	0.0012	0
Diss_Organic_C	Diss_Organic_C	1	0.81134	0.20964	3.87	0.0006	44.46646
Total_Organic_C	Total_Organic_C	1	-0.06723	0.01638	-4.10	0.0003	3.82665
z1		1	21.11521	3.88105	5.44	<.0001	1803.35034
TSS_z1		1	0.00015222	0.00004756	3.20	0.0035	9.34441
Total_P_z1		1	-0.48864	0.14168	-3.45	0.0019	251.79771
Diss_P_z1		1	-0.27794	0.14482	-1.92	0.0656	39.72078
Diss_P_z2		1	-3.73534	1.28059	-2.92	0.0070	11899
Total_Organic_P_z1		1	0.45554	0.22877	1.99	0.0567	170.47614
Total_Organic_P_z2		1	-0.31962	0.08348	-3.83	0.0007	64.85859
Diss_Organic_P_z2		1	8.39783	2.75740	3.05	0.0051	13804
Diss_Organic_C_z1		1	-1.15476	0.24279	-4.76	<.0001	1363.63162

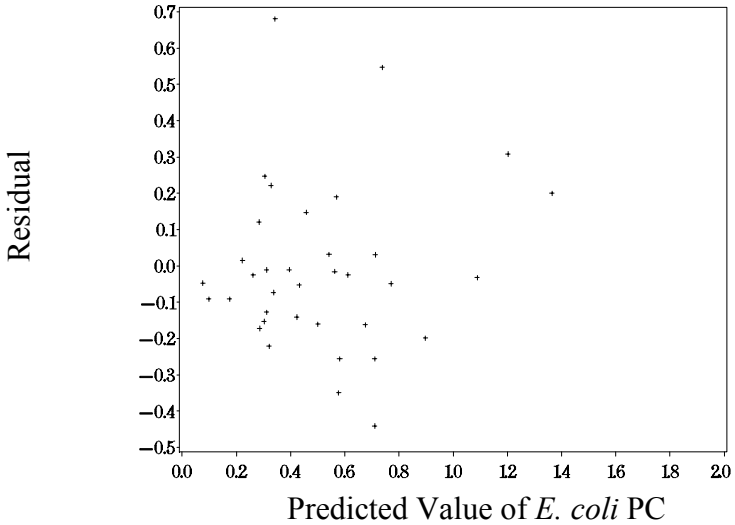


Figure E.1. Residual plots of predicted *E. coli* partitioning coefficient concentration for all three soil types.

E.1.2. Enterococci partitioning coefficient: full model

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	21.79170	1.98106	22.81	<.0001
Error	29	2.51916	0.08687		
Corrected Total	40	24.31086			

Root MSE	0.29473	R-Square	0.8964
Dependent Mean	0.81827	Adj R-Sq	0.8571
Coeff Var	36.01886		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Variance Inflation
Intercept	Intercept	1	-0.94187	0.38796	-2.43	0.0216	0
TSS	TSS	1	0.00077745	0.00016359	4.75	<.0001	56.46735
z1		1	18.57816	2.15747	8.61	<.0001	475.71612
z2		1	1.26955	0.48286	2.63	0.0135	24.74545
TSS_z1		1	-0.00063635	0.00017058	-3.73	0.0008	100.59880
TSS_z2		1	-0.00075304	0.00017777	-4.24	0.0002	59.48931
Total_P_z1		1	-1.01389	0.15192	-6.67	<.0001	262.56041
P_ratio_z1		1	-1.37963	0.54953	-2.51	0.0179	104.43648
Total_Organic_P_z1		1	0.76631	0.25566	3.00	0.0055	188.24882
Organic_P_ratio_z1		1	2.36197	0.55850	4.23	0.0002	114.73770
Total_Organic_C_z1		1	-0.90573	0.16996	-5.33	<.0001	781.11498
Organic_C_ratio_z1		1	14.44640	2.97406	4.86	<.0001	40.27882

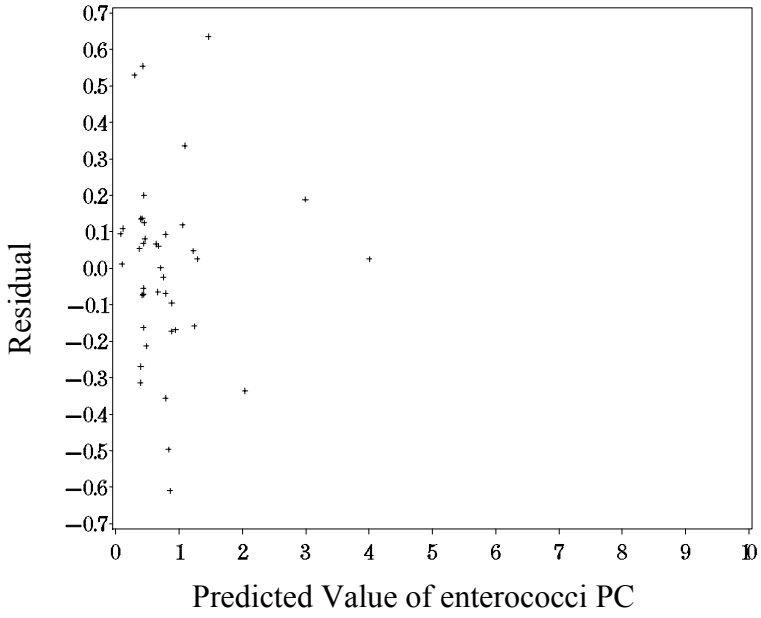


Figure E.2. Residual plots of predicted enterococci partitioning coefficient concentration for all three soil types.

E.1.3. ln E. coli total concentration: full model

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	6816.71571	1363.34314	223.28	<.0001
Error	83	506.78682	6.10587		
Corrected Total	88	7323.50253			

Root MSE	2.47100	R-Square	0.9308
Dependent Mean	10.33297	Adj R-Sq	0.9266
Coeff Var	23.91378		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Variance Inflation
Intercept	Intercept	1	-6.92961	0.78127	-8.87	<.0001	0
Total_P	Total_P	1	0.32350	0.10077	3.21	0.0019	3.55228
Diss_Organic_C	Diss_Organic_C	1	1.19672	0.06574	18.20	<.0001	2.20862
z2		1	5.33296	1.27157	4.19	<.0001	5.26642
Total_P_z2		1	-0.22609	0.11400	-1.98	0.0506	7.83461
Total_Organic_C_z1		1	0.42762	0.04897	8.73	<.0001	1.29645

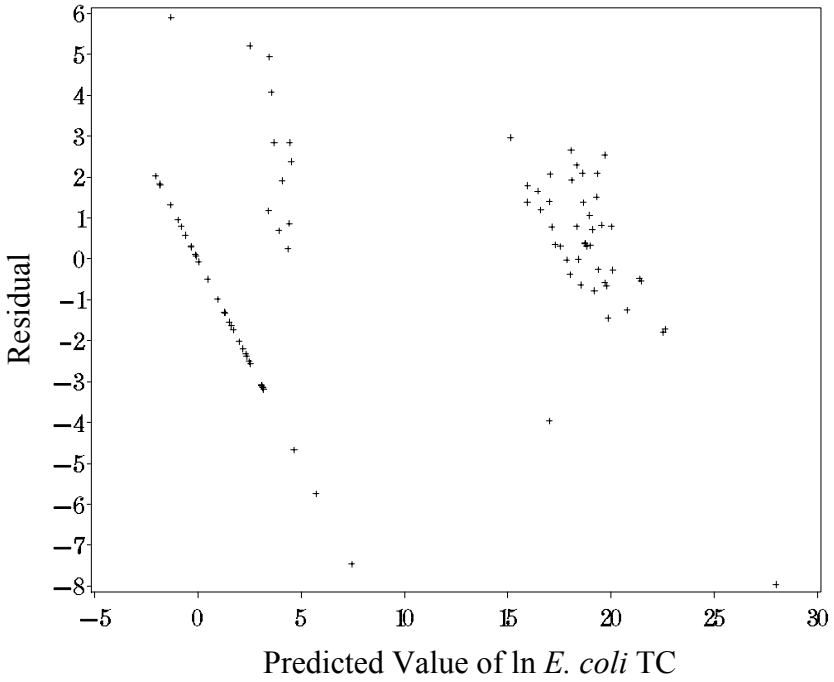


Figure E.3. Residual plots of predicted ln *E. coli* total concentration for all three soil types.

E.1.4. ln enterococci total concentration: full model

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	3224.40887	403.05111	135.16	<.0001
Error	80	238.55535	2.98194		
Corrected Total	88	3462.96422			

Root MSE	1.72683	R-Square	0.9311
Dependent Mean	9.46543	Adj R-Sq	0.9242
Coeff Var	18.24355		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Variance Inflation
Intercept	Intercept	1	0.97240	0.59198	1.64	0.1044	0
Diss_Organic_C	Diss_Organic_C	1	0.90990	0.04907	18.54	<.0001	2.52018
z1		1	-4.65642	0.79719	-5.84	<.0001	4.23841
Total_P_z2		1	-3.40860	1.37297	-2.48	0.0151	2326.92077
Total_Organic_P_z2		1	6.82788	2.88599	2.37	0.0204	2525.31741
Diss_Organic_P_z2		1	1.21106	0.61796	1.96	0.0535	25.94652
Total_Organic_C_z1		1	0.33689	0.06175	5.46	<.0001	4.22125
Total_Organic_C_z2		1	0.50089	0.17706	2.83	0.0059	73.38727
Diss_Organic_C_z2		1	-1.10629	0.31148	-3.55	0.0006	99.74499

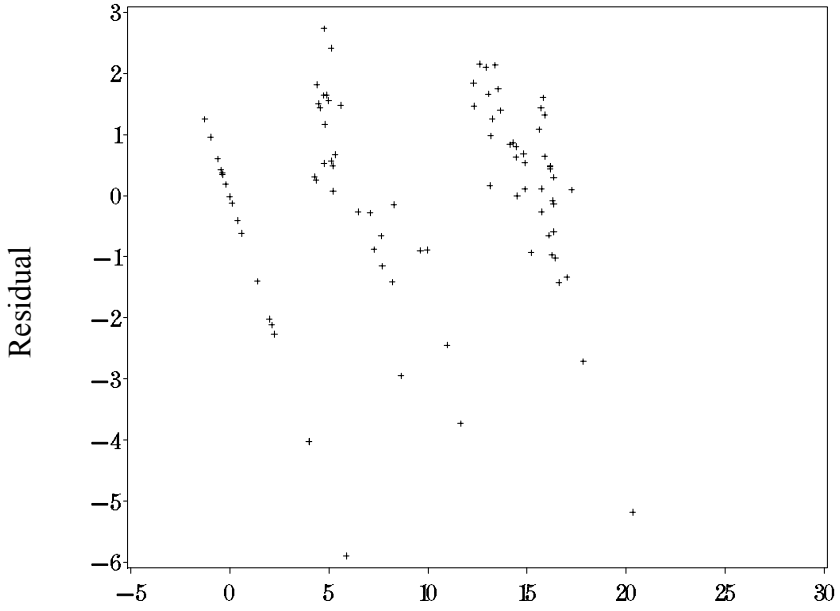


Figure E.4. Residual plots of predicted ln enterococci total concentration concentration for all three soil types.

E.2. Bacterial attachment between soils for each particle size

Table E.1. Bacterial attachment between soils for each particle size. P values showing statistically significant differences between *E. coli* and enterococci associated with sediments retained by the three particle categories, 500 µm, 63 µm, and 8 µm (for each soil type) and also comparing differences between E. coli and enterococci attached to different soil types, Eunola loamy fine sand, Grosclose silt loam, and Levy silty clay loam (for each particle categories).

Soil type	Particle Size µm	*E. coli* *P* value	enterococci *P* value
Eunola loamy fine sand	>500 - 63	< 0.0001	< 0.0001
	63 - 8	< 0.0001	0.0001
	8 - 500	< 0.0001	< 0.0001
Grosclose silt loam	>500 - 63	0.0002	< 0.0001
	63 - 8	0.0001	0.0235
	8 - 500	< 0.0001	< 0.0001
Levy silty clay loam	>500 - 63	< 0.0001	< 0.0001
	63 - 8	0.3844	0.9994
	8 - 500	< 0.0001	< 0.0001
Particle Size	**Soil type**	***E. coli*** ***P* value**	**enterococci *P* value**
>500 µm	Eunola - Grosclose	1.0000	0.9956
	Grosclose - Levy	0.0065	< 0.0001
	Eunola - Levy	0.0215	< 0.0001
63 µm	Eunola - Grosclose	0.9180	0.6826
	Grosclose - Levy	< 0.0001	< 0.0001
	Eunola - Levy	< 0.0001	< 0.0001
8 µm	Eunola - Grosclose	0.1027	0.0384
	Grosclose - Levy	0.0027	0.0003
	Eunola - Levy	0.9340	0.8874

Appendix F. Transport Plot Study Data: Well Manged Pastureland

Table F.1. Transport Plot Study Bacteria, TSS, and Nutrient Results

Sample No.	Time after start of runoff (min)	Runoff volume	Q (cfs)	*E. coli* TC (cfu/100 mL)	*E. coli* PC	*E. coli* PAF	ENT TC (cfu/100 mL)	ENT PC	ENT PAF	TSS (mg/L)	TDP (mg/L)	TP (mg/L)	DOP (mg/L)	TOP (mg/L)	DOC (mg/L)	TOC (mg/L)
P2-1	0	0.00060	0.00001	2100000	0.002	0.002	800000	0.033	0.032	579	3.64	3.90	1.82	1.95	15.40	15.40
P2-2	6	0.01604	0.00004	1800000	0.028	0.027	200000	0.582	0.368	341	3.50	3.86	1.75	1.93	16.60	16.70
P2-3	16	0.62646	0.00104	2100000	0.008	0.008	300000	0.085	0.079	193	3.37	3.84	1.68	1.92	16.30	16.40
P2-4	26	1.48625	0.00248	1500000	0.039	0.037	100000	0.047	0.045	165	3.20	3.71	1.58	1.86	15.80	16.50
P2-5	36	1.95498	0.00326	1800000	0.017	0.016	111000	0.079	0.073	111	3.18	3.69	1.66	1.87	16.50	16.50
P2-6	46	2.15220	0.00359	1500000	0.056	0.053	1400000	0.113	0.102	93	2.82	3.60	1.41	1.80	14.80	15.70
P2-7	56	2.35744	0.00393	1200000	0.044	0.042	200000	0.035	0.034	100	2.60	3.65	1.29	1.82	15.30	16.30
P2-8	66	2.76482	0.00461	2000000	0.010	0.010	1000	0.039	0.037	78	2.54	3.60	1.27	1.80	14.90	15.70
P2-9	76	2.96524	0.00494	900000	0.024	0.023	1500000	0.003	0.003	87	2.37	3.59	1.19	1.87	15.70	15.90
P2-10	83	2.96524	0.00494	1600000	0.026	0.026	2200000	0.010	0.010	79	2.70	3.20	1.51	1.58	14.40	16.00
P2-11	88	0.22742	0.00076	600000	0.001	0.001	1100000	0.194	0.162	60	2.67	3.11	1.45	1.59	15.20	16.20
P3-1	0	0.03363	0.000561	1200000			700000			21	4.20	6.85	2.10	3.43	13.80	15.70
P3-2	7	0.36176	0.000861	900000	0.0117	0.0116	57000	0.208	0.172	19	3.93	5.40	1.97	2.64	15.50	16.00
P3-3	17	0.66215	0.001104	900000	0.0063	0.0063	900000	0.371	0.271	24	3.92	5.40	1.96	2.70	15.00	15.60
P3-4	27	1.05943	0.001766	200000			1000			579	3.78	5.15	1.89	2.40	14.00	15.40
P3-5	37	0.78477	0.001308	400000			49000			341	3.75	4.90	1.88	2.45	14.00	15.20
P3-6	47	1.11519	0.001859	400000	0.0238	0.0232	700000			193	3.60	4.80	1.80	2.37	13.70	14.60
P3-7	57	1.11519	0.001859	700000	0.0021	0.0021	44000			165	3.46	3.51	1.73	1.75	13.70	14.50
P3-8	64	1.11519	0.001859	500000	0.0012	0.0012	1100000	0.118	0.105	111	3.08	3.38	1.54	1.68	12.20	14.70
P3-9	69	0.09000	0.0003	400000	0.0523	0.0497	34000			93	3.60	3.95	1.80	1.97	12.80	15.30
P4-1	0	0.03363	0.000561	1200000	0.0141	0.0139	1200000			100	5.10	7.40	2.55	3.70	16.90	16.90
P4-2	4	0.20672	0.000861	300000	0.0850	0.0783	400000	0.473	0.321	78	4.50	5.95	2.25	2.99	16.60	16.80
P4-3	14	0.56511	0.0009	200000	0.0008	0.0008	68000	0.018	0.018	87	3.69	5.95	1.85	3.01	16.10	16.40
P4-4	24	0.61342	0.0010	300000	0.0109	0.0108	57000	0.851	0.460	79	3.60	5.40	1.82	2.71	13.90	16.40
P4-5	34	0.87643	0.0015	300000	0.0242	0.0237	52000	0.011	0.011	60	3.72	4.50	1.91	2.30	14.10	14.40
P4-6	44	0.97697	0.0016	300000	0.0359	0.0346	78000	0.099	0.090	533	3.56	3.86	1.85	1.93	14.50	14.80
P4-7	51	0.97697	0.0016	100000	0.0066	0.0066	53000	0.006	0.006	143	3.45	3.59	1.75	1.83	14.70	15.10
P4-8	56	0.09000	0.0003	93000	0.0278	0.0271	49000	0.005	0.005	38	3.41	3.50	1.69	1.75	13.60	15.60

Sample #	Time after start of runoff (min)	Runoff volume	Q (cfs)	*E. coli* TC (cfu/100 mL)	*E. coli* PC	*E. coli* PAF	ENT TC (cfu/100 mL)	ENT PC	ENT PAF	TSS (mg/L)	TDP (mg/L)	TP (mg/L)	DOP (mg/L)	TOP (mg/L)	DOC (mg/L)	TOC (mg/L)
P5-1	0	0.01461	0.000244	200000	0.5218	0.3429	90000	0.458	0.314	67	2.62	5.30	1.31	2.64	16.20	17.10
P5-2	6	0.27016	0.0008	700000	0.1430	0.1251	56000	0.144	0.126	36	1.29	4.50	0.65	2.25	15.90	16.30
P5-3	16	0.75440	0.0013	600000	0.2123	0.1751	61000	0.016	0.015	46	2.07	4.30	1.05	2.14	9.91	16.30
P5-4	26	1.30500	0.0022	700000	0.0436	0.0418	60000	0.148	0.129	44	0.76	3.90	0.46	1.96	15.70	16.10
P5-5	36	1.66989	0.0028	300000	0.2310	0.1876	60000	0.212	0.175	41	0.35	3.67	0.18	1.84	16.00	16.30
P5-6	43	1.04983	0.0017	600000	0.0303	0.0294	65000	0.499	0.333	241	0.28	3.25	0.15	2.63	15.20	16.20
P5-7	48	0.42377	0.001413	400000	0.0935	0.0855	51000	0.152	0.132	171	0.26	3.21	0.13	2.61	14.90	16.60

Table F.2. Transport plot study preferential attachment to particulates

Sample no.	Screen Size	*E.coli* cfu/mg	Enterococci cfu/mg	Sample no.	Screen Size	*E.coli* cfu/mg	Enterococci cfu/mg	Sample no.	Screen Size	*E.coli* cfu/mg	Enterococci cfu/mg	Sample no.	Screen Size	*E.coli* cfu/mg	Enterococci cfu/mg
P2-1	500	2325	525	P3-1	500	433	674	P4-1	500		20	P5-1	500	1904	
	63	29	1167		63	117	190		63	87	127		63	270	450
	8	1045	591		8	2082	1388		8		991		8	1080	170
P2-2	500	90	76	P3-2	500		80	P4-2	500			P5-2	500	700	280
	63	147	295		63	498	208		63				63	469	
	8	655	2531		8	525	875		8				8	3321	515
P2-3	500			P3-3	500			P4-3	500			P5-3	500		
	63		1150		63	552	828		63				63	1522	174
	8	1187	687		8				8				8	4541	
P2-4	500			P3-4	500			P4-4	500			P5-4	500		
	63	180	2160		63				63	360	600		63	1091	764
	8	1708	2733		8				8		113		8	568	1364
P2-5	500			P3-5	500			P4-5	500			P5-5	500	1635	102
	63		660		63				63				63	60	148
	8				8				8				8	4992	588
P2-6	500			P3-6	500			P4-6	500			P5-6	500	187	
	63				63				63	584	531		63	59	76
	8				8				8	153			8	1808	1154
P2-7	500			P3-7	500			P4-7	500			P5-7	500		
	63				63				63	159	265		63	1021	333
	8				8				8				8	682	375
P2-8	500			P3-8	500			P4-8	500						
	63				63				63						
	8				8				8						
P2-9	500			P3-9	500										
	63	667	583		63	763	678								
	8				8	2140	2345								
P2-10	500	383													
	63	27	135												
	8														
P2-11	500														
	63														
	8														

Appendix G. Supplemental Statistical Data for Transport Plot Study: Well Managed Pastureland

G.1. Bacterial partitioning related to flow regime

G.1.1. E. coli Total Concentrations

G.1.1.1. Test for Normality

Tests for Normality (rising)

Test	--Statistic---		-----p Value------	
Shapiro-Wilk	W	0.913976	Pr < W	0.0876
Kolmogorov-Smirnov	D	0.136102	Pr > D	>0.1500
Cramer-von Mises	W-Sq	0.066848	Pr > W-Sq	>0.2500
Anderson-Darling	A-Sq	0.4966	Pr > A-Sq	0.1958

```
Stem Leaf                     #  Boxplot                        Normal Probability Plot
   2 11                       2     |     2250000+                                  * ++*++++
   1 5588                     4  +-----+         |                              * *+*+++++++
   1 222                      3  |  +  |  1250000+                         +*+*+++
   0 6799                     4  *-----*         |                    ++++*+*
   0 222334                   6  +-----+   250000+        *  +*+++*+*+  *
     ----+----+----+----+                        +----+----+----+----+----+----+----+----+----+----+
 Multiply Stem.Leaf by 10**+6                         -2        -1         0        +1        +2
```

Tests for Normality (peak)

Test	--Statistic---		-----p Value------	
Shapiro-Wilk	W	0.771755	Pr < W	0.0066
Kolmogorov-Smirnov	D	0.241242	Pr > D	0.0959
Cramer-von Mises	W-Sq	0.145271	Pr > W-Sq	0.0227
Anderson-Darling	A-Sq	0.907768	Pr > A-Sq	0.0136

```
Stem Leaf                     #  Boxplot                        Normal Probability Plot
   2 0                        1     *     2250000+                                    *
   1                                      |                                       +++++++++
   1                                   1250000+                              +++++++++
   0 5779                     4  +--+--+         |                      +++*++*+++  *
   0 13334                    5  *-----*   250000+          *   +*++++++* *
     ----+----+----+----+                        +----+----+----+----+----+----+----+----+----+----+
 Multiply Stem.Leaf by 10**+6                         -2        -1         0        +1        +2
```

Tests for Normality (recession)

Test		--Statistic---	-----p Value------	
Shapiro-Wilk	W	0.814108	Pr < W	0.0784
Kolmogorov-Smirnov	D	0.345296	Pr > D	0.0237
Cramer-von Mises	W-Sq	0.113835	Pr > W-Sq	0.0561
Anderson-Darling	A-Sq	0.618914	Pr > A-Sq	0.0570

```
Stem Leaf                     #  Boxplot                        Normal Probability Plot
  16 0                        1     *     1700000+                                  *      +++
  14                                        |                                        ++++
  12                                        |                                    ++++
  10                                        |                                ++++
   8                                 900000+                            ++++
   6 00                       2  +--+--+     |                       ++*+  *
   4 00                       2  *-----*     |                   * ++*+
   2                                         |                ++++
   0 9                        1     0      100000+          * +++
     ----+----+----+----+                        +----+----+----+----+----+----+----+----+----+----+
 Multiply Stem.Leaf by 10**+5                        -2        -1         0        +1        +2
```

G.1.1.2. Kruskal-Wallis rank test

Wilcoxon Scores (Rank Sums) for Variable Ecoli_total
Classified by Variable stage

stage	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
peak	10	149.50	180.0	27.284295	14.950000
recession	6	90.00	108.0	22.762364	15.000000
rising	19	390.50	342.0	30.087045	20.552632

Average scores were used for ties.

Kruskal-Wallis Test

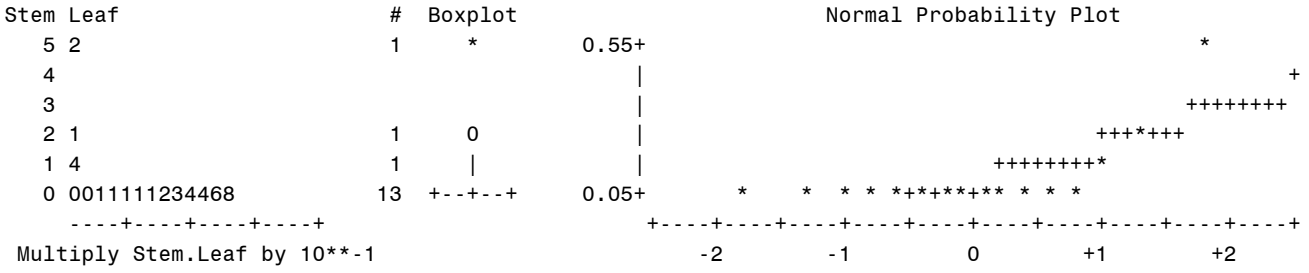
Chi-Square	2.5986
DF	2
Pr > Chi-Square	0.2727

G.1.2. E. coli Partitioning Coefficient

G.1.2.1. Test for Normality

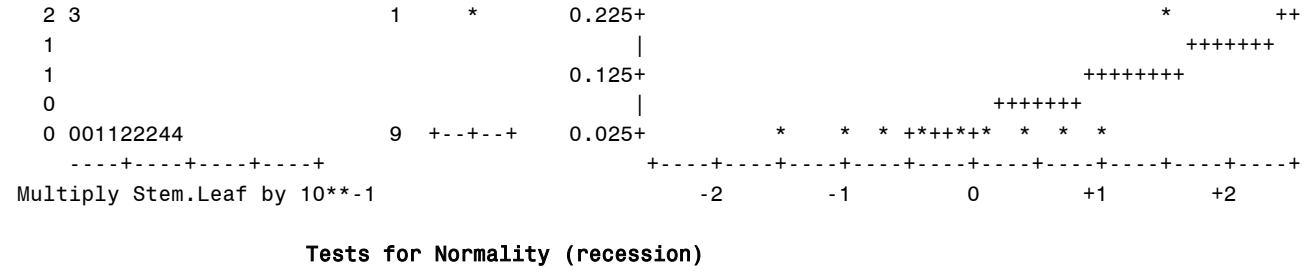
Tests for Normality (rising)

Test		--Statistic---	-----p Value------
Shapiro-Wilk	W	0.587744	Pr < W <0.0001
Kolmogorov-Smirnov	D	0.3075	Pr > D <0.0100
Cramer-von Mises	W-Sq	0.463588	Pr > W-Sq <0.0050
Anderson-Darling	A-Sq	2.497252	Pr > A-Sq <0.0050



Tests for Normality (peak)

Test		--Statistic---	-----p Value------
Shapiro-Wilk	W	0.558008	Pr < W <0.0001
Kolmogorov-Smirnov	D	0.380333	Pr > D <0.0100
Cramer-von Mises	W-Sq	0.355614	Pr > W-Sq <0.0050
Anderson-Darling	A-Sq	1.901796	Pr > A-Sq <0.0050



Tests for Normality (recession)

Test		--Statistic---	-----p Value------
Shapiro-Wilk	W	0.906249	Pr < W 0.4122
Kolmogorov-Smirnov	D	0.27014	Pr > D >0.1500
Cramer-von Mises	W-Sq	0.070428	Pr > W-Sq 0.2365
Anderson-Darling	A-Sq	0.390274	Pr > A-Sq >0.2500

```
Stem Leaf                     #  Boxplot                        Normal Probability Plot
   8 3                        1     0        0.09+                                  *++++++
   6                                |                                        +++++++
   4 2                        1  +-----+     0.05+                          +++++*
   2 680                      3  *--+--*         |                   *+++*++ *
   0 1                        1     |        0.01+            *+++++++
     ----+----+----+----+                      +----+----+----+----+----+----+----+----+----+----+
 Multiply Stem.Leaf by 10**-2                      -2        -1         0        +1        +2
```

G.1.2.2. Kruskal-Wallis rank test

Wilcoxon Scores (Rank Sums) for Variable Ecoli_ratio
Classified by Variable stage

stage	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
peak	10	144.0	165.0	24.596748	14.400000
recession	6	110.0	99.0	20.712315	18.333333
rising	16	274.0	264.0	26.532998	17.125000

Kruskal-Wallis Test

Chi-Square	0.8013
DF	2
Pr > Chi-Square	0.6699

G.1. 3. E. coli Particulate Attached Fraction

G.1.3.1. Test for Normality

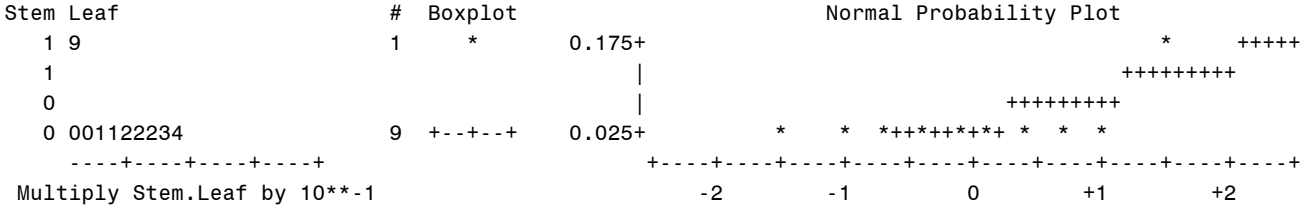
Tests for Normality (rising)

Test		--Statistic---	-----p Value------	
Shapiro-Wilk	W	0.667502	Pr < W	<0.0001
Kolmogorov-Smirnov	D	0.279195	Pr > D	<0.0100
Cramer-von Mises	W-Sq	0.366147	Pr > W-Sq	<0.0050
Anderson-Darling	A-Sq	1.994573	Pr > A-Sq	<0.0050

```
Stem Leaf                     #  Boxplot                        Normal Probability Plot
   3 4                        1     *       0.325+                                    *
   2                                |                                              ++++
   2                                |                                        ++++++
   1 8                        1     0       0.175+                              ++*++
   1 3                        1     |           |                          ++++++*
   0 58                       2  +--+--+        |                     ++++++ * *
   0 00111112344             11  *-----*    0.025+       *    *  * * *+*+** ** *
     ----+----+----+----+                      +----+----+----+----+----+----+----+----+----+----+
 Multiply Stem.Leaf by 10**-1                      -2        -1         0        +1        +2
```

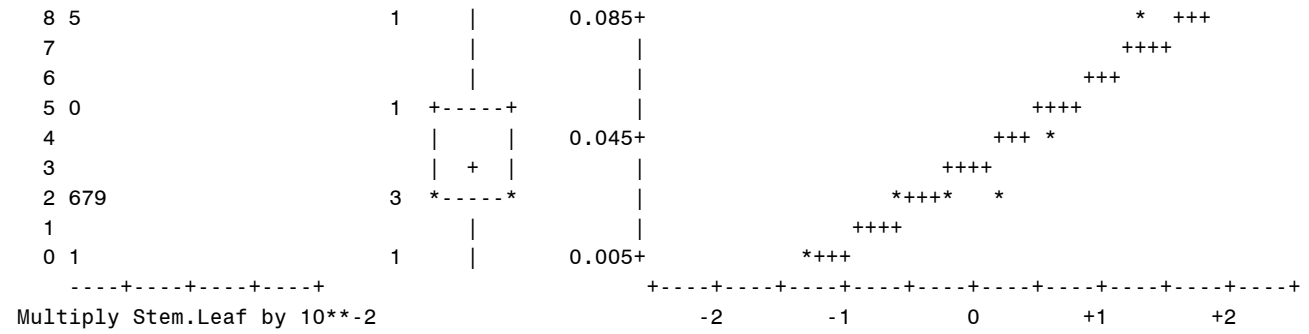
Tests for Normality (peak)

Test		--Statistic---	-----p Value------	
Shapiro-Wilk	W	0.595248	Pr < W	<0.0001
Kolmogorov-Smirnov	D	0.353986	Pr > D	<0.0100
Cramer-von Mises	W-Sq	0.309159	Pr > W-Sq	<0.0050
Anderson-Darling	A-Sq	1.694964	Pr > A-Sq	<0.0050



Tests for Normality (recession)

Test		--Statistic---	-----p Value------	
Shapiro-Wilk	W	0.917888	Pr < W	0.4903
Kolmogorov-Smirnov	D	0.263226	Pr > D	>0.1500
Cramer-von Mises	W-Sq	0.066078	Pr > W-Sq	>0.2500
Anderson-Darling	A-Sq	0.364246	Pr > A-Sq	>0.2500



G.1.3.2. Kruskal-Wallis rank test

Wilcoxon Scores (Rank Sums) for Variable Ecoli_percent
Classified by Variable stage

stage	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
peak	10	144.0	165.0	24.596748	14.400000
recession	6	110.0	99.0	20.712315	18.333333
rising	16	274.0	264.0	26.532998	17.125000

Kruskal-Wallis Test

Chi-Square	0.8013
DF	2
Pr > Chi-Square	0.6699

G.1.4. Enterococci Total Concentrations

G.1.4.1. Test for Normality

Tests for Normality (rising)

Test	--Statistic---		-----p Value------	
Shapiro-Wilk	W	0.764404	Pr < W	0.0004
Kolmogorov-Smirnov	D	0.272668	Pr > D	<0.0100
Cramer-von Mises	W-Sq	0.355526	Pr > W-Sq	<0.0050
Anderson-Darling	A-Sq	1.897448	Pr > A-Sq	<0.0050

```
Stem Leaf                     #  Boxplot                        Normal Probability Plot
  14 0                        1     |     1500000+                                    *      +
  12 0                        1     |            |                                 *     +++++
  10                              |            |                                   ++++
   8 00                       2     |            |                              * *++++
   6 0                        1  +-----+         |                            +*+++
   4 0                        1  |     |         |                         +++*
   2 000                      3  |  +  |         |                      ++++* **
   0 0566667901              10  *-----*   100000+       *   *  * *+++*** **
     ----+----+----+----+                        +----+----+----+----+----+----+----+----+----+----+
 Multiply Stem.Leaf by 10**+5                        -2        -1         0        +1        +2
```

Tests for Normality (peak)

Test	--Statistic---		-----p Value------	
Shapiro-Wilk	W	0.6897	Pr < W	0.0007
Kolmogorov-Smirnov	D	0.401786	Pr > D	<0.0100
Cramer-von Mises	W-Sq	0.295291	Pr > W-Sq	<0.0050
Anderson-Darling	A-Sq	1.513677	Pr > A-Sq	<0.0050

```
Stem Leaf                     #  Boxplot                        Normal Probability Plot
  14 0                        1     |     1500000+                                    *    +++
  12                                |            |                                     ++++
  10 0                        1     |            |                                  * ++++
   8                                |            |                                 +++
   6 0                        1  +-----+         |                              ++*+
   4                             |     |         |                           ++++
   2                             |  +  |         |                        ++++
   0 0455668                  7  *-----*   100000+           *    *  *+++*  *    *
     ----+----+----+----+                        +----+----+----+----+----+----+----+----+----+----+
 Multiply Stem.Leaf by 10**+5                        -2        -1         0        +1        +2
```

Tests for Normality (recession)

Test		--Statistic---	-----p Value------	
Shapiro-Wilk	W	0.709602	Pr < W	0.0078
Kolmogorov-Smirnov	D	0.384999	Pr > D	<0.0100
Cramer-von Mises	W-Sq	0.163412	Pr > W-Sq	0.0105
Anderson-Darling	A-Sq	0.878373	Pr > A-Sq	0.0097

```
Stem Leaf                     #  Boxplot                        Normal Probability Plot
   2 2                        1     |     2250000+                                  *  ++++++
   1                                |            |                                +++++
   1 1                        1  +-----+  1250000+                             +*++++
   0                             |  +  |         |                          +++++
   0 0011                     4  *-----*   250000+             *      *+++*+  *
     ----+----+----+----+                        +----+----+----+----+----+----+----+----+----+----+
 Multiply Stem.Leaf by 10**+6                        -2        -1         0        +1        +2
```

G.1.4.2. Kruskal-Wallis rank test

Wilcoxon Scores (Rank Sums) for Variable enterococci_total
Classified by Variable stage

stage	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
peak	10	158.50	180.0	27.372700	15.850000
recession	6	97.00	108.0	22.836117	16.166667
rising	19	374.50	342.0	30.184531	19.710526

Average scores were used for ties.

Kruskal-Wallis Test

Chi-Square	1.1629
DF	2
Pr > Chi-Square	0.5591

G.1.5. Enterococci Partitioning Coefficient

G.1.5.1. Test for Normality

Tests for Normality (rising)

Test		--Statistic---	-----p Value------	
Shapiro-Wilk	W	0.822552	Pr < W	0.0073
Kolmogorov-Smirnov	D	0.238988	Pr > D	0.0211
Cramer-von Mises	W-Sq	0.190599	Pr > W-Sq	0.0059
Anderson-Darling	A-Sq	1.058621	Pr > A-Sq	0.0065

```
Stem Leaf                     #  Boxplot                        Normal Probability Plot
   8 5                        1     |        0.85+                                    *     +++
   7                                |           |                                       ++++
   6                                |           |                                  ++++
   5 8                        1     |           |                                +*++
   4 67                       2  +-----+     0.45+                             *+*+
   3 7                        1  |     |         |                          ++*+
   2 1                        1  |  +  |         |                       ++++*
   1 14                       2  *-----*         |                    ++++ * *
   0 2234589                  7  +-----+     0.05+        *    *  *+*+* **
     ----+----+----+----+                          +----+----+----+----+----+----+----+----+----+----+
 Multiply Stem.Leaf by 10**-1                          -2        -1         0        +1        +2
```

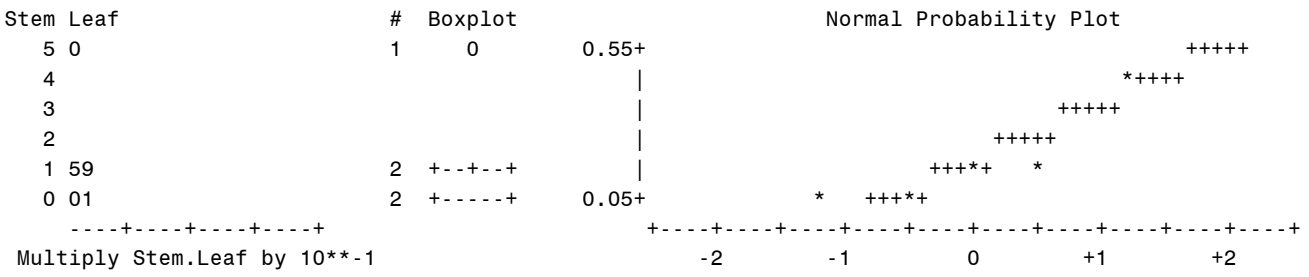
Tests for Normality (peak)

Test		--Statistic---	-----p Value------	
Shapiro-Wilk	W	0.897566	Pr < W	0.2747
Kolmogorov-Smirnov	D	0.200626	Pr > D	>0.1500
Cramer-von Mises	W-Sq	0.056962	Pr > W-Sq	>0.2500
Anderson-Darling	A-Sq	0.371476	Pr > A-Sq	>0.2500

```
Stem Leaf                     #  Boxplot                        Normal Probability Plot
   2 1                        1     |       0.225+                                  * +++++++
   1 5                        1     |           |                                 ++++++
   1 02                       2  +-----+    0.125+                           ++*+++*
   0                             *--+--*         |                      +++++*
   0 0114                     4  +-----+    0.025+           *   +*+++*+ *
     ----+----+----+----+                          +----+----+----+----+----+----+----+----+----+----+
 Multiply Stem.Leaf by 10**-1                          -2        -1         0        +1        +2
```

Tests for Normality (recession)

Test		--Statistic---	-----p Value------	
Shapiro-Wilk	W	0.858144	Pr < W	0.2217
Kolmogorov-Smirnov	D	0.256609	Pr > D	>0.1500
Cramer-von Mises	W-Sq	0.063661	Pr > W-Sq	>0.2500
Anderson-Darling	A-Sq	0.397289	Pr > A-Sq	0.2251



G.1.5.2. Kruskal-Wallis rank test

Wilcoxon Scores (Rank Sums) for Variable enterococci_ratio
Classified by Variable stage

stage	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
peak	8	89.0	116.00	19.663842	11.1250
recession	5	71.0	72.50	16.670833	14.2000
rising	15	246.0	217.50	21.708293	16.4000

Kruskal-Wallis Test

Chi-Square	2.1536
DF	2
Pr > Chi-Square	0.3407

G.1.6. Enterococci Particulate Attached Fraction

G.1.6.1. Test for Normality

Tests for Normality (rising)

Test		--Statistic---	-----p Value------	
Shapiro-Wilk	W	0.867717	Pr < W	0.0312
Kolmogorov-Smirnov	D	0.19783	Pr > D	0.1129
Cramer-von Mises	W-Sq	0.1432	Pr > W-Sq	0.0250
Anderson-Darling	A-Sq	0.806887	Pr > A-Sq	0.0287

```
Stem Leaf                     #  Boxplot                        Normal Probability Plot
   4 6                        1     |       0.475+                                    *  ++++
   4                                |           |                                     +++
   3 7                        1     |           |                                  *++++
   3 12                       2  +-----+    0.325+                               * *+++
   2 7                        1  |     |         |                              * +++
   2                             |     |         |                            ++++
   1 7                        1  |  +  |    0.175+                          +++*
   1 03                       2  *-----*         |                       ++++* *
   0 578                      3  |     |         |                    +++ **
   0 2233                     4  +-----+    0.025+         *    *++*+* *
     ----+----+----+----+                        +----+----+----+----+----+----+----+----+----+----+
         Multiply Stem.Leaf by 10**-1                          -2        -1         0        +1
     +2
```

Tests for Normality (peak)

Test		--Statistic---	-----p Value------	
Shapiro-Wilk	W	0.90087	Pr < W	0.2942
Kolmogorov-Smirnov	D	0.192028	Pr > D	>0.1500
Cramer-von Mises	W-Sq	0.057055	Pr > W-Sq	>0.2500
Anderson-Darling	A-Sq	0.364648	Pr > A-Sq	>0.2500

```
Stem Leaf                     #  Boxplot                        Normal Probability Plot
  16 5                        1     |        0.17+                                  *+++
  14                               |           |                                 +++
  12 9                        1     |           |                              +*+
  10 5                        1  +-----+        |                           *++
   8 0                        1  |     |     0.09+                        *++
   6                             *--+--*         |                     +++
   4                             |     |         |                  +++
   2 7                        1  |     |         |               +++  *
   0 361                      3  +-----+     0.01+           *   +*+  *
     ----+----+----+----+                        +----+----+----+----+----+----+----+----+----+----+
 Multiply Stem.Leaf by 10**-2                          -2        -1         0        +1        +2
```

Tests for Normality (recession)

Test		--Statistic---	-----p Value------	
Shapiro-Wilk	W	0.89825	Pr < W	0.4003
Kolmogorov-Smirnov	D	0.211222	Pr > D	>0.1500
Cramer-von Mises	W-Sq	0.046722	Pr > W-Sq	>0.2500
Anderson-Darling	A-Sq	0.312726	Pr > A-Sq	>0.2500

```
Stem Leaf                     #  Boxplot                        Normal Probability Plot
   3 3                        1     |       0.325+                                  *++++
   2                                |           |                                 +++
   2                                |           |                              ++++
   1 6                        1  +-----+    0.175+                          +++*
   1 3                        1  *--+--*         |                       ++*+
   0                             |     |         |                    +++
   0 01                       2  +-----+    0.025+              *  ++++*
     ----+----+----+----+                        +----+----+----+----+----+----+----+----+----+----+
 Multiply Stem.Leaf by 10**-1                          -2        -1         0        +1        +2
```

G.1.6.2. Kruskal-Wallis rank test

Wilcoxon Scores (Rank Sums) for Variable enterococci_percent
Classified by Variable stage

stage	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
peak	8	89.0	116.00	19.663842	11.1250
recession	5	71.0	72.50	16.670833	14.2000
rising	15	246.0	217.50	21.708293	16.4000

Kruskal-Wallis Test

Chi-Square	2.1536
DF	2
Pr > Chi-Square	0.3407

G.1.7. Total Phosphorous Concentration

G.1.7.1. Test for Normality

Tests for Normality (rising)

Test	--Statistic---		-----p Value------	
Shapiro-Wilk	W	0.906409	Pr < W	0.0636
Kolmogorov-Smirnov	D	0.175401	Pr > D	0.1250
Cramer-von Mises	W-Sq	0.086876	Pr > W-Sq	0.1608
Anderson-Darling	A-Sq	0.607592	Pr > A-Sq	0.0982

```
Stem Leaf                     #  Boxplot                        Normal Probability Plot
   7 4                        1     |        7.25+                                                *+++++
   6 8                        1     |            |                                            *++++
   6 00                       2     |            |                                       +++++
   5                                |            |                                  +++* *
   5 23444                    5  +-----+         |                              *+**+ *
   4 59                       2  *--+--*         |                         ++**+
   4 3                        1  |     |         |                     ++++*
   3 6677899                  7  +-----+     3.75+       *   * +*+*+* **
     ----+----+----+----+                          +----+----+----+----+----+----+----+----+----+----+----+
                                                      -2        -1         0        +1        +2
```

Tests for Normality (peak)

Test	--Statistic---		-----p Value------	
Shapiro-Wilk	W	0.815793	Pr < W	0.0225
Kolmogorov-Smirnov	D	0.247959	Pr > D	0.0807
Cramer-von Mises	W-Sq	0.156103	Pr > W-Sq	0.0170
Anderson-Darling	A-Sq	0.858748	Pr > A-Sq	0.0186

```
Stem Leaf                     #  Boxplot                      Normal Probability Plot
  48 0                        1     0          4.9+                                  *     +++++
  46                                |                                              ++++
  44 0                        1     0             |                              *  ++++
  42                                |                                         +++++
  40                                |          4.1+                      ++++
  38 60                       2  +--+--+          |                    ++++*  *
  36 07                       2  *-----*          |                 ++++* *
  34 199                      3  +-----+          |              *+++*
  32 8                        1     |          3.3+          * ++++
     ----+----+----+----+                          +----+----+----+----+----+----+----+----+----+----+
 Multiply Stem.Leaf by 10**-1                          -2        -1         0        +1        +2
```

Tests for Normality (recession)

Test		--Statistic---	-----p Value------	
Shapiro-Wilk	W	0.804783	Pr < W	0.0649
Kolmogorov-Smirnov	D	0.315957	Pr > D	0.0596
Cramer-von Mises	W-Sq	0.107343	Pr > W-Sq	0.0721
Anderson-Darling	A-Sq	0.608053	Pr > A-Sq	0.0624

```
Stem Leaf                     #  Boxplot                      Normal Probability Plot
  39 5                        1     |          3.95+                                 *   ++++
  38                                |             |                                 +++
  37                                |             |                              +++
  36                                |             |                           +++
  35 0                        1  +-----+          3.55+                     +*+
  34                             |     |             |                  ++++
  33                             |  +  |             |               +++
  32 015                      3  *-----*             |            *+++*   *
  31 1                        1     |          3.15+          *    +++
     ----+----+----+----+                          +----+----+----+----+----+----+----+----+----+----+
 Multiply Stem.Leaf by 10**-1                          -2        -1         0        +1        +2
```

G.1.7.2. Kruskal-Wallis rank test

Wilcoxon Scores (Rank Sums) for Variable Total_P
Classified by Variable stage

stage	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
peak	10	138.0	180.0	27.366943	13.800000
recession	6	37.0	108.0	22.831314	6.166667
rising	19	455.0	342.0	30.178182	23.947368

Average scores were used for ties.

Kruskal-Wallis Test

Chi-Square	16.1046
DF	2
Pr > Chi-Square	0.0003

Wilcoxon Scores (Rank Sums) for Variable Total_P
Classified by Variable stage

stage	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
peak	10	106.0	85.0	9.212763	10.60
recession	6	30.0	51.0	9.212763	5.00

Average scores were used for ties.

Wilcoxon Two-Sample Test

Statistic	30.0000
Normal Approximation	
Z	-2.2252
One-Sided Pr < Z	0.0130
Two-Sided Pr > |Z|	0.0261
t Approximation	
One-Sided Pr < Z	0.0209
Two-Sided Pr > |Z|	0.0418

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	5.1959
DF	1
Pr > Chi-Square	0.0226

Wilcoxon Scores (Rank Sums) for Variable Total_P
Classified by Variable stage

stage	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
recession	6	28.0	78.0	15.701115	4.666667
rising	19	297.0	247.0	15.701115	15.631579

Average scores were used for ties.

Wilcoxon Two-Sample Test

Statistic	28.0000
Normal Approximation	
Z	-3.1526
One-Sided Pr < Z	0.0008
Two-Sided Pr > |Z|	0.0016
t Approximation	

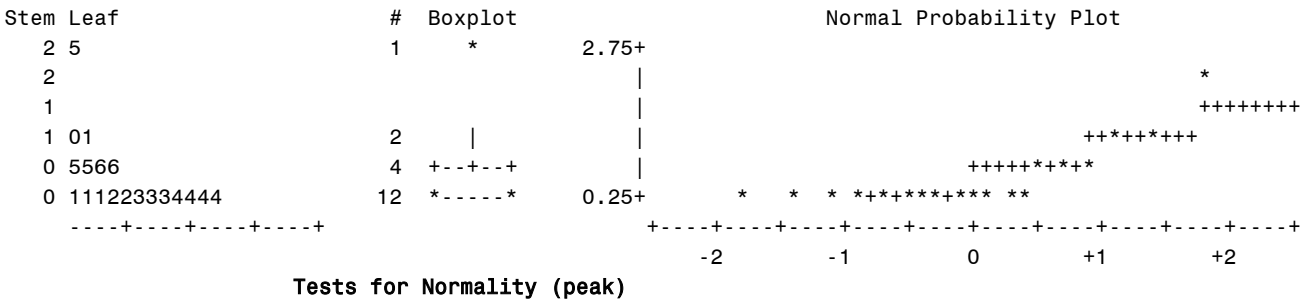
```
One-Sided Pr <  Z      0.0022
Two-Sided Pr > |Z|     0.0043

Z includes a continuity correction of 0.5.


     Kruskal-Wallis Test

Chi-Square            10.1410
DF                          1
Pr > Chi-Square        0.0015


           Wilcoxon Scores (Rank Sums) for Variable Total_P
                     Classified by Variable stage

                      Sum of      Expected       Std Dev          Mean
stage        N        Scores      Under H0      Under H0         Score
-----------------------------------------------------------------------
peak        10          87.0         150.0     21.767638      8.700000
rising      19         348.0         285.0     21.767638     18.315789

                 Average scores were used for ties.


  Wilcoxon Two-Sample Test

Statistic             87.0000

Normal Approximation
Z                     -2.8712
One-Sided Pr <  Z      0.0020
Two-Sided Pr > |Z|     0.0041

t Approximation
One-Sided Pr <  Z      0.0039
Two-Sided Pr > |Z|     0.0077

Z includes a continuity correction of 0.5.


     Kruskal-Wallis Test

Chi-Square             8.3764
DF                          1
Pr > Chi-Square        0.0038
```

G.1.8. Phosphorous Partitioning Ratio

G.1.8.1. Test for Normality

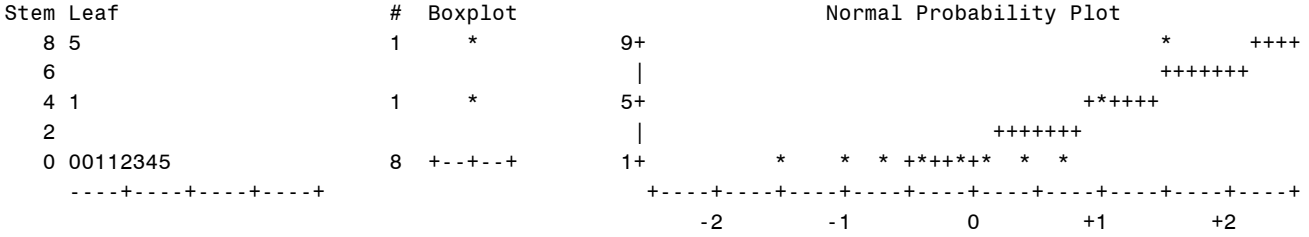
Tests for Normality (rising)

Test		--Statistic---	-----p Value------	
Shapiro-Wilk	W	0.675384	Pr < W	<0.0001
Kolmogorov-Smirnov	D	0.260829	Pr > D	<0.0100
Cramer-von Mises	W-Sq	0.347961	Pr > W-Sq	<0.0050
Anderson-Darling	A-Sq	1.991595	Pr > A-Sq	<0.0050



Tests for Normality (peak)

Test		--Statistic---	-----p Value------	
Shapiro-Wilk	W	0.568335	Pr < W	<0.0001
Kolmogorov-Smirnov	D	0.430411	Pr > D	<0.0100
Cramer-von Mises	W-Sq	0.397184	Pr > W-Sq	<0.0050
Anderson-Darling	A-Sq	2.013412	Pr > A-Sq	<0.0050



Tests for Normality (recession)

Test		--Statistic---		-----p Value------
Shapiro-Wilk	W	0.664547	Pr < W	0.0026
Kolmogorov-Smirnov	D	0.403302	Pr > D	<0.0100
Cramer-von Mises	W-Sq	0.188886	Pr > W-Sq	<0.0050
Anderson-Darling	A-Sq	1.023641	Pr > A-Sq	<0.0050

```
Stem Leaf                     #  Boxplot                        Normal Probability Plot
  10 63                       2  +-----+       11+                                 *     +*+
   8                             |     |         |                                  +++++
   6                             |     |         |                                +++
   4                             |     |         |                            ++++
   2                             |  +  |         |                        ++++
   0 0122                      4  *-----*        1+             *      *++ *   *
     ----+----+----+----+                          +----+----+----+----+----+----+----+----+----+----+----+
                                                      -2        -1         0        +1        +2
```

G.1.8.2. Kruskal-Wallis rank test

Wilcoxon Scores (Rank Sums) for Variable P_ratio
Classified by Variable stage

stage	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
peak	10	162.0	180.0	27.386128	16.200000
recession	6	102.0	108.0	22.847319	17.000000
rising	19	366.0	342.0	30.199338	19.263158

Kruskal-Wallis Test

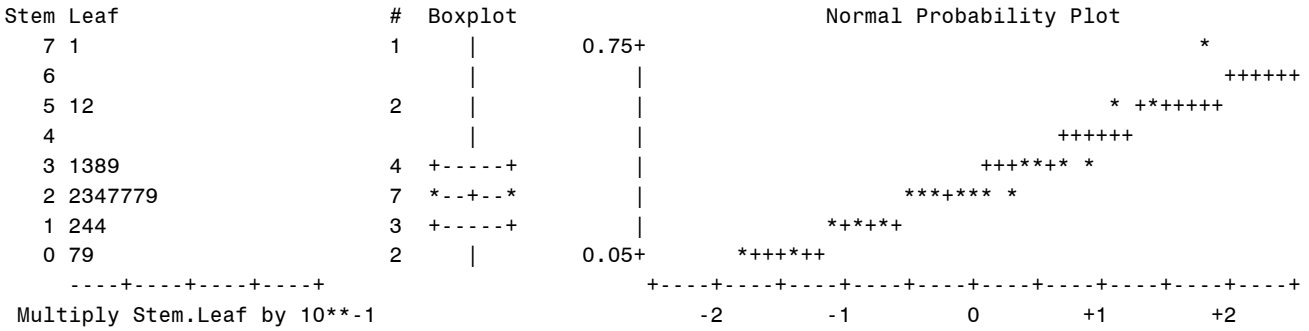
Chi-Square	0.6544
DF	2
Pr > Chi-Square	0.7209

G.1.9. Phosphorous Particulate Attached Fraction

G.1.9.1. Test for Normality

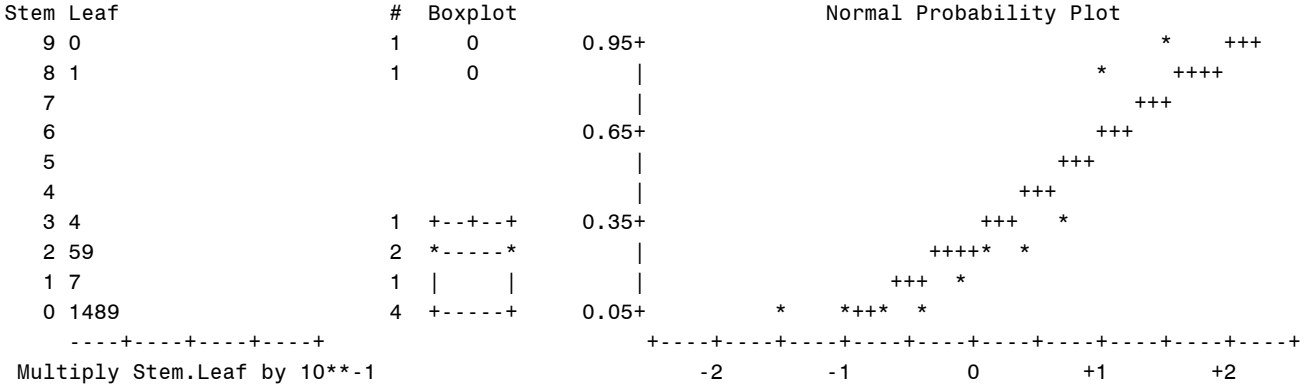
Tests for Normality (rising)

Test		--Statistic---		-----p Value------
Shapiro-Wilk	W	0.928474	Pr < W	0.1623
Kolmogorov-Smirnov	D	0.13621	Pr > D	>0.1500
Cramer-von Mises	W-Sq	0.072258	Pr > W-Sq	0.2491
Anderson-Darling	A-Sq	0.462446	Pr > A-Sq	0.2351



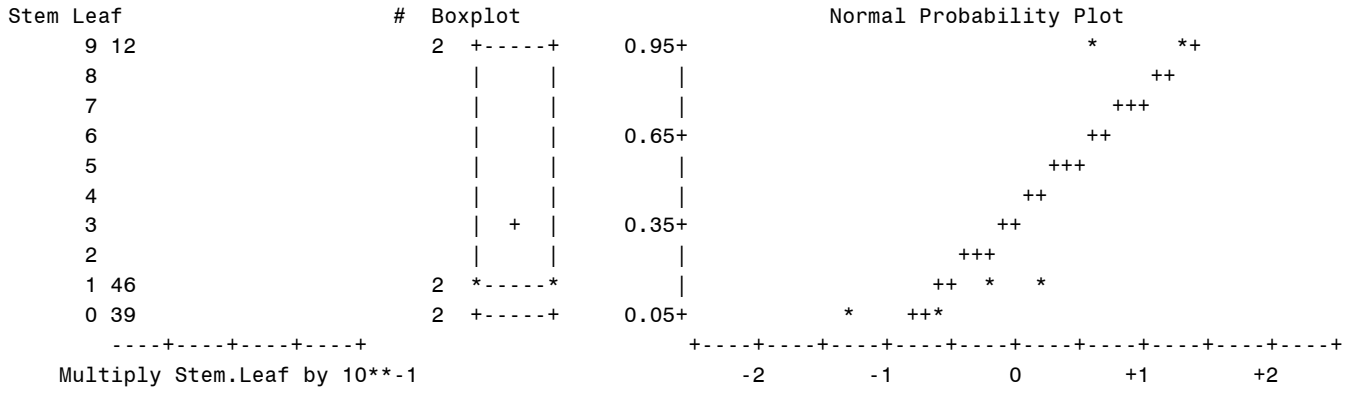
Tests for Normality (peak)

Test	--Statistic---		-----p Value------	
Shapiro-Wilk	W	0.811635	Pr < W	0.0201
Kolmogorov-Smirnov	D	0.247822	Pr > D	0.0810
Cramer-von Mises	W-Sq	0.135272	Pr > W-Sq	0.0318
Anderson-Darling	A-Sq	0.807425	Pr > A-Sq	0.0238



Tests for Normality (recession)

Test	--Statistic---		-----p Value------	
Shapiro-Wilk	W	0.730105	Pr < W	0.0126
Kolmogorov-Smirnov	D	0.363632	Pr > D	0.0141
Cramer-von Mises	W-Sq	0.145525	Pr > W-Sq	0.0202
Anderson-Darling	A-Sq	0.816073	Pr > A-Sq	0.0161



G.1.9.2. Kruskal-Wallis rank test

Wilcoxon Scores (Rank Sums) for Variable P_percent
Classified by Variable stage

stage	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
peak	10	162.0	180.0	27.386128	16.200000
recession	6	102.0	108.0	22.847319	17.000000
rising	19	366.0	342.0	30.199338	19.263158

Kruskal-Wallis Test

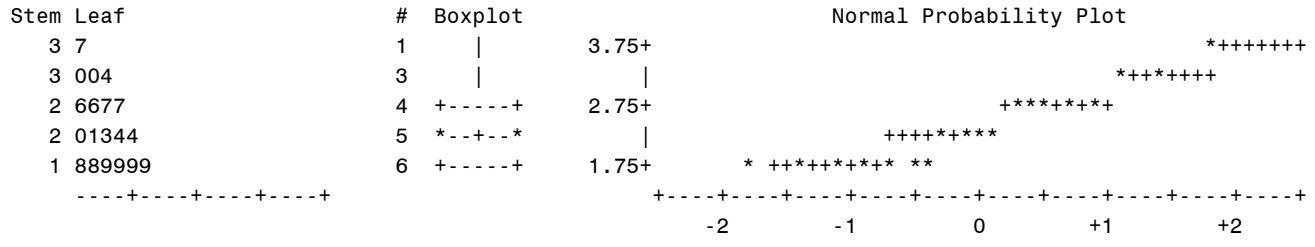
Chi-Square	0.6544
DF	2
Pr > Chi-Square	0.7209

G.1.10. Total Organic Phosphorous Concentration

G.1.10.1. Test for Normality

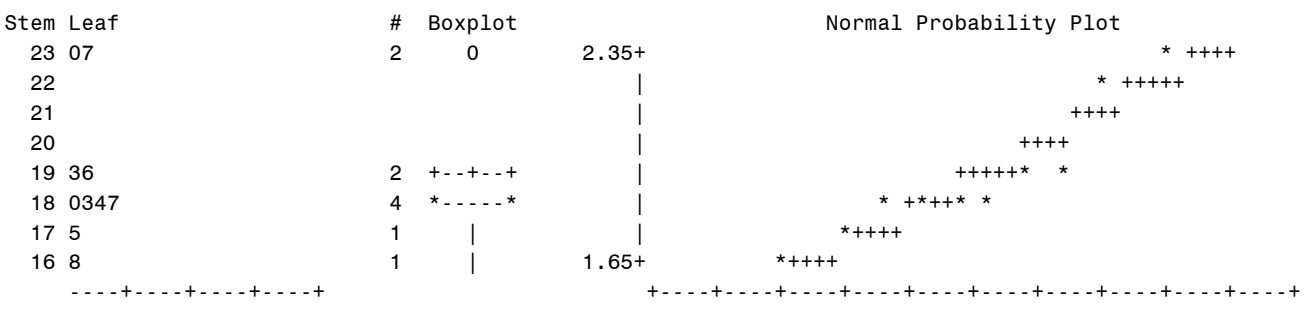
Tests for Normality (rising)

Test	--Statistic---		-----p Value------	
Shapiro-Wilk	W	0.907592	Pr < W	0.0669
Kolmogorov-Smirnov	D	0.171085	Pr > D	0.1473
Cramer-von Mises	W-Sq	0.081168	Pr > W-Sq	0.1953
Anderson-Darling	A-Sq	0.580777	Pr > A-Sq	0.1169



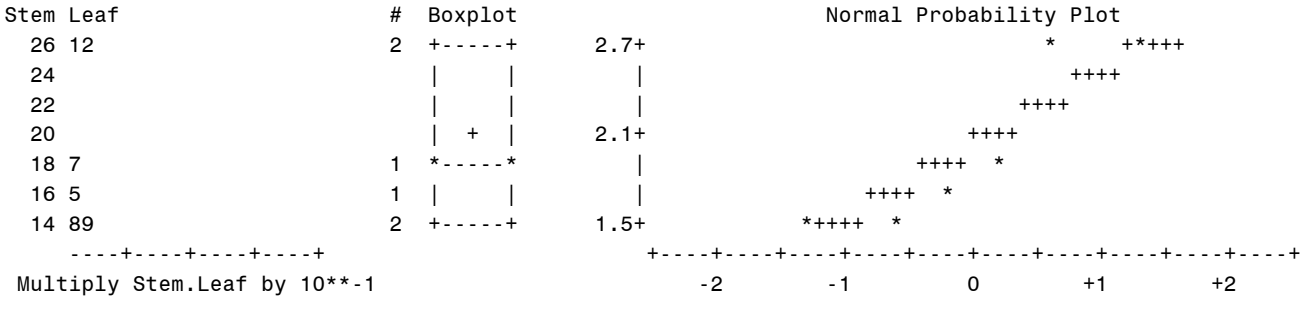
Tests for Normality (peak)

Test		--Statistic---	-----p Value------	
Shapiro-Wilk	W	0.837139	Pr < W	0.0408
Kolmogorov-Smirnov	D	0.259018	Pr > D	0.0558
Cramer-von Mises	W-Sq	0.135513	Pr > W-Sq	0.0315
Anderson-Darling	A-Sq	0.766545	Pr > A-Sq	0.0316



Tests for Normality (recession)

Test		--Statistic---	-----p Value------	
Shapiro-Wilk	W	0.818546	Pr < W	0.0857
Kolmogorov-Smirnov	D	0.221675	Pr > D	>0.1500
Cramer-von Mises	W-Sq	0.0789	Pr > W-Sq	0.1825
Anderson-Darling	A-Sq	0.507185	Pr > A-Sq	0.1205



G.1.10.2. Kruskal-Wallis rank test

```
        Wilcoxon Scores (Rank Sums) for Variable Total_Organic_P
                      Classified by Variable stage

                           Sum of      Expected       Std Dev          Mean
stage           N         Scores      Under H0      Under H0         Score
-------------------------------------------------------------------------
peak           10          123.0         180.0     27.386128     12.300000
recession       6           80.0         108.0     22.847319     13.333333
rising         19          427.0         342.0     30.199338     22.473684

   Kruskal-Wallis Test

Chi-Square         7.9603
DF                      2
Pr > Chi-Square    0.0187

        Wilcoxon Scores (Rank Sums) for Variable Total_Organic_P
                      Classified by Variable stage

                           Sum of      Expected       Std Dev          Mean
stage           N         Scores      Under H0      Under H0         Score
-------------------------------------------------------------------------
peak           10           85.0          85.0      9.219544          8.50
recession       6           51.0          51.0      9.219544          8.50

  Wilcoxon Two-Sample Test

Statistic             51.0000

Normal Approximation
Z                      0.0000
One-Sided Pr <  Z      0.5000
Two-Sided Pr > |Z|     1.0000

t Approximation
One-Sided Pr <  Z      0.5000
Two-Sided Pr > |Z|     1.0000

Z includes a continuity correction of 0.5.

     Kruskal-Wallis Test

Chi-Square             0.0000
DF                          1
Pr > Chi-Square        1.0000

        Wilcoxon Scores (Rank Sums) for Variable Total_Organic_P
                      Classified by Variable stage

                           Sum of      Expected       Std Dev          Mean
stage           N         Scores      Under H0      Under H0         Score
-------------------------------------------------------------------------
recession       6           50.0          78.0     15.716234      8.333333
rising         19          275.0         247.0     15.716234     14.473684
```

Wilcoxon Two-Sample Test

Statistic	50.0000

Normal Approximation

Z	-1.7498
One-Sided Pr < Z	0.0401
Two-Sided Pr > |Z|	0.0802

t Approximation

One-Sided Pr < Z	0.0465
Two-Sided Pr > |Z|	0.0929

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	3.1741
DF	1
Pr > Chi-Square	0.0748

Wilcoxon Scores (Rank Sums) for Variable Total_Organic_P
Classified by Variable stage

stage	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
peak	10	93.0	150.0	21.794495	9.30
rising	19	342.0	285.0	21.794495	18.00

Wilcoxon Two-Sample Test

Statistic	93.0000

Normal Approximation

Z	-2.5924
One-Sided Pr < Z	0.0048
Two-Sided Pr > |Z|	0.0095

t Approximation

One-Sided Pr < Z	0.0075
Two-Sided Pr > |Z|	0.0150

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	6.8400
DF	1
Pr > Chi-Square	0.0089

G.1.11. Organic Phosphorous Partitioning Ratio

G.1.11.1. Test for Normality

Tests for Normality (rising)

Test		--Statistic---	-----p Value------	
Shapiro-Wilk	W	0.677032	Pr < W	<0.0001
Kolmogorov-Smirnov	D	0.251977	Pr > D	<0.0100
Cramer-von Mises	W-Sq	0.337236	Pr > W-Sq	<0.0050
Anderson-Darling	A-Sq	1.955028	Pr > A-Sq	<0.0050

```
Stem Leaf                     #  Boxplot                        Normal Probability Plot
   2 5                        1     *        2.75+
   2                                 |                                                *
   1                                 |                                                 +++++++
   1 00                       2     |            |                                 ++*++*++++
   0 5566                     4  +--+--+         |                           +++++++*+*
   0 111123333344            12  *-----*     0.25+       *   *  * *+*+***+*** ***
     ----+----+----+----+                        +----+----+----+----+----+----+----+----+----+----+----+
                                                    -2        -1         0        +1        +2
```

Tests for Normality (peak)

Test		--Statistic---	-----p Value------	
Shapiro-Wilk	W	0.5543	Pr < W	<0.0001
Kolmogorov-Smirnov	D	0.414091	Pr > D	<0.0100
Cramer-von Mises	W-Sq	0.392133	Pr > W-Sq	<0.0050
Anderson-Darling	A-Sq	2.028532	Pr > A-Sq	<0.0050

```
Stem Leaf                     #  Boxplot                        Normal Probability Plot
   8 2                        1     *           9+                                    *       +++
   6                                 |            |                                   +++++++
   4                                 |           5+                              +++++++
   2 2                        1     *            |                         ++++++ *
   0 00012346                 8  +--+--+         1+          *    *  *  *++*+*+ *  *
     ----+----+----+----+                          +----+----+----+----+----+----+----+----+----+----+----+
                                                      -2        -1         0        +1        +2
```

Tests for Normality (recession)

Test		--Statistic---	-----p Value------	
Shapiro-Wilk	W	0.66669	Pr < W	0.0027
Kolmogorov-Smirnov	D	0.40588	Pr > D	<0.0100
Cramer-von Mises	W-Sq	0.190431	Pr > W-Sq	<0.0050
Anderson-Darling	A-Sq	1.021372	Pr > A-Sq	<0.0050

```
Stem Leaf                     #  Boxplot                       Normal Probability Plot
      1 79                    2  +-----+     17.5+                                *   +++*+
      1                          |     |         |                              +++++
      0                          |  +  |         |                          ++++++
      0 0000                  4  *-----*      2.5+             *      *+++*   *
        ----+----+----+----+                        +----+----+----+----+----+----+----+----+----+----+----+
    Multiply Stem.Leaf by 10**+1                        -2        -1         0        +1        +2
```

G.1.11.2. Kruskal-Wallis rank test

Wilcoxon Scores (Rank Sums) for Variable Organic_P_ratio
Classified by Variable stage

stage	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
peak	10	163.0	180.0	27.386128	16.300000
recession	6	91.0	108.0	22.847319	15.166667
rising	19	376.0	342.0	30.199338	19.789474

Kruskal-Wallis Test

Chi-Square	1.3134
DF	2
Pr > Chi-Square	0.5186

G.1.12. Organic Phosphorous Particulate Attached Fraction

G.1.12.1. Test for Normality

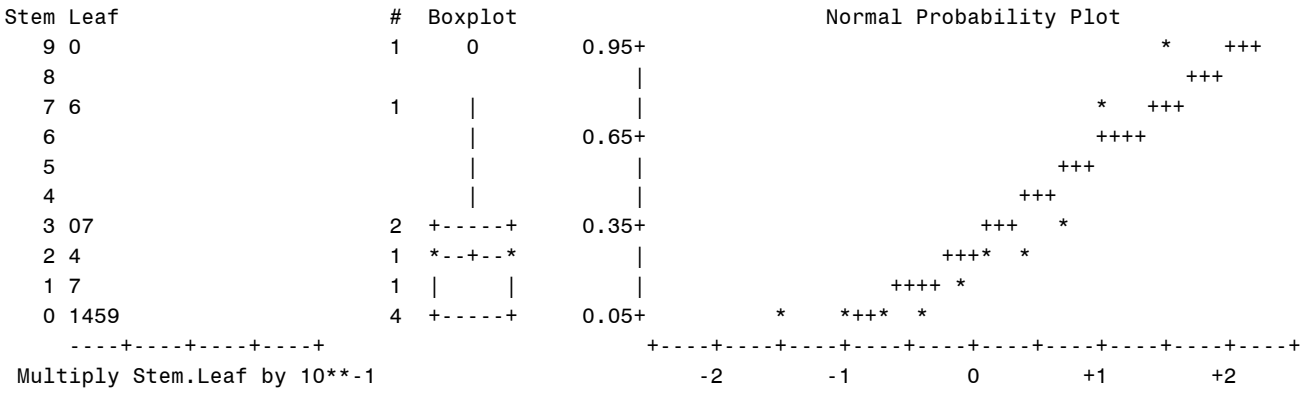
Tests for Normality (rising)

Test		--Statistic---	-----p Value------		
Shapiro-Wilk	W	0.929317	Pr < W	0.1682	
Kolmogorov-Smirnov	D	0.129902	Pr > D	>0.1500	
Cramer-von Mises	W-Sq	0.063812	Pr > W-Sq	>0.2500	
Anderson-Darling	A-Sq	0.430043	Pr > A-Sq	>0.2500	

```
  Stem Leaf                     #  Boxplot                       Normal Probability Plot
     7 1                        1     |        0.75+                                      *
     6                                |           |                                     ++++++
     5 01                       2     |           |                                  *  *+++++
     4                                |           |                               ++++++
     3 1389                     4  +-----+        |                          +++***+ *
     2 1235679                  7  *--+--*        |                     ***+*** *
     1 0125                     4  +-----+        |                *+*+*+
     0 7                        1     |        0.05+       *+++*++
       ----+----+----+----+                        +----+----+----+----+----+----+----+----+----+----+----+
    Multiply Stem.Leaf by 10**-1                        -2        -1         0        +1        +2
```

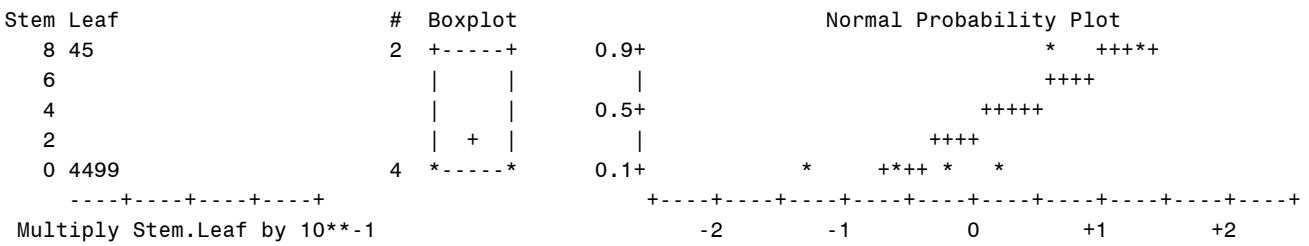
Tests for Normality (peak)

Test		--Statistic---	-----p Value------	
Shapiro-Wilk	W	0.826359	Pr < W	0.0302
Kolmogorov-Smirnov	D	0.207354	Pr > D	>0.1500
Cramer-von Mises	W-Sq	0.120463	Pr > W-Sq	0.0494
Anderson-Darling	A-Sq	0.729747	Pr > A-Sq	0.0399



Tests for Normality (recession)

Test		--Statistic---	-----p Value------	
Shapiro-Wilk	W	0.675891	Pr < W	0.0034
Kolmogorov-Smirnov	D	0.388835	Pr > D	<0.0100
Cramer-von Mises	W-Sq	0.175477	Pr > W-Sq	0.0074
Anderson-Darling	A-Sq	0.973186	Pr > A-Sq	0.0051



G.1.12.2. Kruskal-Wallis rank test

Wilcoxon Scores (Rank Sums) for Variable Organic_P_percent
Classified by Variable stage

stage	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
peak	10	163.0	180.0	27.386128	16.300000
recession	6	91.0	108.0	22.847319	15.166667
rising	19	376.0	342.0	30.199338	19.789474

Kruskal-Wallis Test

Chi-Square	1.3134
DF	2
Pr > Chi-Square	0.5186

G.1.13. Total Organic Carbon Concentration

G.1.13.1. Test for Normality

Tests for Normality (rising)

Test	--Statistic---		-----p Value------	
Shapiro-Wilk	W	0.94479	Pr < W	0.3210
Kolmogorov-Smirnov	D	0.211409	Pr > D	0.0239
Cramer-von Mises	W-Sq	0.096163	Pr > W-Sq	0.1204
Anderson-Darling	A-Sq	0.495029	Pr > A-Sq	0.1976

```
Stem Leaf                     #  Boxplot                        Normal Probability Plot
  17 1                        1     |       17.25+                                    +++*++++++
  16 55789                    5  +-----+         |                                *+*+*+*+*+*
  16 0333444                  7  *--+--*    16.25+                        **++*++*+*+
  15 677                      3  +-----+         |                  +++*+*+++
  15 244                      3     |       15.25+    +++*+++*+
     ----+----+----+----+                          +----+----+----+----+----+----+----+----+----+----+----+
                                                      -2        -1         0        +1        +2
```

Tests for Normality (peak)

Test	--Statistic---		-----p Value------	
Shapiro-Wilk	W	0.882505	Pr < W	0.1394
Kolmogorov-Smirnov	D	0.215402	Pr > D	>0.1500
Cramer-von Mises	W-Sq	0.088319	Pr > W-Sq	0.1433
Anderson-Darling	A-Sq	0.513187	Pr > A-Sq	0.1484

```
Stem Leaf                     #  Boxplot                       Normal Probability Plot
     16 13                    2     |       16.25+                                  *++++++++
     15 79                    2  +-----+         |                              *+++*+++
     15 1                     1  *--+--*    15.25+                         +++*+++
     14 5678                  4  +-----+         |                  *++++++* *
     14 4                     1     |       14.25+           +*+++++
        ----+----+----+----+                        +----+----+----+----+----+----+----+----+----+----+----+
                                                       -2        -1         0        +1        +2
```

Tests for Normality (recession)

Test		--Statistic---		-----p Value------	
Shapiro-Wilk	W	0.959598	Pr < W		0.8166
Kolmogorov-Smirnov	D	0.180915	Pr > D		>0.1500
Cramer-von Mises	W-Sq	0.039799	Pr > W-Sq		>0.2500
Anderson-Darling	A-Sq	0.230677	Pr > A-Sq		>0.2500

```
   Stem Leaf                     #  Boxplot                       Normal Probability Plot
    166 0                        1     |        16.7+                                    *++++
    164                          1     |           |                                  +++++
    162 00                       2  +-----+         |                             *  +*++
    160 0                        1  *-----*         |                          *  ++++
    158                          1  |  +  |         |                        ++++
    156 0                        1  +-----+         |                   ++*++
    154                          1     |           |              ++++
    152 0                        1     |        15.3+          +++*
        ----+----+----+----+                        +----+----+----+----+----+----+----+----+----+----+----+
    Multiply Stem.Leaf by 10**-1                        -2        -1         0        +1        +2
```

G.1.13.2. Kruskal-Wallis rank test

Wilcoxon Scores (Rank Sums) for Variable Total_Organic_C
Classified by Variable stage

stage	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
peak	10	93.50	180.0	27.341983	9.350000
recession	6	109.00	108.0	22.810491	18.166667
rising	19	427.50	342.0	30.150658	22.500000

Average scores were used for ties.

Kruskal-Wallis Test

Chi-Square	10.8267
DF	2
Pr > Chi-Square	0.0045

Wilcoxon Scores (Rank Sums) for Variable Total_Organic_C
Classified by Variable stage

stage	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
peak	10	67.0	85.0	9.212763	6.70
recession	6	69.0	51.0	9.212763	11.50

Average scores were used for ties.

Wilcoxon Two-Sample Test

Statistic	69.0000

Normal Approximation

Z	1.8995
One-Sided Pr > Z	0.0287
Two-Sided Pr > |Z|	0.0575

t Approximation

One-Sided Pr > Z	0.0384
Two-Sided Pr > |Z|	0.0769

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	3.8174
DF	1
Pr > Chi-Square	0.0507

Wilcoxon Scores (Rank Sums) for Variable Total_Organic_C
Classified by Variable stage

stage	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
recession	6	61.0	78.0	15.673864	10.166667
rising	19	264.0	247.0	15.673864	13.894737

Average scores were used for ties.

Wilcoxon Two-Sample Test

Statistic	61.0000

Normal Approximation

Z	-1.0527
One-Sided Pr < Z	0.1462
Two-Sided Pr > |Z|	0.2925

t Approximation

One-Sided Pr < Z	0.1515
Two-Sided Pr > |Z|	0.3030

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	1.1764
DF	1
Pr > Chi-Square	0.2781

Wilcoxon Scores (Rank Sums) for Variable Total_Organic_C
Classified by Variable stage

stage	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
peak	10	81.50	150.0	21.740747	8.150000
rising	19	353.50	285.0	21.740747	18.605263

Average scores were used for ties.

Wilcoxon Two-Sample Test

Statistic	81.5000

Normal Approximation

Z	-3.1278
One-Sided Pr < Z	0.0009
Two-Sided Pr > |Z|	0.0018

t Approximation

One-Sided Pr < Z	0.0020
Two-Sided Pr > |Z|	0.0041

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

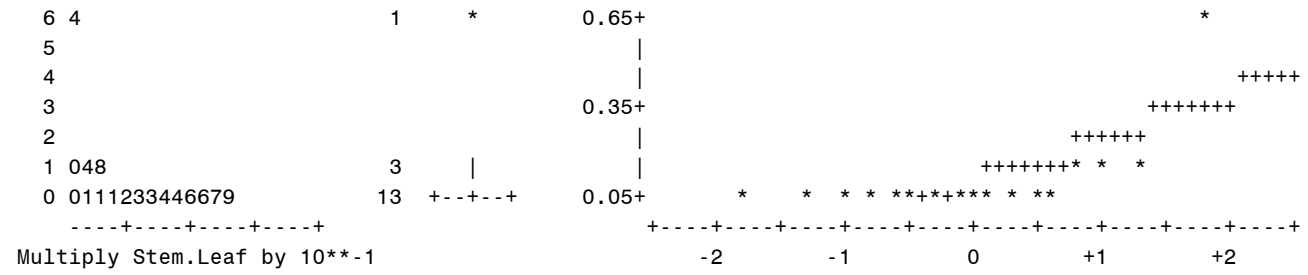
Chi-Square	9.9273
DF	1
Pr > Chi-Square	0.0016

G.1.14. Organic Carbon Partitioning Coefficient

G.1.14.1. Test for Normality

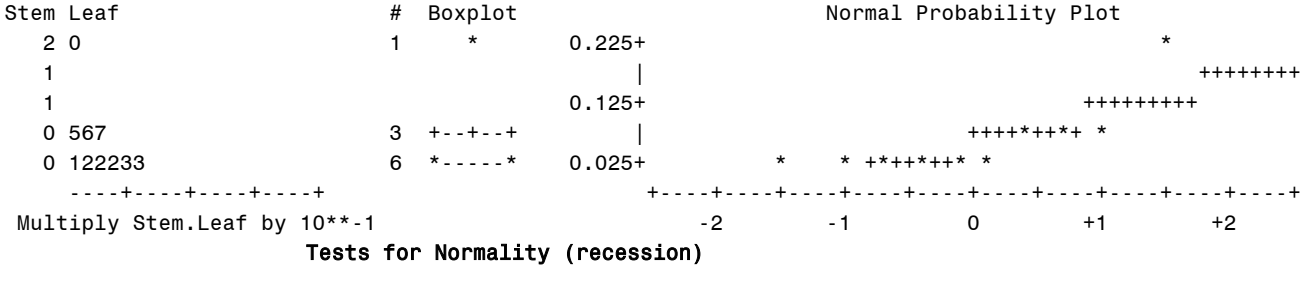
Tests for Normality (rising)

Test		--Statistic---	-----p Value------
Shapiro-Wilk	W	0.541508	Pr < W <0.0001
Kolmogorov-Smirnov	D	0.294763	Pr > D <0.0100
Cramer-von Mises	W-Sq	0.493487	Pr > W-Sq <0.0050
Anderson-Darling	A-Sq	2.756108	Pr > A-Sq <0.0050



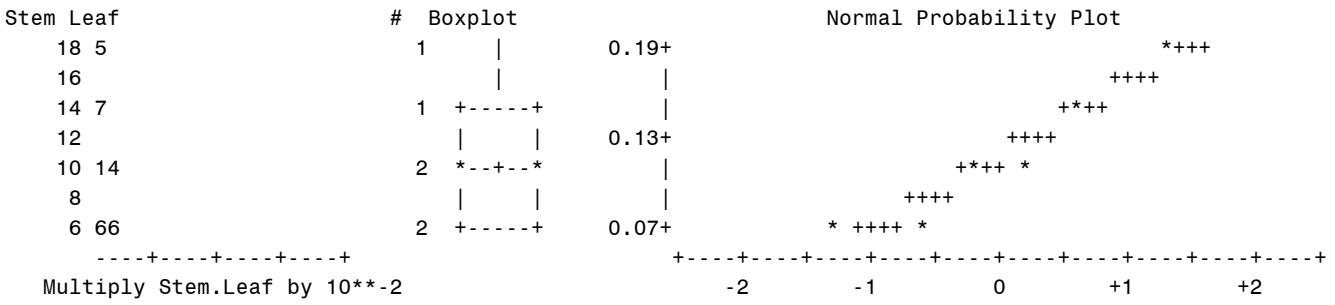
Tests for Normality (peak)

Test		--Statistic---	-----p Value------
Shapiro-Wilk	W	0.644152	Pr < W 0.0002
Kolmogorov-Smirnov	D	0.297931	Pr > D 0.0130
Cramer-von Mises	W-Sq	0.251652	Pr > W-Sq <0.0050
Anderson-Darling	A-Sq	1.45006	Pr > A-Sq <0.0050



Tests for Normality (recession)

Test		--Statistic---	-----p Value------
Shapiro-Wilk	W	0.917914	Pr < W 0.4905
Kolmogorov-Smirnov	D	0.186202	Pr > D >0.1500
Cramer-von Mises	W-Sq	0.041409	Pr > W-Sq >0.2500
Anderson-Darling	A-Sq	0.282968	Pr > A-Sq >0.2500



G.1.14.2. Kruskal-Wallis rank test

Wilcoxon Scores (Rank Sums) for Variable Organic_C_ratio
Classified by Variable stage

stage	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
peak	10	139.0	170.0	25.525630	13.900000
recession	6	158.0	102.0	21.422495	26.333333
rising	17	264.0	289.0	27.758564	15.529412

Average scores were used for ties.

Kruskal-Wallis Test

Chi-Square	7.0122
DF	2
Pr > Chi-Square	0.0300

Wilcoxon Scores (Rank Sums) for Variable Organic_C_ratio
Classified by Variable stage

stage	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
peak	10	61.0	85.0	9.212763	6.10
recession	6	75.0	51.0	9.212763	12.50

Average scores were used for ties.

Wilcoxon Two-Sample Test

Statistic	75.0000
Normal Approximation	
Z	2.5508
One-Sided Pr > Z	0.0054
Two-Sided Pr > |Z|	0.0107
t Approximation	
One-Sided Pr > Z	0.0111
Two-Sided Pr > |Z|	0.0222

Z includes a continuity correction of 0.5.

```
     Kruskal-Wallis Test

Chi-Square              6.7865
DF                           1
Pr > Chi-Square         0.0092

        Wilcoxon Scores (Rank Sums) for Variable Organic_C_ratio
                      Classified by Variable stage

                          Sum of      Expected       Std Dev          Mean
stage            N        Scores      Under H0      Under H0         Score
-------------------------------------------------------------------------
recession        6         104.0          72.0     14.279328     17.333333
rising          17         172.0         204.0     14.279328     10.117647

                   Average scores were used for ties.

   Wilcoxon Two-Sample Test

Statistic              104.0000

Normal Approximation
Z                       2.2060
One-Sided Pr >  Z       0.0137
Two-Sided Pr > |Z|      0.0274

t Approximation
One-Sided Pr >  Z       0.0191
Two-Sided Pr > |Z|      0.0381

Z includes a continuity correction of 0.5.

     Kruskal-Wallis Test

Chi-Square              5.0221
DF                           1
Pr > Chi-Square         0.0250

       Wilcoxon Scores (Rank Sums) for Variable Organic_C_ratio
                     Classified by Variable stage

                      Sum of      Expected       Std Dev          Mean
stage          N      Scores      Under H0      Under H0         Score
-----------------------------------------------------------------------
peak          10         133.0         140.0     19.916492     13.300000
rising        17         245.0         238.0     19.916492     14.411765

   Wilcoxon Two-Sample Test

Statistic              133.0000

Normal Approximation
Z                      -0.3264
One-Sided Pr <  Z       0.3721
Two-Sided Pr > |Z|      0.7441

t Approximation
```

```
One-Sided Pr <  Z       0.3734
Two-Sided Pr > |Z|      0.7468

Z includes a continuity correction of 0.5.


     Kruskal-Wallis Test

Chi-Square              0.1235
DF                           1
Pr > Chi-Square         0.7252
```

G.1.15. Organic Carbon Particulate Attached Fraction

G.1.15.1. Test for Normality

Tests for Normality (rising)

```
Test                  --Statistic---    -----p Value------

Shapiro-Wilk          W     0.663672    Pr < W     <0.0001
Kolmogorov-Smirnov    D     0.241803    Pr > D     <0.0100
Cramer-von Mises      W-Sq  0.306906    Pr > W-Sq  <0.0050
Anderson-Darling      A-Sq  1.838598    Pr > A-Sq  <0.0050
```

```
Stem Leaf                     #  Boxplot                        Normal Probability Plot
   3 9                        1     *       0.375+                                    *
   3                                            |                                      +
   2                                            |                                  +++++
   2                                            |                              ++++++
   1 5                        1     |           |                            ++++*
   1 2                        1     |           |                         +++++ *
   0 56689                    5  +--+--+        |                      +++*+* ** *
   0 011122344                9  *-----*    0.025+       *    *  * *+**+* **
     ----+----+----+----+                        +----+----+----+----+----+----+----+----+----+----+
 Multiply Stem.Leaf by 10**-1                        -2        -1         0        +1        +2
```

Tests for Normality (peak)

```
Test                  --Statistic---    -----p Value------

Shapiro-Wilk          W     0.679316    Pr < W      0.0005
Kolmogorov-Smirnov    D      0.27022    Pr > D      0.0377
Cramer-von Mises      W-Sq  0.217483    Pr > W-Sq  <0.0050
Anderson-Darling      A-Sq   1.28733    Pr > A-Sq  <0.0050
```

```
Stem Leaf                     #  Boxplot                      Normal Probability Plot
     16 0                     1     *        0.17+                                    *         +
     14                                          |                                  ++++
     12                                          |                                +++++
     10                                          |                              ++++
      8                                       0.09+                           ++++
      6 2                     1     |             |                        +++++  *
      4 15                    2  +--+--+          |                     ++++ *  *
      2 0156                  4  *-----*          |                  * +*++* *
      0 38                    2     |          0.01+          *    *++++
        ----+----+----+----+                       +----+----+----+----+----+----+----+----+----+----+
    Multiply Stem.Leaf by 10**-2                        -2        -1         0        +1        +2
```

Tests for Normality (recession)

Test		--Statistic---	-----p Value------	
Shapiro-Wilk	W	0.921792	Pr < W	0.5184
Kolmogorov-Smirnov	D	0.186358	Pr > D	>0.1500
Cramer-von Mises	W-Sq	0.039829	Pr > W-Sq	>0.2500
Anderson-Darling	A-Sq	0.274304	Pr > A-Sq	>0.2500

```
   Stem Leaf                     #  Boxplot                      Normal Probability Plot
     16 3                     1     |        0.17+                                  * +++++
     14                             |           |                                +++++
     12 8                     1  +-----+        |                             +*+++
     10 02                    2  *--+--*        |                          * ++*++
      8                          |     |        |                       +++++
      6 22                    2  +-----+     0.07+             *  ++++*
        ----+----+----+----+                       +----+----+----+----+----+----+----+----+----+----+
    Multiply Stem.Leaf by 10**-2                        -2        -1         0        +1        +2
```

G.1.15.2. Kruskal-Wallis rank test

Wilcoxon Scores (Rank Sums) for Variable Organic_C_percent
Classified by Variable stage

stage	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
peak	10	139.0	170.0	25.525630	13.900000
recession	6	158.0	102.0	21.422495	26.333333
rising	17	264.0	289.0	27.758564	15.529412

Average scores were used for ties.

Kruskal-Wallis Test

Chi-Square	7.0122
DF	2
Pr > Chi-Square	0.0300

Wilcoxon Scores (Rank Sums) for Variable Organic_C_percent
Classified by Variable stage

stage	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
peak	10	61.0	85.0	9.212763	6.10
recession	6	75.0	51.0	9.212763	12.50

Average scores were used for ties.

Wilcoxon Two-Sample Test

Statistic	75.0000

Normal Approximation

Z	2.5508
One-Sided Pr > Z	0.0054
Two-Sided Pr > |Z|	0.0107

t Approximation

One-Sided Pr > Z	0.0111
Two-Sided Pr > |Z|	0.0222

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	6.7865
DF	1
Pr > Chi-Square	0.0092

Wilcoxon Scores (Rank Sums) for Variable Organic_C_percent
Classified by Variable stage

stage	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
recession	6	104.0	72.0	14.279328	17.333333
rising	17	172.0	204.0	14.279328	10.117647

Average scores were used for ties.

Wilcoxon Two-Sample Test

Statistic	104.0000

Normal Approximation

Z	2.2060
One-Sided Pr > Z	0.0137
Two-Sided Pr > |Z|	0.0274

t Approximation

One-Sided Pr > Z	0.0191
Two-Sided Pr > |Z|	0.0381

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	5.0221
DF	1
Pr > Chi-Square	0.0250

Wilcoxon Scores (Rank Sums) for Variable Organic_C_percent
Classified by Variable stage

stage	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
peak	10	133.0	140.0	19.916492	13.300000
rising	17	245.0	238.0	19.916492	14.411765

Wilcoxon Two-Sample Test

Statistic	133.0000

Normal Approximation

Z	-0.3264
One-Sided Pr < Z	0.3721
Two-Sided Pr > |Z|	0.7441

t Approximation

One-Sided Pr < Z	0.3734
Two-Sided Pr > |Z|	0.7468

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	0.1235
DF	1
Pr > Chi-Square	0.7252

G.2. Multiple Regression Analysis Details for Transport Plot Study

G.2.1. ln E. coli Total Concentrations

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	4.26755	2.13377	3.29	0.0504
Error	32	20.77999	0.64937		
Corrected Total	34	25.04754			

Root MSE	0.80584	R-Square	0.1704
Dependent Mean	13.31202	Adj R-Sq	0.1185
Coeff Var	6.05346		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Variance Inflation
Intercept	Intercept	1	10.75398	1.45571	7.39	<.0001	0
Q	Q	1	204.92114	102.17949	2.01	0.0534	1.00114
Diss_Organic_C	Diss_Organic_C	1	0.14864	0.09727	1.53	0.1363	1.00114

G.2.2. ln E. coli Partitioning Coefficient

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	21.05554	10.52777	5.44	0.0099
Error	29	56.14880	1.93617		
Corrected Total	31	77.20435			

Root MSE	1.39146	R-Square	0.2727
Dependent Mean	-3.89200	Adj R-Sq	0.2226
Coeff Var	-35.75184		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Variance Inflation
Intercept	Intercept	1	-12.76484	5.69824	-2.24	0.0329	0
Diss_Organic_P	Diss_Organic_P	1	-0.98128	0.43208	-2.27	0.0307	1.04530
Total_Organic_C	Total_Organic_C	1	0.64799	0.34727	1.87	0.0722	1.04530

G.2.3. ln E. coli Particulate Attached Fraction

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	18.97177	9.48589	5.25	0.0113
Error	29	52.41567	1.80744		
Corrected Total	31	71.38744			

Root MSE	1.34441	R-Square	0.2658
Dependent Mean	-3.94394	Adj R-Sq	0.2151
Coeff Var	-34.08797		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Variance Inflation
Intercept	Intercept	1	-12.25969	5.50556	-2.23	0.0339	0
Diss_Organic_P	Diss_Organic_P	1	-0.93808	0.41747	-2.25	0.0324	1.04530
Total_Organic_C	Total_Organic_C	1	0.60900	0.33553	1.82	0.0799	1.04530

G.2.4. ln enterococci Total Concentrations

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	7.81253	3.90627	1.24	0.3034
Error	32	100.93589	3.15425		
Corrected Total	34	108.74843			

Root MSE	1.77602	R-Square	0.0718
Dependent Mean	11.76932	Adj R-Sq	0.0138
Coeff Var	15.09026		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Variance Inflation
Intercept	Intercept	1	12.28519	0.45212	27.17	<.0001	0
TSS	TSS	1	-0.00248	0.00202	-1.23	0.2286	1.00293
P_ratio	P_ratio	1	-0.10877	0.10359	-1.05	0.3016	1.00293

G.2.5. ln enterococci Partitioning Coefficient

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	20.05945	10.02973	5.40	0.0112
Error	25	46.40536	1.85621		
Corrected Total	27	66.46481			

Root MSE	1.36243	R-Square	0.3018
Dependent Mean	-2.59661	Adj R-Sq	0.2460
Coeff Var	-52.46947		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	Intercept	1	-17.08801	6.50353	-2.63	0.0145
Q	Q	1	-374.09066	181.41334	-2.06	0.0497
Total_Organic_C	Total_Organic_C	1	0.94883	0.40362	2.35	0.0269

G.2.6. ln enterococci Particulate Attached Fraction

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	15.98455	7.99228	5.07	0.0142
Error	25	39.44484	1.57779		
Corrected Total	27	55.42939			

Root MSE	1.25610	R-Square	0.2884
Dependent Mean	-2.74738	Adj R-Sq	0.2314
Coeff Var	-45.72004		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	Intercept	1	-15.71882	5.99598	-2.62	0.0147
Q	Q	1	-332.88903	167.25544	-1.99	0.0576
Total_Organic_C	Total_Organic_C	1	0.84909	0.37212	2.28	0.0313

G.2.7. ln E. coli unattached

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	122.01253	30.50313	37.28	<.0001
Error	21	17.18153	0.81817		
Corrected Total	25	139.19406			

Root MSE	0.90453	R-Square	0.8766
Dependent Mean	24.41661	Adj R-Sq	0.8531
Coeff Var	3.70455		

Parameter Estimates

Variable	Label	DF	Estimate	Error	t Value	Pr > |t|	Inflation
Intercept	Intercept	1	21.20195	0.36124	58.69	<.0001	0
TP	TP	1	182.70696	61.84728	2.95	0.0076	930.71561
DOC	DOC	1	-25.87730	13.69611	-1.89	0.0727	862.71623
Sq_TP		1	-1348.53595	454.26719	-2.97	0.0073	4337.82407
Sq_DOC		1	63.90247	23.91053	2.67	0.0142	4128.74874

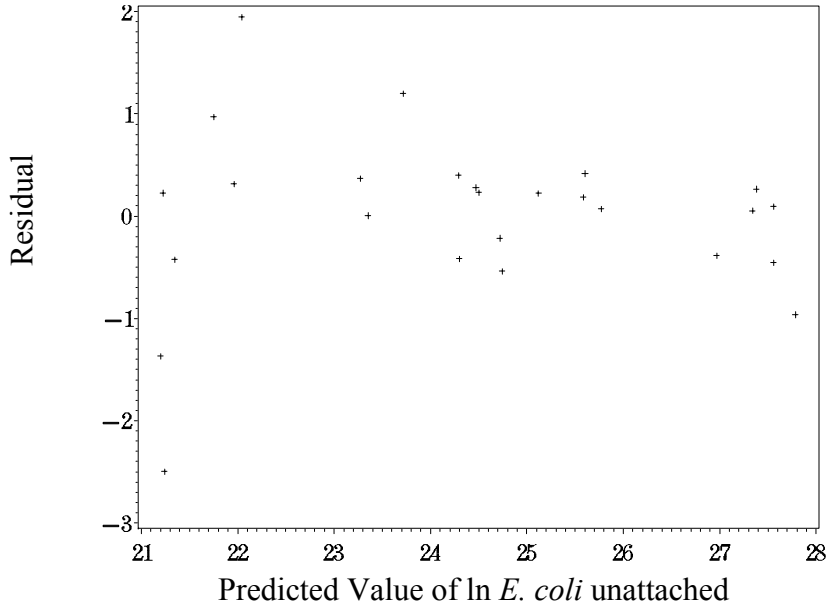


Figure G.1. Residual plots of predicted ln *E. coli* unattached loading rates in runoff from well managed pastureland.

G.2.8. ln E. coli attached

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	152.90198	30.58040	31.39	<.0001
Error	20	19.48602	0.97430		
Corrected Total	25	172.38800			

Root MSE	0.98707	R-Square	0.8870
Dependent Mean	21.10797	Adj R-Sq	0.8587
Coeff Var	4.67628		
Coeff Var	6.15515		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Variance Inflation
Intercept	Intercept	1	15.86963	0.55292	28.70	<.0001	0
TSS	TSS	1	3.64728	0.53167	6.86	<.0001	52.55137
P_ratio	P_ratio	1	4.47340	0.81274	5.50	<.0001	26.56764
Sq_TSS		1	-0.80135	0.16495	-4.86	<.0001	313.20356
Sq_P_ratio		1	-1.04504	0.21611	-4.84	0.0001	30.74792
Sq_TOC		1	17.99062	4.23415	4.25	0.0004	130.24061

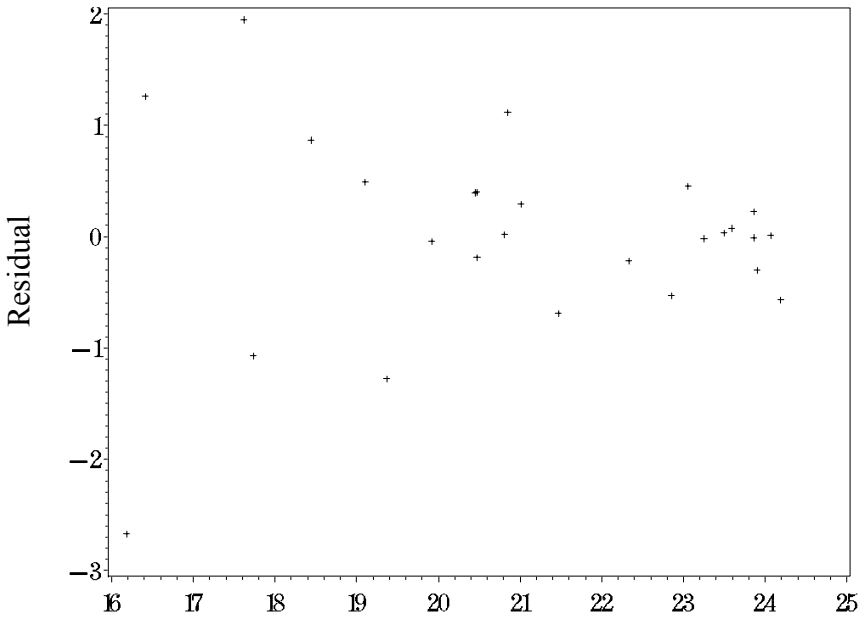


Figure G.2. Residual plots of predicted ln *E. coli* attached loading rates in runoff from well managed pastureland.

G.2.9. ln enterococci attached

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	108.08188	54.04094	41.78	<.0001
Error	23	29.75171	1.29355		
Corrected Total	25	137.83359			

Root MSE	1.13734	R-Square	0.7841
Dependent Mean	20.48983	Adj R-Sq	0.7654
Coeff Var	5.55078		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Variance Inflation
Intercept	Intercept	1	17.69332	0.39666	44.61	<.0001	0
TP	TP	1	55.05166	8.36244	6.58	<.0001	10.76218
Sq_TP		1	-123.38681	28.45092	-4.34	0.0002	10.76218

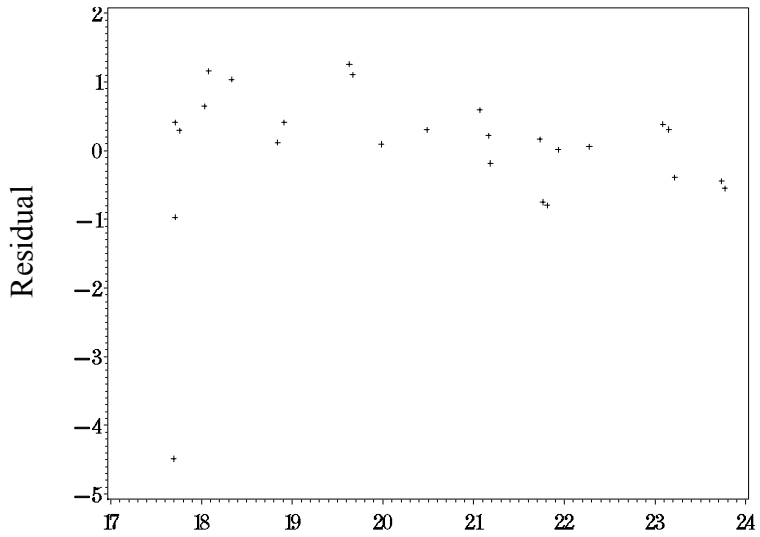


Figure G.3. Residual plots of predicted ln enterococci unattached loading rates in runoff from well managed pastureland.

G.2.10. ln enterococci unattached

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	209.90184	41.98037	44.82	<.0001
Error	20	18.73232	0.93662		
Corrected Total	25	228.63417			

Root MSE	0.96779	R-Square	0.9181
Dependent Mean	22.54861	Adj R-Sq	0.8976
Coeff Var	4.29201		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Variance Inflation
Intercept	Intercept	1	17.78876	0.51108	34.81	<.0001	0
TSS	TSS	1	3.97339	0.50114	7.93	<.0001	48.56902
OP_ratio	OP_ratio	1	2.40251	0.68371	3.51	0.0022	20.56133
Sq_TSS		1	-0.85769	0.15583	-5.50	<.0001	290.78105
Sq_OP_ratio		1	-0.65584	0.16746	-3.92	0.0009	23.01293
Sq_TOC		1	19.77649	4.01994	4.92	<.0001	122.11962

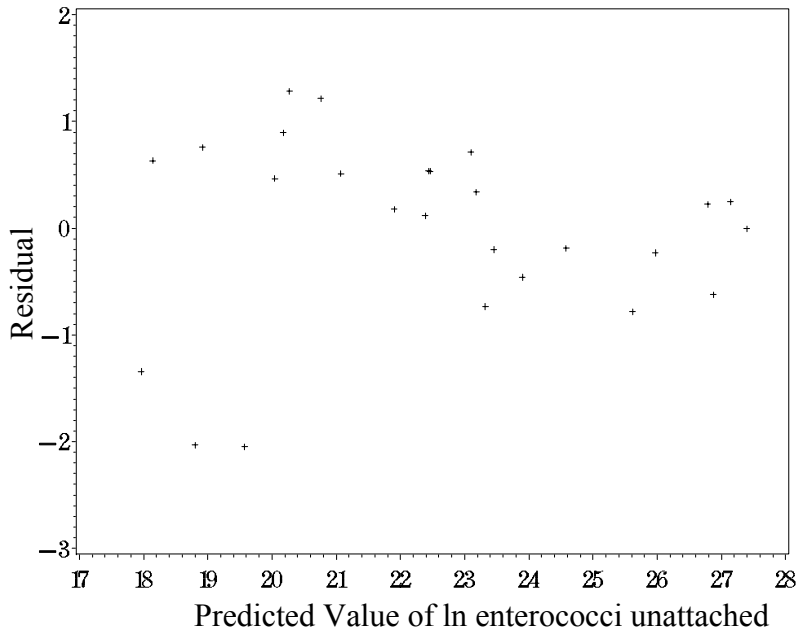


Figure G.4. Residual plots of predicted ln enterococci attached loading rates in runoff from well managed pastureland.

G.2.11. ln E. coli loading ratio

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	19.80222	9.90111	18.13	<.0001
Error	23	12.56046	0.54611		
Corrected Total	25	32.36267			

Root MSE	0.73899	R-Square	0.6119
Dependent Mean	-3.30864	Adj R-Sq	0.5781
Coeff Var	-22.33515		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Variance Inflation
Intercept	Intercept	1	-4.51577	0.26082	-17.31	<.0001	0
OP_ratio	OP_ratio	1	2.81015	0.61752	4.55	0.0001	28.76677
Sq_P_ratio		1	-0.58460	0.15649	-3.74	0.0011	28.76677

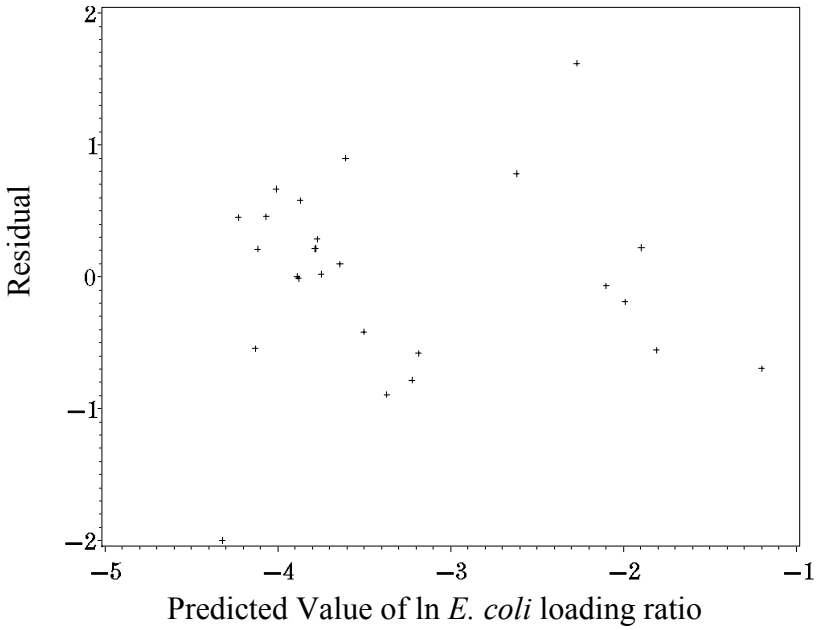


Figure G.5. Residual plots of predicted ln *E. coli* loading ratio in runoff from well managed pastureland.

G.2.12. ln enterococci loading ratio

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	18.44090	9.22045	11.98	0.0003
Error	23	17.70402	0.76974		
Corrected Total	25	36.14492			

Root MSE	0.87735	R-Square	0.5102
Dependent Mean	-2.05878	Adj R-Sq	0.4676
Coeff Var	-42.61499		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Variance Inflation
Intercept	Intercept	1	-1.18123	0.24879	-4.75	<.0001	0
TSS	TSS	1	-0.31641	0.06640	-4.77	<.0001	1.03752
Sq_OC_ratio		1	-10.84372	5.41033	-2.00	0.0570	1.03752

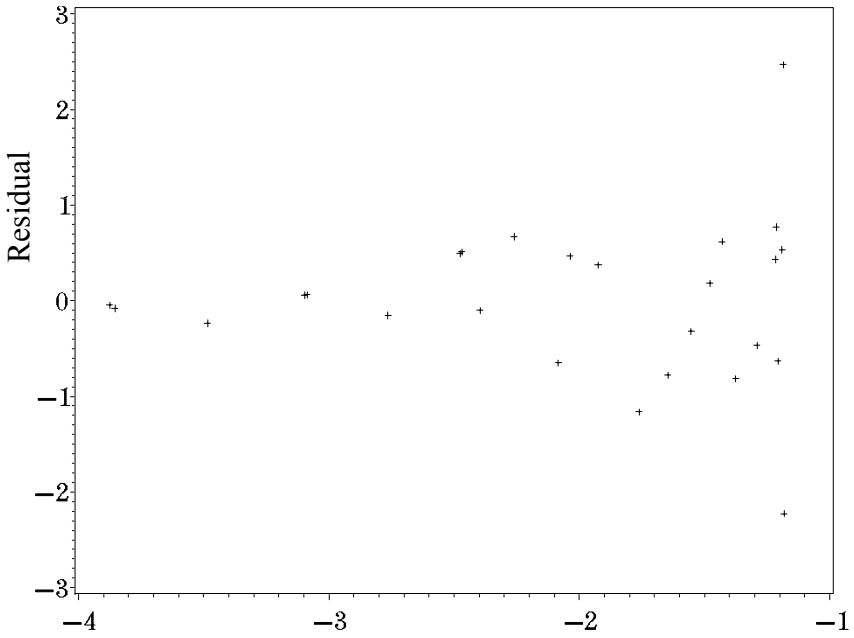


Figure G.6. Residual plots of predicted ln enterococci loading ratio in runoff from well managed pastureland.

Appendix H. Transport Plot Study Data: Comparison of Well-managed and Poorly-managed Pastureland

Table H.1. Transport Plot Study Bacteria, TSS, and Nutrient Results

Sample No.	Treatment	Time after runoff start	Runoff volume	Q (cfs)	*E. coli* TC (cfu/100 mL)	*E. coli* PC	*E. coli* PAF	ENT TC (cfu/100 mL)	ENT PC	ENT PAF	TSS (mg/L)	TDP (mg/L)	TP (mg/L)	DOP (mg/L)	TOP (mg/L)	DOC (mg/L)	TOC (mg/L)
P2-1	high veg.	1	0.05	0.00085	17400	0.0004	0.0004	2700	0.0024	0.0024	29	6.55	6.75	2.83	3.48	15.47	15.70
P2-2	high veg.	11	1.12	0.00186	19600	0.0002	0.0002	3800	0.0135	0.0133	18	6.36	6.70	3.18	3.45	16.50	18.14
P2-3	high veg.	21	1.35	0.00224	12900	0.0001	0.0001	3700	0.0011	0.0011	7	5.88	5.90	2.72	3.05	16.40	17.70
P2-4	high veg.	31	1.54	0.00257	10400	0.0004	0.0004	3900	0.0001	0.0001	10	5.80	5.96	2.84	3.08	15.30	16.71
P2-5	high veg.	41	1.63	0.00272	5200	0.0002	0.0002	2200	0.0103	0.0102	7	5.54	5.90	2.76	3.05	16.30	17.09
P2-6	high veg.	51	1.93	0.00321	7200	0.0006	0.0006	1700	0.0204	0.0200	6	4.86	4.92	2.33	2.56	15.90	17.60
P2-7	high veg.	61	2.07	0.00345	10400	0.0000	0.0000	3400	0.0056	0.0056	3	4.15	4.56	1.98	2.39	15.50	16.60
P2-8	high veg.	71	2.29	0.00382	6100	0.0001	0.0001	3100	0.0008	0.0008	7	4.74	5.06	2.33	2.63	15.60	15.77
P2-9	high veg.	81	2.12	0.00353	7000	0.0007	0.0007	3200			10	3.41	4.68	1.70	2.44	15.40	15.95
P2-10	high veg.	91	2.35	0.00392	5700			3400	0.0006	0.0006	11	4.45	4.82	2.11	2.42	15.00	17.47
P2-11	high veg.	101	2.73	0.00456	6600	0.0002	0.0002	4900			15	4.10	5.67	1.95	2.94	14.60	15.48
P2-12	high veg.	105	1.06	0.00441	6800	0.0002	0.0002	4500			11	2.37	3.77	1.07	2.00	15.40	16.37
P3-1	high veg.	1	0.03	0.00056	7800	0.0003	0.0003	1800	0.0856	0.0789	157	5.75	6.86	2.88	3.63	15.00	16.18
P3-2	high veg.	11	0.52	0.00086	10900	0.0015	0.0015	2700	0.0117	0.0116	151	4.88	5.85	2.44	3.00	15.30	16.18
P3-3	high veg.	21	0.66	0.00110	6700	0.0009	0.0009	2900	0.0009	0.0009	31	3.97	5.65	1.98	2.94	15.10	16.18
P3-4	high veg.	31	1.06	0.00177	4200	0.0002	0.0002	4100	0.0007	0.0007	14	3.83	4.22	1.92	2.22	15.00	15.80
P3-5	high veg.	41	1.09	0.00181	6000	0.0020	0.0020	4000	0.0009	0.0009	65	3.80	4.65	1.88	2.43	14.98	15.10
P3-6	high veg.	51	1.12	0.00186	5100	0.0014	0.0014	4100	0.0006	0.0006	29	3.65	3.78	1.77	2.00	12.91	14.50
P3-7	high veg.	55	0.27	0.00114	6500	0.0010	0.0010	4500			6	3.00	3.73	1.49	1.98	15.30	21.20
P4-1	low veg.	1	0.07	0.00109	5500	0.0014	0.0014	2200	0.0016	0.0016	173	6.90	7.65	3.45	3.93	14.90	16.30
P4-2	low veg.	11	1.01	0.00168	4200	0.0002	0.0002	2300	0.0046	0.0046	134	4.67	6.35	2.33	3.28	14.90	19.19
P4-3	low veg.	21	1.46	0.00244	3700	0.0027	0.0027	2400			141	5.54	6.15	2.76	3.19	14.90	15.25
P4-4	low veg.	31	2.02	0.00336	4900	0.0214	0.0210	5500	0.0073	0.0072	1029	5.67	5.86	2.78	3.04	15.70	16.53
P4-5	low veg.	41	2.23	0.00372	7200	0.0089	0.0088	4200	0.0124	0.0122	1123	5.40	5.63	2.69	2.92	15.70	16.39
P4-6	low veg.	51	2.29	0.00382	4600	0.0179	0.0176	4200	0.0006	0.0006	842	5.34	6.85	2.67	3.64	15.51	15.80
P4-7	low veg.	61	2.49	0.00415	6800	0.2991	0.2302	4800	0.0142	0.0140	2405	5.15	6.94	2.57	3.68	15.60	15.96
P4-8	low veg.	71	2.42	0.00404	5300	0.0150	0.0148	4900	0.0045	0.0045	895	4.80	6.23	2.39	3.23	15.30	15.79
P4-9	low veg.	75	0.77	0.00321	7100	0.0001	0.0001	4100	0.0008	0.0008	453	2.28	5.48	1.13	2.84	15.60	15.83

Sample No.	Treatment	Time after runoff start	Runoff volume	Q (cfs)	*E. coli* TC (cfu/100 mL)	*E. coli* PC	*E. coli* PAF	ENT TC (cfu/100 mL)	ENT PC	ENT PAF	TSS (mg/L)	TDP (mg/L)	TP (mg/L)	DOP (mg/L)	TOP (mg/L)	DOC (mg/L)	TOC (mg/L)
P5-1	low veg.	1	0.01	0.00024	10700	0.0009	0.0009	900	0.0008	0.0008	134	5.24	5.47	2.62	2.84	13.70	18.12
P5-2	low veg.	11	0.18	0.00030	9100	0.0005	0.0005	1400	0.0034	0.0034	137	3.93	5.99	1.96	3.11	12.80	15.92
P5-3	low veg.	21	0.20	0.00034	7900	0.0012	0.0012	1400	0.0003	0.0003	103	3.77	4.65	1.88	2.44	12.70	13.00
P5-4	low veg.	31	0.24	0.00040	10100	0.1033	0.0936	4900			344	4.75	5.18	2.37	2.69	13.40	14.20
P5-5	low veg.	41	0.76	0.00126	9300	0.1458	0.1272	4400	0.0052	0.0051	695	4.67	5.25	2.33	2.73	12.20	15.00
P5-6	low veg.	51	1.09	0.00181	5400	0.0114	0.0113	3200	0.0136	0.0134	946	4.77	6.77	2.38	3.50	14.20	14.80
P5-7	low veg.	61	1.16	0.00194	6300	0.0022	0.0022	2700	0.0007	0.0007	785	4.84	5.96	2.42	3.09	14.70	14.90
P5-8	low veg.	71	1.26	0.00211	7700	0.0009	0.0009	2700	0.0036	0.0036	345	4.36	5.55	2.17	2.89	13.50	15.00
P5-9	low veg.	81	1.26	0.00211	4400	0.0230	0.0225	4500	0.0101	0.0100	2720	4.40	6.75	2.19	3.49	13.40	14.20
P5-10	low veg.	91	1.35	0.00224	6300	0.0009	0.0009	5900	0.1286	0.1140	283	4.15	5.33	2.08	2.78	13.10	13.30
P5-11	low veg.	95	0.34	0.00141	7000	0.0006	0.0006	4200	0.0251	0.0245	208	4.00	4.25	1.99	2.23	14.20	14.40

Table H.2. Transport plot study preferential attachment to particulates

Sample no.	Screen Size	*E.coli* cfu/mg	Enterococci cfu/mg	Sample no.	Screen Size	*E.coli* cfu/mg	Enterococci cfu/mg	Sample no.	Screen Size	*E.coli* cfu/mg	Enterococci cfu/mg	Sample no.	Screen Size	*E.coli* cfu/mg	Enterococci cfu/mg
P2-1	500			P3-1	500	42	372	P4-1	500	1		P5-1	500		
	63				63	10			63		2		63		
	8				8		19		8				8		
P2-2	500			P3-2	500		17	P4-2	500	23	229	P5-2	500		
	63				63	21			63	25	231		63		24
	8				8	172	10		8		23		8		
P2-3	500			P3-3	500		7	P4-3	500			P5-3	500		
	63				63	33			63	48			63	82	12
	8				8				8				8	186	
P2-4	500			P3-4	500	111		P4-4	500		19	P5-4	500	25	
	63				63	30	23		63	3	3		63	8	
	8				8	15			8	53	11		8	4645	
P2-5	500			P3-5	500			P4-5	500	3	3	P5-5	500	20	41
	63				63	32			63	6	1		63	4	3
	8				8		11		8	54	26		8	2750	
P2-6	500			P3-6	500		85	P4-6	500			P5-6	500		
	63				63				63	1	1		63	2	11
	8				8	70	14		8	68			8	29	23
P2-7	500			P3-7	500			P4-7	500	2	8	P5-7	500	3	2
	63				63				63	578	9		63		1
	8				8				8	39	141		8		
P2-8	500							P4-8	500	2	1	P5-8	500	4	7
	63								63	1	2		63	1	1
	8								8	60	12		8		12
P2-9	500							P4-9	500			P5-9	500	6	4
	63								63	1			63	9	11
	8								8				8	86	16
P2-10	500											P5-10	500	117	59
	63												63	6	6
	8												8	14	71

Sample no.	Screen Size	*E.coli* cfu/mg	Enterococci cfu/mg	Sample no.	Screen Size	*E.coli* cfu/mg	Enterococci cfu/mg	Sample no.	Screen Size	*E.coli* cfu/mg	Enterococci cfu/mg	Sample no.	Screen Size	*E.coli* cfu/mg	Enterococci cfu/mg
P2-11	500											P5-11	500	2	6
	63												63	5	193
	8												8		
P2-12	500														
	63														
	8														

Appendix I. Supplemental Statistical Data for Transport Plot Study: Comparison of Well-managed and Poorly-managed Pastureland

I.1. Bacterial TC and PC Related to Flow Regime and Vegetative Cover

I.1.1. ln E. coli Total Concentration Full Model

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	1	33	0.45	0.5053
stage	2	33	3.65	0.0369
Treatment*stage	2	33	1.88	**0.1690**

Least Squares Means

Effect	stage	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment		50	8.7804	0.09502	33	92.40	<.0001
Treatment		100	8.8716	0.09655	33	91.88	<.0001
stage	peak		8.6660	0.09257	33	93.61	<.0001
stage	recession		8.8314	0.1664	33	53.08	<.0001
stage	rising		8.9805	0.07095	33	126.57	<.0001
Treatment*stage	peak	50	8.6861	0.1258	33	69.06	<.0001
Treatment*stage	recession	50	8.8608	0.2353	33	37.65	<.0001
Treatment*stage	rising	50	8.7943	0.1003	33	87.65	<.0001
Treatment*stage	peak	100	8.6460	0.1359	33	63.64	<.0001
Treatment*stage	recession	100	8.8021	0.2353	33	37.41	<.0001
Treatment*stage	rising	100	9.1667	0.1003	33	91.36	<.0001

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Estimate	Standard Error	DF	t Value
Treatment		50		100	-0.09124	0.1355	33	-0.67
stage	peak		recession		-0.1654	0.1904	33	-0.87
stage	peak		rising		-0.3145	0.1166	33	-2.70
stage	recession		rising		-0.1491	0.1809	33	-0.82
Treatment*stage	peak	50	recession	50	-0.1747	0.2668	33	-0.65
Treatment*stage	peak	50	rising	50	-0.1083	0.1609	33	-0.67
Treatment*stage	peak	50	peak	100	0.04005	0.1851	33	0.22
Treatment*stage	peak	50	recession	100	-0.1161	0.2668	33	-0.43
Treatment*stage	peak	50	rising	100	-0.4807	0.1609	33	-2.99
Treatment*stage	recession	50	rising	50	0.06643	0.2558	33	0.26
Treatment*stage	recession	50	peak	100	0.2147	0.2717	33	0.79
Treatment*stage	recession	50	recession	100	0.05864	0.3328	33	0.18
Treatment*stage	recession	50	rising	100	-0.3060	0.2558	33	-1.20
Treatment*stage	rising	50	peak	100	0.1483	0.1689	33	0.88
Treatment*stage	rising	50	recession	100	-0.00779	0.2558	33	-0.03
Treatment*stage	rising	50	rising	100	-0.3724	0.1419	33	-2.62
Treatment*stage	peak	100	recession	100	-0.1561	0.2717	33	-0.57
Treatment*stage	peak	100	rising	100	-0.5207	0.1689	33	-3.08
Treatment*stage	recession	100	rising	100	-0.3646	0.2558	33	-1.43

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Pr > |t|	Adjustment	Adj P
Treatment		50		100	0.5053	Tukey-Kramer	0.5053
stage	peak		recession		0.3913	Tukey-Kramer	0.6635
stage	peak		rising		0.0109	Tukey-Kramer	0.0287
stage	recession		rising		0.4157	Tukey-Kramer	0.6909
Treatment*stage	peak	50	recession	50	0.5172	Tukey-Kramer	0.9856
Treatment*stage	peak	50	rising	50	0.5057	Tukey-Kramer	0.9838
Treatment*stage	peak	50	peak	100	0.8301	Tukey-Kramer	0.9999
Treatment*stage	peak	50	recession	100	0.6664	Tukey-Kramer	0.9978
Treatment*stage	peak	50	rising	100	0.0053	Tukey-Kramer	0.0543
Treatment*stage	recession	50	rising	50	0.7967	Tukey-Kramer	0.9998
Treatment*stage	recession	50	peak	100	0.4350	Tukey-Kramer	0.9672
Treatment*stage	recession	50	recession	100	0.8612	Tukey-Kramer	1.0000
Treatment*stage	recession	50	rising	100	0.2402	Tukey-Kramer	0.8355
Treatment*stage	rising	50	peak	100	0.3862	Tukey-Kramer	0.9491
Treatment*stage	rising	50	recession	100	0.9759	Tukey-Kramer	1.0000
Treatment*stage	rising	50	rising	100	0.0130	Tukey-Kramer	0.1196
Treatment*stage	peak	100	recession	100	0.5695	Tukey-Kramer	0.9921
Treatment*stage	peak	100	rising	100	0.0041	Tukey-Kramer	0.0435
Treatment*stage	recession	100	rising	100	0.1635	Tukey-Kramer	0.7118

I.1.2. ln E. coli Total Concentration Reduced Model

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	1	35	3.06	0.0891
stage	2	35	3.30	0.0488

Least Squares Means

Effect	stage	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment		50	8.7334	0.08746	35	99.86	<.0001
Treatment		100	8.9246	0.08919	35	100.06	<.0001
stage	peak		8.6749	0.09468	35	91.63	<.0001
stage	recession		8.8314	0.1705	35	51.79	<.0001
stage	rising		8.9805	0.07271	35	123.52	<.0001

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment		50		100	-0.1912	0.1093	35	-1.75	0.0891
stage	peak		recession		-0.1565	0.1950	35	-0.80	0.4277
stage	peak		rising		-0.3056	0.1194	35	-2.56	0.0149
stage	recession		rising		-0.1491	0.1854	35	-0.80	0.4266

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Adjustment	Adj P
Treatment		50		100	Tukey-Kramer	0.0891
stage	peak		recession		Tukey-Kramer	0.7040
stage	peak		rising		Tukey-Kramer	0.0387
stage	recession		rising		Tukey-Kramer	0.7029

I.1.3. ln E. coli Partitioning Coefficient Full Model

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	1	32	4.79	0.0360
stage	2	32	2.80	0.0756
Treatment*stage	2	32	2.03	**0.1474**

Least Squares Means

Effect	stage	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment		50	-6.2633	0.4891	32	-12.81	<.0001
Treatment		100	-7.8065	0.5078	32	-15.37	<.0001
stage	peak		-5.9628	0.5015	32	-11.89	<.0001
stage	recession		-8.2032	0.8565	32	-9.58	<.0001
stage	rising		-6.9387	0.3652	32	-19.00	<.0001
Treatment*stage	peak	50	-4.5737	0.6475	32	-7.06	<.0001
Treatment*stage	recession	50	-8.6512	1.2113	32	-7.14	<.0001
Treatment*stage	rising	50	-5.5649	0.5165	32	-10.77	<.0001
Treatment*stage	peak	100	-7.3519	0.7661	32	-9.60	<.0001
Treatment*stage	recession	100	-7.7552	1.2113	32	-6.40	<.0001
Treatment*stage	rising	100	-8.3124	0.5165	32	-16.09	<.0001

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Estimate	Standard Error	DF	t Value
Treatment		50		100	1.5432	0.7051	32	2.19
stage	peak		recession		2.2404	0.9925	32	2.26
stage	peak		rising		0.9759	0.6204	32	1.57
stage	recession		rising		-1.2645	0.9311	32	-1.36
Treatment*stage	peak	50	recession	50	4.0775	1.3735	32	2.97
Treatment*stage	peak	50	rising	50	0.9912	0.8282	32	1.20
Treatment*stage	peak	50	peak	100	2.7782	1.0030	32	2.77
Treatment*stage	peak	50	recession	100	3.1815	1.3735	32	2.32
Treatment*stage	peak	50	rising	100	3.7387	0.8282	32	4.51
Treatment*stage	recession	50	rising	50	-3.0862	1.3168	32	-2.34
Treatment*stage	recession	50	peak	100	-1.2993	1.4332	32	-0.91
Treatment*stage	recession	50	recession	100	-0.8960	1.7130	32	-0.52
Treatment*stage	recession	50	rising	100	-0.3388	1.3168	32	-0.26
Treatment*stage	rising	50	peak	100	1.7870	0.9239	32	1.93
Treatment*stage	rising	50	recession	100	2.1903	1.3168	32	1.66
Treatment*stage	rising	50	rising	100	2.7475	0.7304	32	3.76
Treatment*stage	peak	100	recession	100	0.4033	1.4332	32	0.28
Treatment*stage	peak	100	rising	100	0.9605	0.9239	32	1.04
Treatment*stage	recession	100	rising	100	0.5572	1.3168	32	0.42

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Pr > |t|	Adjustment	Adj P
Treatment		50		100	0.0360	Tukey-Kramer	0.0360
stage	peak		recession		0.0310	Tukey-Kramer	0.0768
stage	peak		rising		0.1256	Tukey-Kramer	0.2718
stage	recession		rising		0.1839	Tukey-Kramer	0.3745
Treatment*stage	peak	50	recession	50	0.0056	Tukey-Kramer	0.0574
Treatment*stage	peak	50	rising	50	0.2402	Tukey-Kramer	0.8351
Treatment*stage	peak	50	peak	100	0.0093	Tukey-Kramer	0.0889
Treatment*stage	peak	50	recession	100	0.0271	Tukey-Kramer	0.2172
Treatment*stage	peak	50	rising	100	<.0001	Tukey-Kramer	0.0011
Treatment*stage	recession	50	rising	50	0.0255	Tukey-Kramer	0.2068
Treatment*stage	recession	50	peak	100	0.3714	Tukey-Kramer	0.9420
Treatment*stage	recession	50	recession	100	0.6045	Tukey-Kramer	0.9948
Treatment*stage	recession	50	rising	100	0.7986	Tukey-Kramer	0.9998
Treatment*stage	rising	50	peak	100	0.0620	Tukey-Kramer	0.4010
Treatment*stage	rising	50	recession	100	0.1060	Tukey-Kramer	0.5647
Treatment*stage	rising	50	rising	100	0.0007	Tukey-Kramer	0.0082
Treatment*stage	peak	100	recession	100	0.7802	Tukey-Kramer	0.9997
Treatment*stage	peak	100	rising	100	0.3063	Tukey-Kramer	0.9009
Treatment*stage	recession	100	rising	100	0.6750	Tukey-Kramer	0.9981

I.1.4. ln E. coli Partitioning Coefficient Reduced Model

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	1	34	16.99	0.0002
stage	2	34	2.77	0.0768

Least Squares Means

Effect	stage	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment		50	-5.8385	0.4525	34	-12.90	<.0001
Treatment		100	-8.2086	0.4723	34	-17.38	<.0001
stage	peak		-5.9288	0.5116	34	-11.59	<.0001
stage	recession		-8.2032	0.8822	34	-9.30	<.0001
stage	rising		-6.9387	0.3762	34	-18.45	<.0001

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment		50		100	2.3701	0.5750	34	4.12	0.0002
stage	peak		recession		2.2744	1.0198	34	2.23	0.0324
stage	peak		rising		1.0099	0.6350	34	1.59	0.1210
stage	recession		rising		-1.2645	0.9590	34	-1.32	0.1961

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Adjustment	Adj P
Treatment		50		100	Tukey-Kramer	0.0002
stage	peak		recession		Tukey-Kramer	0.0804
stage	peak		rising		Tukey-Kramer	0.2635
stage	recession		rising		Tukey-Kramer	0.3949

I.1.5. Enterococci Total Concentration Full Model

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	1	33	0.01	0.9365
stage	2	33	8.10	0.0014
Treatment*stage	2	33	0.40	**0.6753**

Least Squares Means

Effect	stage	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment		50	3817.53	298.66	33	12.78	<.0001
Treatment		100	3783.33	303.47	33	12.47	<.0001
stage	peak		4203.57	290.96	33	14.45	<.0001
stage	recession		4325.00	522.99	33	8.27	<.0001
stage	rising		2872.73	223.00	33	12.88	<.0001
Treatment*stage	peak	50	4457.14	395.34	33	11.27	<.0001
Treatment*stage	recession	50	4150.00	739.62	33	5.61	<.0001
Treatment*stage	rising	50	2845.45	315.37	33	9.02	<.0001
Treatment*stage	peak	100	3950.00	427.02	33	9.25	<.0001
Treatment*stage	recession	100	4500.00	739.62	33	6.08	<.0001
Treatment*stage	rising	100	2900.00	315.37	33	9.20	<.0001

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Estimate	Standard Error	DF	t Value
Treatment		50		100	34.1991	425.79	33	0.08
stage	peak		recession		-121.43	598.48	33	-0.20
stage	peak		rising		1330.84	366.59	33	3.63
stage	recession		rising		1452.27	568.55	33	2.55
Treatment*stage	peak	50	recession	50	307.14	838.65	33	0.37
Treatment*stage	peak	50	rising	50	1611.69	505.72	33	3.19
Treatment*stage	peak	50	peak	100	507.14	581.93	33	0.87
Treatment*stage	peak	50	recession	100	-42.8571	838.65	33	-0.05
Treatment*stage	peak	50	rising	100	1557.14	505.72	33	3.08
Treatment*stage	recession	50	rising	50	1304.55	804.05	33	1.62
Treatment*stage	recession	50	peak	100	200.00	854.04	33	0.23
Treatment*stage	recession	50	recession	100	-350.00	1045.98	33	-0.33
Treatment*stage	recession	50	rising	100	1250.00	804.05	33	1.55
Treatment*stage	rising	50	peak	100	-1104.55	530.85	33	-2.08
Treatment*stage	rising	50	recession	100	-1654.55	804.05	33	-2.06
Treatment*stage	rising	50	rising	100	-54.5455	446.01	33	-0.12
Treatment*stage	peak	100	recession	100	-550.00	854.04	33	-0.64
Treatment*stage	peak	100	rising	100	1050.00	530.85	33	1.98
Treatment*stage	recession	100	rising	100	1600.00	804.05	33	1.99

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Pr > |t|	Adjustment	Adj P
Treatment		50		100	0.9365	Tukey-Kramer	0.9365
stage	peak		recession		0.8405	Tukey-Kramer	0.9776
stage	peak		rising		0.0009	Tukey-Kramer	0.0027
stage	recession		rising		0.0154	Tukey-Kramer	0.0399
Treatment*stage	peak	50	recession	50	0.7165	Tukey-Kramer	0.9991
Treatment*stage	peak	50	rising	50	0.0031	Tukey-Kramer	0.0340
Treatment*stage	peak	50	peak	100	0.3898	Tukey-Kramer	0.9507
Treatment*stage	peak	50	recession	100	0.9596	Tukey-Kramer	1.0000
Treatment*stage	peak	50	rising	100	0.0042	Tukey-Kramer	0.0439
Treatment*stage	recession	50	rising	50	0.1142	Tukey-Kramer	0.5902
Treatment*stage	recession	50	peak	100	0.8163	Tukey-Kramer	0.9999
Treatment*stage	recession	50	recession	100	0.7400	Tukey-Kramer	0.9994
Treatment*stage	recession	50	rising	100	0.1296	Tukey-Kramer	0.6328
Treatment*stage	rising	50	peak	100	0.0453	Tukey-Kramer	0.3217
Treatment*stage	rising	50	recession	100	0.0476	Tukey-Kramer	0.3334
Treatment*stage	rising	50	rising	100	0.9034	Tukey-Kramer	1.0000
Treatment*stage	peak	100	recession	100	0.5240	Tukey-Kramer	0.9866
Treatment*stage	peak	100	rising	100	0.0563	Tukey-Kramer	0.3759
Treatment*stage	recession	100	rising	100	0.0549	Tukey-Kramer	0.3694

I.1.6. Enterococci Total Concentration Reduced Model

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	1	35	0.10	0.7597
stage	2	35	8.55	0.0010

Least Squares Means

Effect	stage	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment		50	3856.42	263.58	35	14.63	<.0001
Treatment		100	3754.84	268.81	35	13.97	<.0001
stage	peak		4219.17	285.34	35	14.79	<.0001
stage	recession		4325.00	513.91	35	8.42	<.0001
stage	rising		2872.73	219.13	35	13.11	<.0001

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment		50		100	101.58	329.49	35	0.31	0.7597
stage	peak		recession		-105.83	587.81	35	-0.18	0.8582
stage	peak		rising		1346.44	359.78	35	3.74	0.0007
stage	recession		rising		1452.27	558.67	35	2.60	0.0136

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Adjustment	Adj P
Treatment		50		100	Tukey-Kramer	0.7597
stage	peak		recession		Tukey-Kramer	0.9823
stage	peak		rising		Tukey-Kramer	0.0018
stage	recession		rising		Tukey-Kramer	0.0354

I.1.7. ln Enterococci Partitioning Coefficient Full Model

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	1	28	2.51	0.1245
stage	2	28	0.06	0.9413
Treatment*stage	1	28	5.47	**0.0267**

Least Squares Means

Effect	stage	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment		50	-5.4244	0.4671	28	-11.61	<.0001
Treatment		100	Non-est	.	.	.	.
stage	peak		-6.0356	0.5058	28	-11.93	<.0001
stage	recession		Non-est	.	.	.	.
stage	rising		-5.8191	0.3627	28	-16.04	<.0001
Treatment*stage	peak	50	-4.8149	0.6100	28	-7.89	<.0001
Treatment*stage	recession	50	-5.4043	1.1412	28	-4.74	<.0001
Treatment*stage	rising	50	-6.0541	0.5380	28	-11.25	<.0001
Treatment*stage	peak	100	-7.2564	0.8069	28	-8.99	<.0001
Treatment*stage	rising	100	-5.5840	0.4866	28	-11.48	<.0001

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Estimate	Standard Error	DF	t Value
Treatment		50		100	Non-est	.	.	.
stage	peak		recession		Non-est	.	.	.
stage	peak		rising		-0.2166	0.6224	28	-0.35
stage	recession		rising		Non-est	.	.	.
Treatment*stage	peak	50	recession	50	0.5895	1.2940	28	0.46
Treatment*stage	peak	50	rising	50	1.2392	0.8133	28	1.52
Treatment*stage	peak	50	peak	100	2.4415	1.0115	28	2.41
Treatment*stage	peak	50	rising	100	0.7692	0.7803	28	0.99
Treatment*stage	recession	50	rising	50	0.6498	1.2616	28	0.52
Treatment*stage	recession	50	peak	100	1.8521	1.3976	28	1.33
Treatment*stage	recession	50	rising	100	0.1797	1.2406	28	0.14
Treatment*stage	rising	50	peak	100	1.2023	0.9698	28	1.24
Treatment*stage	rising	50	rising	100	-0.4701	0.7254	28	-0.65
Treatment*stage	peak	100	rising	100	-1.6724	0.9423	28	-1.77

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Pr > |t|	Adjustment	Adj P
Treatment		50		100	.	Tukey-Kramer	.
stage	peak		recession		.	Tukey-Kramer	.
stage	peak		rising		0.7305	Tukey-Kramer	0.7305
stage	recession		rising		.	Tukey-Kramer	.
Treatment*stage	peak	50	recession	50	0.6522	Tukey-Kramer	0.9906
Treatment*stage	peak	50	rising	50	0.1388	Tukey-Kramer	0.5563
Treatment*stage	peak	50	peak	100	0.0226	Tukey-Kramer	0.1412
Treatment*stage	peak	50	rising	100	0.3327	Tukey-Kramer	0.8594
Treatment*stage	recession	50	rising	50	0.6106	Tukey-Kramer	0.9851
Treatment*stage	recession	50	peak	100	0.1958	Tukey-Kramer	0.6783
Treatment*stage	recession	50	rising	100	0.8859	Tukey-Kramer	0.9999
Treatment*stage	rising	50	peak	100	0.2254	Tukey-Kramer	0.7287
Treatment*stage	rising	50	rising	100	0.5222	Tukey-Kramer	0.9656
Treatment*stage	peak	100	rising	100	0.0868	Tukey-Kramer	0.4075

I.2. TSS and Nutrient TC and PC Related to Flow Regime and Vegetative Cover

I.2.1. ln Total Suspended Solids Full Model

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	1	33	72.25	<.0001
stage	2	33	2.25	0.1213
Treatment*stage	2	33	1.14	**0.3331**

Least Squares Means

Effect	stage	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment		50	6.0698	0.2856	33	21.25	<.0001
Treatment		100	2.6087	0.2902	33	8.99	<.0001
stage	peak		4.8757	0.2783	33	17.52	<.0001
stage	recession		3.9108	0.5002	33	7.82	<.0001
stage	rising		4.2312	0.2133	33	19.84	<.0001
Treatment*stage	peak	50	6.8199	0.3781	33	18.04	<.0001
Treatment*stage	recession	50	5.7267	0.7073	33	8.10	<.0001
Treatment*stage	rising	50	5.6628	0.3016	33	18.78	<.0001
Treatment*stage	peak	100	2.9315	0.4084	33	7.18	<.0001
Treatment*stage	recession	100	2.0948	0.7073	33	2.96	0.0056
Treatment*stage	rising	100	2.7996	0.3016	33	9.28	<.0001

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Estimate	Standard Error	DF	t Value
Treatment		50		100	3.4611	0.4072	33	8.50
stage	peak		recession		0.9649	0.5724	33	1.69
stage	peak		rising		0.6445	0.3506	33	1.84
stage	recession		rising		-0.3205	0.5437	33	-0.59
Treatment*stage	peak	50	recession	50	1.0931	0.8020	33	1.36
Treatment*stage	peak	50	rising	50	1.1570	0.4836	33	2.39
Treatment*stage	peak	50	peak	100	3.8883	0.5565	33	6.99
Treatment*stage	peak	50	recession	100	4.7250	0.8020	33	5.89
Treatment*stage	peak	50	rising	100	4.0202	0.4836	33	8.31
Treatment*stage	recession	50	rising	50	0.06387	0.7689	33	0.08
Treatment*stage	recession	50	peak	100	2.7952	0.8168	33	3.42
Treatment*stage	recession	50	recession	100	3.6319	1.0003	33	3.63
Treatment*stage	recession	50	rising	100	2.9271	0.7689	33	3.81
Treatment*stage	rising	50	peak	100	2.7313	0.5077	33	5.38
Treatment*stage	rising	50	recession	100	3.5680	0.7689	33	4.64
Treatment*stage	rising	50	rising	100	2.8632	0.4265	33	6.71
Treatment*stage	peak	100	recession	100	0.8367	0.8168	33	1.02
Treatment*stage	peak	100	rising	100	0.1319	0.5077	33	0.26
Treatment*stage	recession	100	rising	100	-0.7048	0.7689	33	-0.92

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Pr > |t|	Adjustment	Adj P
Treatment		50		100	<.0001	Tukey-Kramer	<.0001
stage	peak		recession		0.1012	Tukey-Kramer	0.2256
stage	peak		rising		0.0750	Tukey-Kramer	0.1730
stage	recession		rising		0.5596	Tukey-Kramer	0.8267
Treatment*stage	peak	50	recession	50	0.1821	Tukey-Kramer	0.7480
Treatment*stage	peak	50	rising	50	0.0226	Tukey-Kramer	0.1883
Treatment*stage	peak	50	peak	100	<.0001	Tukey-Kramer	<.0001
Treatment*stage	peak	50	recession	100	<.0001	Tukey-Kramer	<.0001
Treatment*stage	peak	50	rising	100	<.0001	Tukey-Kramer	<.0001
Treatment*stage	recession	50	rising	50	0.9343	Tukey-Kramer	1.0000
Treatment*stage	recession	50	peak	100	0.0017	Tukey-Kramer	0.0191
Treatment*stage	recession	50	recession	100	0.0009	Tukey-Kramer	0.0112
Treatment*stage	recession	50	rising	100	0.0006	Tukey-Kramer	0.0070
Treatment*stage	rising	50	peak	100	<.0001	Tukey-Kramer	<.0001
Treatment*stage	rising	50	recession	100	<.0001	Tukey-Kramer	0.0007
Treatment*stage	rising	50	rising	100	<.0001	Tukey-Kramer	<.0001
Treatment*stage	peak	100	recession	100	0.3131	Tukey-Kramer	0.9063
Treatment*stage	peak	100	rising	100	0.7966	Tukey-Kramer	0.9998
Treatment*stage	recession	100	rising	100	0.3660	Tukey-Kramer	0.9394

I.2.2. ln Total Suspended Solids Reduced Model

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	1	35	103.97	<.0001
stage	2	35	2.38	0.1075

Least Squares Means

Effect	stage	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment		50	5.9883	0.2575	35	23.25	<.0001
Treatment		100	2.7057	0.2626	35	10.30	<.0001
stage	peak		4.8990	0.2788	35	17.57	<.0001
stage	recession		3.9108	0.5021	35	7.79	<.0001
stage	rising		4.2312	0.2141	35	19.76	<.0001

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment		50		100	3.2826	0.3219	35	10.20	<.0001
stage	peak		recession		0.9882	0.5743	35	1.72	0.0941
stage	peak		rising		0.6678	0.3515	35	1.90	0.0657
stage	recession		rising		-0.3205	0.5458	35	-0.59	0.5609

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Adjustment	Adj P
Treatment		50		100	Tukey-Kramer	<.0001
stage	peak		recession		Tukey-Kramer	0.2118
stage	peak		rising		Tukey-Kramer	0.1538
stage	recession		rising		Tukey-Kramer	0.8279

I.2.3. ln Total Phosphorous Full Model

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	1	33	12.33	0.0013
stage	2	33	10.36	0.0003
Treatment*stage	2	33	4.91	**0.0136**

Least Squares Means

Effect	stage	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment		50	1.7209	0.03729	33	46.15	<.0001
Treatment		100	1.5343	0.03789	33	40.49	<.0001
stage	peak		1.6714	0.03633	33	46.01	<.0001
stage	recession		1.4479	0.06530	33	22.17	<.0001
stage	rising		1.7635	0.02784	33	63.33	<.0001
Treatment*stage	peak	50	1.8165	0.04936	33	36.80	<.0001
Treatment*stage	recession	50	1.5740	0.09235	33	17.04	<.0001
Treatment*stage	rising	50	1.7722	0.03938	33	45.01	<.0001
Treatment*stage	peak	100	1.5263	0.05332	33	28.63	<.0001
Treatment*stage	recession	100	1.3217	0.09235	33	14.31	<.0001
Treatment*stage	rising	100	1.7548	0.03938	33	44.56	<.0001

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Estimate	Standard Error	DF	t Value
Treatment		50		100	0.1867	0.05316	33	3.51
stage	peak		recession		0.2235	0.07473	33	2.99
stage	peak		rising		-0.09209	0.04577	33	-2.01
stage	recession		rising		-0.3156	0.07099	33	-4.45
Treatment*stage	peak	50	recession	50	0.2425	0.1047	33	2.32
Treatment*stage	peak	50	rising	50	0.04430	0.06315	33	0.70
Treatment*stage	peak	50	peak	100	0.2903	0.07266	33	3.99
Treatment*stage	peak	50	recession	100	0.4948	0.1047	33	4.73
Treatment*stage	peak	50	rising	100	0.06177	0.06315	33	0.98
Treatment*stage	recession	50	rising	50	-0.1982	0.1004	33	-1.97
Treatment*stage	recession	50	peak	100	0.04773	0.1066	33	0.45
Treatment*stage	recession	50	recession	100	0.2523	0.1306	33	1.93
Treatment*stage	recession	50	rising	100	-0.1808	0.1004	33	-1.80
Treatment*stage	rising	50	peak	100	0.2460	0.06628	33	3.71
Treatment*stage	rising	50	recession	100	0.4505	0.1004	33	4.49
Treatment*stage	rising	50	rising	100	0.01747	0.05569	33	0.31
Treatment*stage	peak	100	recession	100	0.2045	0.1066	33	1.92
Treatment*stage	peak	100	rising	100	-0.2285	0.06628	33	-3.45
Treatment*stage	recession	100	rising	100	-0.4330	0.1004	33	-4.31

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Pr > |t|	Adjustment	Adj P
Treatment		50		100	0.0013	Tukey-Kramer	0.0013
stage	peak		recession		0.0052	Tukey-Kramer	0.0141
stage	peak		rising		0.0524	Tukey-Kramer	0.1251
stage	recession		rising		<.0001	Tukey-Kramer	0.0003
Treatment*stage	peak	50	recession	50	0.0269	Tukey-Kramer	0.2165
Treatment*stage	peak	50	rising	50	0.4879	Tukey-Kramer	0.9805
Treatment*stage	peak	50	peak	100	0.0003	Tukey-Kramer	0.0042
Treatment*stage	peak	50	recession	100	<.0001	Tukey-Kramer	0.0005
Treatment*stage	peak	50	rising	100	0.3351	Tukey-Kramer	0.9216
Treatment*stage	recession	50	rising	50	0.0567	Tukey-Kramer	0.3778
Treatment*stage	recession	50	peak	100	0.6574	Tukey-Kramer	0.9975
Treatment*stage	recession	50	recession	100	0.0620	Tukey-Kramer	0.4019
Treatment*stage	recession	50	rising	100	0.0809	Tukey-Kramer	0.4793
Treatment*stage	rising	50	peak	100	0.0008	Tukey-Kramer	0.0091
Treatment*stage	rising	50	recession	100	<.0001	Tukey-Kramer	0.0011
Treatment*stage	rising	50	rising	100	0.7557	Tukey-Kramer	0.9996
Treatment*stage	peak	100	recession	100	0.0638	Tukey-Kramer	0.4096
Treatment*stage	peak	100	rising	100	0.0016	Tukey-Kramer	0.0179
Treatment*stage	recession	100	rising	100	0.0001	Tukey-Kramer	0.0018

I.2.4. Total Dissolved Phosphorous Full Model

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	1	33	1.29	0.2638
stage	2	33	16.65	<.0001
Treatment*stage	2	33	2.98	**0.0648**

Least Squares Means

Effect	stage	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment		50	4.3058	0.2144	33	20.08	<.0001
Treatment		100	3.9582	0.2179	33	18.17	<.0001
stage	peak		4.3367	0.2089	33	20.76	<.0001
stage	recession		2.9125	0.3755	33	7.76	<.0001
stage	rising		5.1468	0.1601	33	32.15	<.0001
Treatment*stage	peak	50	4.8000	0.2838	33	16.91	<.0001
Treatment*stage	recession	50	3.1400	0.5310	33	5.91	<.0001
Treatment*stage	rising	50	4.9773	0.2264	33	21.98	<.0001
Treatment*stage	peak	100	3.8733	0.3066	33	12.64	<.0001
Treatment*stage	recession	100	2.6850	0.5310	33	5.06	<.0001
Treatment*stage	rising	100	5.3164	0.2264	33	23.48	<.0001

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Estimate	Standard Error	DF	t Value
Treatment		50		100	0.3475	0.3057	33	1.14
stage	peak		recession		1.4242	0.4296	33	3.31
stage	peak		rising		-0.8102	0.2632	33	-3.08
stage	recession		rising		-2.2343	0.4082	33	-5.47
Treatment*stage	peak	50	recession	50	1.6600	0.6021	33	2.76
Treatment*stage	peak	50	rising	50	-0.1773	0.3631	33	-0.49
Treatment*stage	peak	50	peak	100	0.9267	0.4178	33	2.22
Treatment*stage	peak	50	recession	100	2.1150	0.6021	33	3.51
Treatment*stage	peak	50	rising	100	-0.5164	0.3631	33	-1.42
Treatment*stage	recession	50	rising	50	-1.8373	0.5772	33	-3.18
Treatment*stage	recession	50	peak	100	-0.7333	0.6131	33	-1.20
Treatment*stage	recession	50	recession	100	0.4550	0.7509	33	0.61
Treatment*stage	recession	50	rising	100	-2.1764	0.5772	33	-3.77
Treatment*stage	rising	50	peak	100	1.1039	0.3811	33	2.90
Treatment*stage	rising	50	recession	100	2.2923	0.5772	33	3.97
Treatment*stage	rising	50	rising	100	-0.3391	0.3202	33	-1.06
Treatment*stage	peak	100	recession	100	1.1883	0.6131	33	1.94
Treatment*stage	peak	100	rising	100	-1.4430	0.3811	33	-3.79
Treatment*stage	recession	100	rising	100	-2.6314	0.5772	33	-4.56

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Pr > |t|	Adjustment	Adj P
Treatment		50		100	0.2638	Tukey-Kramer	0.2638
stage	peak		recession		0.0022	Tukey-Kramer	0.0062
stage	peak		rising		0.0042	Tukey-Kramer	0.0113
stage	recession		rising		<.0001	Tukey-Kramer	<.0001
Treatment*stage	peak	50	recession	50	0.0094	Tukey-Kramer	0.0906
Treatment*stage	peak	50	rising	50	0.6286	Tukey-Kramer	0.9963
Treatment*stage	peak	50	peak	100	0.0335	Tukey-Kramer	0.2569
Treatment*stage	peak	50	recession	100	0.0013	Tukey-Kramer	0.0151
Treatment*stage	peak	50	rising	100	0.1643	Tukey-Kramer	0.7136
Treatment*stage	recession	50	rising	50	0.0032	Tukey-Kramer	0.0343
Treatment*stage	recession	50	peak	100	0.2402	Tukey-Kramer	0.8355
Treatment*stage	recession	50	recession	100	0.5487	Tukey-Kramer	0.9899
Treatment*stage	recession	50	rising	100	0.0006	Tukey-Kramer	0.0077
Treatment*stage	rising	50	peak	100	0.0066	Tukey-Kramer	0.0667
Treatment*stage	rising	50	recession	100	0.0004	Tukey-Kramer	0.0045
Treatment*stage	rising	50	rising	100	0.2973	Tukey-Kramer	0.8938
Treatment*stage	peak	100	recession	100	0.0612	Tukey-Kramer	0.3981
Treatment*stage	peak	100	rising	100	0.0006	Tukey-Kramer	0.0074
Treatment*stage	recession	100	rising	100	<.0001	Tukey-Kramer	0.0009

I.2.5. Total Dissolved Phosphorous Reduced Model

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	1	35	0.41	0.5257
stage	2	35	14.78	<.0001

Least Squares Means

Effect	stage	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment		50	4.2232	0.2031	35	20.79	<.0001
Treatment		100	4.0604	0.2072	35	19.60	<.0001
stage	peak		4.3660	0.2199	35	19.85	<.0001
stage	recession		2.9125	0.3961	35	7.35	<.0001
stage	rising		5.1468	0.1689	35	30.47	<.0001

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment		50		100	0.1628	0.2539	35	0.64	0.5257
stage	peak		recession		1.4535	0.4530	35	3.21	0.0029
stage	peak		rising		-0.7808	0.2773	35	-2.82	0.0079
stage	recession		rising		-2.2343	0.4306	35	-5.19	<.0001

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Adjustment	Adj P
Treatment		50		100	Tukey-Kramer	0.5257
stage	peak		recession		Tukey-Kramer	0.0078
stage	peak		rising		Tukey-Kramer	0.0211
stage	recession		rising		Tukey-Kramer	<.0001

I.2.6. Total Suspended Phosphorous Full Model

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	1	33	4.12	0.0504
stage	2	33	2.03	0.1480
Treatment*stage	2	33	0.08	**0.9191**

Least Squares Means

Effect	stage	Treatment	Standard Estimate	Error	DF	t Value	Pr > |t|
Treatment		50	1.3550	0.1986	33	6.82	<.0001
Treatment		100	0.7801	0.2018	33	3.86	0.0005
stage	peak		1.0731	0.1935	33	5.55	<.0001
stage	recession		1.3950	0.3478	33	4.01	0.0003
stage	rising		0.7345	0.1483	33	4.95	<.0001
Treatment*stage	peak	50	1.3829	0.2629	33	5.26	<.0001
Treatment*stage	recession	50	1.7250	0.4919	33	3.51	0.0013
Treatment*stage	rising	50	0.9573	0.2098	33	4.56	<.0001
Treatment*stage	peak	100	0.7633	0.2840	33	2.69	0.0112
Treatment*stage	recession	100	1.0650	0.4919	33	2.16	0.0377
Treatment*stage	rising	100	0.5118	0.2098	33	2.44	0.0202

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Standard Estimate	Error	DF	t Value
Treatment		50		100	0.5750	0.2832	33	2.03
stage	peak		recession		-0.3219	0.3981	33	-0.81
stage	peak		rising		0.3385	0.2438	33	1.39
stage	recession		rising		0.6605	0.3781	33	1.75
Treatment*stage	peak	50	recession	50	-0.3421	0.5578	33	-0.61
Treatment*stage	peak	50	rising	50	0.4256	0.3364	33	1.27
Treatment*stage	peak	50	peak	100	0.6195	0.3870	33	1.60
Treatment*stage	peak	50	recession	100	0.3179	0.5578	33	0.57
Treatment*stage	peak	50	rising	100	0.8710	0.3364	33	2.59
Treatment*stage	recession	50	rising	50	0.7677	0.5348	33	1.44
Treatment*stage	recession	50	peak	100	0.9617	0.5680	33	1.69
Treatment*stage	recession	50	recession	100	0.6600	0.6957	33	0.95
Treatment*stage	recession	50	rising	100	1.2132	0.5348	33	2.27
Treatment*stage	rising	50	peak	100	0.1939	0.3531	33	0.55
Treatment*stage	rising	50	recession	100	-0.1077	0.5348	33	-0.20
Treatment*stage	rising	50	rising	100	0.4455	0.2966	33	1.50
Treatment*stage	peak	100	recession	100	-0.3017	0.5680	33	-0.53
Treatment*stage	peak	100	rising	100	0.2515	0.3531	33	0.71
Treatment*stage	recession	100	rising	100	0.5532	0.5348	33	1.03

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Pr > |t|	Adjustment	Adj P
Treatment		50		100	0.0504	Tukey-Kramer	0.0504
stage	peak		recession		0.4245	Tukey-Kramer	0.7003
stage	peak		rising		0.1743	Tukey-Kramer	0.3584
stage	recession		rising		0.0900	Tukey-Kramer	0.2034
Treatment*stage	peak	50	recession	50	0.5438	Tukey-Kramer	0.9893
Treatment*stage	peak	50	rising	50	0.2146	Tukey-Kramer	0.8012
Treatment*stage	peak	50	peak	100	0.1190	Tukey-Kramer	0.6039
Treatment*stage	peak	50	recession	100	0.5726	Tukey-Kramer	0.9923
Treatment*stage	peak	50	rising	100	0.0142	Tukey-Kramer	0.1284
Treatment*stage	recession	50	rising	50	0.1605	Tukey-Kramer	0.7057
Treatment*stage	recession	50	peak	100	0.0999	Tukey-Kramer	0.5458
Treatment*stage	recession	50	recession	100	0.3497	Tukey-Kramer	0.9305
Treatment*stage	recession	50	rising	100	0.0300	Tukey-Kramer	0.2355
Treatment*stage	rising	50	peak	100	0.5865	Tukey-Kramer	0.9935
Treatment*stage	rising	50	recession	100	0.8416	Tukey-Kramer	0.9999
Treatment*stage	rising	50	rising	100	0.1427	Tukey-Kramer	0.6656
Treatment*stage	peak	100	recession	100	0.5989	Tukey-Kramer	0.9945
Treatment*stage	peak	100	rising	100	0.4813	Tukey-Kramer	0.9791
Treatment*stage	recession	100	rising	100	0.3085	Tukey-Kramer	0.9028

I.2.7. Total Suspended Phosphorous Reduced Model

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	1	35	5.85	0.0209
stage	2	35	2.16	0.1310

Least Squares Means

Effect	stage	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment		50	1.3314	0.1737	35	7.67	<.0001
Treatment		100	0.8061	0.1771	35	4.55	<.0001
stage	peak		1.0767	0.1880	35	5.73	<.0001
stage	recession		1.3950	0.3386	35	4.12	0.0002
stage	rising		0.7345	0.1444	35	5.09	<.0001

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment		50		100	0.5253	0.2171	35	2.42	0.0209
stage	peak		recession		-0.3183	0.3873	35	-0.82	0.4168
stage	peak		rising		0.3422	0.2371	35	1.44	0.1578
stage	recession		rising		0.6605	0.3681	35	1.79	0.0814

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Adjustment	Adj P
Treatment		50		100	Tukey-Kramer	0.0209
stage	peak		recession		Tukey-Kramer	0.6922
stage	peak		rising		Tukey-Kramer	0.3303
stage	recession		rising		Tukey-Kramer	0.1863

I.2.8. ln Phosphorous Partitioning Coefficient Full Model

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	1	33	0.89	0.3513
stage	2	33	3.39	0.0459
Treatment*stage	2	33	0.62	**0.5422**

Least Squares Means

Effect	stage	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment		50	-1.5087	0.3150	33	-4.79	<.0001
Treatment		100	-1.9332	0.3201	33	-6.04	<.0001
stage	peak		-1.6677	0.3069	33	-5.43	<.0001
stage	recession		-1.0933	0.5516	33	-1.98	0.0559
stage	rising		-2.4019	0.2352	33	-10.21	<.0001
Treatment*stage	peak	50	-1.4100	0.4170	33	-3.38	0.0019
Treatment*stage	recession	50	-1.2168	0.7801	33	-1.56	0.1283
Treatment*stage	rising	50	-1.8992	0.3326	33	-5.71	<.0001
Treatment*stage	peak	100	-1.9253	0.4504	33	-4.27	0.0002
Treatment*stage	recession	100	-0.9699	0.7801	33	-1.24	0.2225
Treatment*stage	rising	100	-2.9046	0.3326	33	-8.73	<.0001

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Estimate	Standard Error	DF	t Value
Treatment		50		100	0.4246	0.4491	33	0.95
stage	peak		recession		-0.5743	0.6312	33	-0.91
stage	peak		rising		0.7342	0.3867	33	1.90
stage	recession		rising		1.3086	0.5997	33	2.18
Treatment*stage	peak	50	recession	50	-0.1932	0.8846	33	-0.22
Treatment*stage	peak	50	rising	50	0.4892	0.5334	33	0.92
Treatment*stage	peak	50	peak	100	0.5153	0.6138	33	0.84
Treatment*stage	peak	50	recession	100	-0.4402	0.8846	33	-0.50
Treatment*stage	peak	50	rising	100	1.4946	0.5334	33	2.80
Treatment*stage	recession	50	rising	50	0.6824	0.8481	33	0.80
Treatment*stage	recession	50	peak	100	0.7085	0.9008	33	0.79
Treatment*stage	recession	50	recession	100	-0.2469	1.1032	33	-0.22
Treatment*stage	recession	50	rising	100	1.6878	0.8481	33	1.99
Treatment*stage	rising	50	peak	100	0.02607	0.5599	33	0.05
Treatment*stage	rising	50	recession	100	-0.9293	0.8481	33	-1.10
Treatment*stage	rising	50	rising	100	1.0054	0.4704	33	2.14
Treatment*stage	peak	100	recession	100	-0.9554	0.9008	33	-1.06
Treatment*stage	peak	100	rising	100	0.9793	0.5599	33	1.75
Treatment*stage	recession	100	rising	100	1.9347	0.8481	33	2.28

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Pr > |t|	Adjustment	Adj P
Treatment		50		100	0.3513	Tukey-Kramer	0.3513
stage	peak		recession		0.3695	Tukey-Kramer	0.6380
stage	peak		rising		0.0663	Tukey-Kramer	0.1549
stage	recession		rising		0.0363	Tukey-Kramer	0.0892
Treatment*stage	peak	50	recession	50	0.8284	Tukey-Kramer	0.9999
Treatment*stage	peak	50	rising	50	0.3657	Tukey-Kramer	0.9393
Treatment*stage	peak	50	peak	100	0.4072	Tukey-Kramer	0.9578
Treatment*stage	peak	50	recession	100	0.6221	Tukey-Kramer	0.9959
Treatment*stage	peak	50	rising	100	0.0084	Tukey-Kramer	0.0822
Treatment*stage	recession	50	rising	50	0.4268	Tukey-Kramer	0.9646
Treatment*stage	recession	50	peak	100	0.4372	Tukey-Kramer	0.9679
Treatment*stage	recession	50	recession	100	0.8243	Tukey-Kramer	0.9999
Treatment*stage	recession	50	rising	100	0.0549	Tukey-Kramer	0.3692
Treatment*stage	rising	50	peak	100	0.9632	Tukey-Kramer	1.0000
Treatment*stage	rising	50	recession	100	0.2811	Tukey-Kramer	0.8795
Treatment*stage	rising	50	rising	100	0.0401	Tukey-Kramer	0.2939
Treatment*stage	peak	100	recession	100	0.2966	Tukey-Kramer	0.8932
Treatment*stage	peak	100	rising	100	0.0896	Tukey-Kramer	0.5109
Treatment*stage	recession	100	rising	100	0.0291	Tukey-Kramer	0.2302

I.2.9. ln Phosphorous Partitioning Coefficient Reduced Model

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	1	35	4.16	0.0489
stage	2	35	3.44	0.0434

Least Squares Means

Effect	stage	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment		50	-1.3665	0.2799	35	-4.88	<.0001
Treatment		100	-2.0805	0.2854	35	-7.29	<.0001
stage	peak		-1.6753	0.3030	35	-5.53	<.0001
stage	recession		-1.0933	0.5457	35	-2.00	0.0529
stage	rising		-2.4019	0.2327	35	-10.32	<.0001

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment		50		100	0.7139	0.3498	35	2.04	0.0489
stage	peak		recession		-0.5820	0.6241	35	-0.93	0.3575
stage	peak		rising		0.7266	0.3820	35	1.90	0.0654
stage	recession		rising		1.3086	0.5932	35	2.21	0.0340

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Adjustment	Adj P
Treatment		50		100	Tukey-Kramer	0.0489
stage	peak		recession		Tukey-Kramer	0.6237
stage	peak		rising		Tukey-Kramer	0.1531
stage	recession		rising		Tukey-Kramer	0.0841

I.2.10. ln Total Organic Phosphorous Full Model

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	1	33	12.29	0.0013
stage	2	33	9.89	0.0004
Treatment*stage	2	33	5.20	**0.0109**

Least Squares Means

Effect	stage	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment		50	1.0683	0.03649	33	29.27	<.0001
Treatment		100	0.8859	0.03708	33	23.89	<.0001
stage	peak		1.0194	0.03555	33	28.67	<.0001
stage	recession		0.8050	0.06390	33	12.60	<.0001
stage	rising		1.1069	0.02725	33	40.62	<.0001
Treatment*stage	peak	50	1.1667	0.04830	33	24.15	<.0001
Treatment*stage	recession	50	0.9231	0.09037	33	10.21	<.0001
Treatment*stage	rising	50	1.1151	0.03853	33	28.94	<.0001
Treatment*stage	peak	100	0.8721	0.05217	33	16.71	<.0001
Treatment*stage	recession	100	0.6869	0.09037	33	7.60	<.0001
Treatment*stage	rising	100	1.0987	0.03853	33	28.51	<.0001

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Estimate	Standard Error	DF	t Value
Treatment		50		100	0.1824	0.05202	33	3.51
stage	peak		recession		0.2144	0.07312	33	2.93
stage	peak		rising		-0.08751	0.04479	33	-1.95
stage	recession		rising		-0.3019	0.06947	33	-4.35
Treatment*stage	peak	50	recession	50	0.2436	0.1025	33	2.38
Treatment*stage	peak	50	rising	50	0.05161	0.06179	33	0.84
Treatment*stage	peak	50	peak	100	0.2946	0.07110	33	4.14
Treatment*stage	peak	50	recession	100	0.4798	0.1025	33	4.68
Treatment*stage	peak	50	rising	100	0.06795	0.06179	33	1.10
Treatment*stage	recession	50	rising	50	-0.1920	0.09824	33	-1.95
Treatment*stage	recession	50	peak	100	0.05097	0.1043	33	0.49
Treatment*stage	recession	50	recession	100	0.2362	0.1278	33	1.85
Treatment*stage	recession	50	rising	100	-0.1757	0.09824	33	-1.79
Treatment*stage	rising	50	peak	100	0.2430	0.06486	33	3.75
Treatment*stage	rising	50	recession	100	0.4282	0.09824	33	4.36
Treatment*stage	rising	50	rising	100	0.01633	0.05449	33	0.30
Treatment*stage	peak	100	recession	100	0.1852	0.1043	33	1.78
Treatment*stage	peak	100	rising	100	-0.2266	0.06486	33	-3.49
Treatment*stage	recession	100	rising	100	-0.4119	0.09824	33	-4.19

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Pr > |t|	Adjustment	Adj P
Treatment		50		100	0.0013	Tukey-Kramer	0.0013
stage	peak		recession		0.0061	Tukey-Kramer	0.0163
stage	peak		rising		0.0593	Tukey-Kramer	0.1399
stage	recession		rising		0.0001	Tukey-Kramer	0.0004
Treatment*stage	peak	50	recession	50	0.0234	Tukey-Kramer	0.1936
Treatment*stage	peak	50	rising	50	0.4096	Tukey-Kramer	0.9586
Treatment*stage	peak	50	peak	100	0.0002	Tukey-Kramer	0.0028
Treatment*stage	peak	50	recession	100	<.0001	Tukey-Kramer	0.0006
Treatment*stage	peak	50	rising	100	0.2795	Tukey-Kramer	0.8780
Treatment*stage	recession	50	rising	50	0.0592	Tukey-Kramer	0.3891
Treatment*stage	recession	50	peak	100	0.6284	Tukey-Kramer	0.9963
Treatment*stage	recession	50	recession	100	0.0735	Tukey-Kramer	0.4504
Treatment*stage	recession	50	rising	100	0.0830	Tukey-Kramer	0.4869
Treatment*stage	rising	50	peak	100	0.0007	Tukey-Kramer	0.0083
Treatment*stage	rising	50	recession	100	0.0001	Tukey-Kramer	0.0016
Treatment*stage	rising	50	rising	100	0.7663	Tukey-Kramer	0.9996
Treatment*stage	peak	100	recession	100	0.0851	Tukey-Kramer	0.4947
Treatment*stage	peak	100	rising	100	0.0014	Tukey-Kramer	0.0159
Treatment*stage	recession	100	rising	100	0.0002	Tukey-Kramer	0.0025

I.2.11. Dissolved Organic Phosphorous Full Model

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	1	33	2.53	0.1209
stage	2	33	17.52	<.0001
Treatment*stage	2	33	2.85	**0.0719**

Least Squares Means

Effect	stage	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment		50	2.1450	0.1026	33	20.91	<.0001
Treatment		100	1.9123	0.1042	33	18.35	<.0001
stage	peak		2.1396	0.09993	33	21.41	<.0001
stage	recession		1.4215	0.1796	33	7.91	<.0001
stage	rising		2.5249	0.07659	33	32.97	<.0001
Treatment*stage	peak	50	2.3920	0.1358	33	17.62	<.0001
Treatment*stage	recession	50	1.5625	0.2540	33	6.15	<.0001
Treatment*stage	rising	50	2.4806	0.1083	33	22.90	<.0001
Treatment*stage	peak	100	1.8872	0.1467	33	12.87	<.0001
Treatment*stage	recession	100	1.2805	0.2540	33	5.04	<.0001
Treatment*stage	rising	100	2.5691	0.1083	33	23.72	<.0001

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Estimate	Standard Error	DF	t Value
Treatment		50		100	0.2328	0.1462	33	1.59
stage	peak		recession		0.7181	0.2055	33	3.49
stage	peak		rising		-0.3853	0.1259	33	-3.06
stage	recession		rising		-1.1034	0.1953	33	-5.65
Treatment*stage	peak	50	recession	50	0.8295	0.2880	33	2.88
Treatment*stage	peak	50	rising	50	-0.08864	0.1737	33	-0.51
Treatment*stage	peak	50	peak	100	0.5048	0.1999	33	2.53
Treatment*stage	peak	50	recession	100	1.1115	0.2880	33	3.86
Treatment*stage	peak	50	rising	100	-0.1771	0.1737	33	-1.02
Treatment*stage	recession	50	rising	50	-0.9181	0.2761	33	-3.32
Treatment*stage	recession	50	peak	100	-0.3247	0.2933	33	-1.11
Treatment*stage	recession	50	recession	100	0.2820	0.3592	33	0.79
Treatment*stage	recession	50	rising	100	-1.0066	0.2761	33	-3.65
Treatment*stage	rising	50	peak	100	0.5935	0.1823	33	3.26
Treatment*stage	rising	50	recession	100	1.2001	0.2761	33	4.35
Treatment*stage	rising	50	rising	100	-0.08845	0.1532	33	-0.58
Treatment*stage	peak	100	recession	100	0.6067	0.2933	33	2.07
Treatment*stage	peak	100	rising	100	-0.6819	0.1823	33	-3.74
Treatment*stage	recession	100	rising	100	-1.2886	0.2761	33	-4.67

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Pr > |t|	Adjustment	Adj P
Treatment		50		100	0.1209	Tukey-Kramer	0.1209
stage	peak		recession		0.0014	Tukey-Kramer	0.0038
stage	peak		rising		0.0044	Tukey-Kramer	0.0119
stage	recession		rising		<.0001	Tukey-Kramer	<.0001
Treatment*stage	peak	50	recession	50	0.0069	Tukey-Kramer	0.0693
Treatment*stage	peak	50	rising	50	0.6132	Tukey-Kramer	0.9954
Treatment*stage	peak	50	peak	100	0.0165	Tukey-Kramer	0.1458
Treatment*stage	peak	50	recession	100	0.0005	Tukey-Kramer	0.0061
Treatment*stage	peak	50	rising	100	0.3153	Tukey-Kramer	0.9080
Treatment*stage	recession	50	rising	50	0.0022	Tukey-Kramer	0.0243
Treatment*stage	recession	50	peak	100	0.2763	Tukey-Kramer	0.8750
Treatment*stage	recession	50	recession	100	0.4380	Tukey-Kramer	0.9682
Treatment*stage	recession	50	rising	100	0.0009	Tukey-Kramer	0.0108
Treatment*stage	rising	50	peak	100	0.0026	Tukey-Kramer	0.0288
Treatment*stage	rising	50	recession	100	0.0001	Tukey-Kramer	0.0016
Treatment*stage	rising	50	rising	100	0.5675	Tukey-Kramer	0.9919
Treatment*stage	peak	100	recession	100	0.0465	Tukey-Kramer	0.3279
Treatment*stage	peak	100	rising	100	0.0007	Tukey-Kramer	0.0084
Treatment*stage	recession	100	rising	100	<.0001	Tukey-Kramer	0.0006

I.2.12. Dissolved Organic Phosphorous Reduced Model

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	1	35	1.47	0.2342
stage	2	35	15.67	<.0001

Least Squares Means

Effect	stage	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment		50	2.1065	0.09688	35	21.74	<.0001
Treatment		100	1.9599	0.09881	35	19.84	<.0001
stage	peak		2.1534	0.1049	35	20.53	<.0001
stage	recession		1.4215	0.1889	35	7.53	<.0001
stage	rising		2.5249	0.08054	35	31.35	<.0001

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment		50		100	0.1466	0.1211	35	1.21	0.2342
stage	peak		recession		0.7319	0.2161	35	3.39	0.0018
stage	peak		rising		-0.3715	0.1322	35	-2.81	0.0081
stage	recession		rising		-1.1034	0.2053	35	-5.37	<.0001

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Adjustment	Adj P
Treatment		50		100	Tukey-Kramer	0.2342
stage	peak		recession		Tukey-Kramer	0.0049
stage	peak		rising		Tukey-Kramer	0.0215
stage	recession		rising		Tukey-Kramer	<.0001

I.2.13. Suspended Organic Phosphorous Full Model

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	1	33	2.87	0.0997
stage	2	33	1.79	0.1827
Treatment*stage	2	33	0.28	**0.7580**

Least Squares Means

Effect	stage	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment		50	0.8016	0.09966	33	8.04	<.0001
Treatment		100	0.5609	0.1013	33	5.54	<.0001
stage	peak		0.6796	0.09709	33	7.00	<.0001
stage	recession		0.8400	0.1745	33	4.81	<.0001
stage	rising		0.5241	0.07441	33	7.04	<.0001
Treatment*stage	peak	50	0.8381	0.1319	33	6.35	<.0001
Treatment*stage	recession	50	0.9730	0.2468	33	3.94	0.0004
Treatment*stage	rising	50	0.5935	0.1052	33	5.64	<.0001
Treatment*stage	peak	100	0.5210	0.1425	33	3.66	0.0009
Treatment*stage	recession	100	0.7070	0.2468	33	2.86	0.0072
Treatment*stage	rising	100	0.4547	0.1052	33	4.32	0.0001

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Estimate	Standard Error	DF	t Value
Treatment		50		100	0.2407	0.1421	33	1.69
stage	peak		recession		-0.1604	0.1997	33	-0.80
stage	peak		rising		0.1554	0.1223	33	1.27
stage	recession		rising		0.3159	0.1897	33	1.66
Treatment*stage	peak	50	recession	50	-0.1349	0.2798	33	-0.48
Treatment*stage	peak	50	rising	50	0.2446	0.1688	33	1.45
Treatment*stage	peak	50	peak	100	0.3171	0.1942	33	1.63
Treatment*stage	peak	50	recession	100	0.1311	0.2798	33	0.47
Treatment*stage	peak	50	rising	100	0.3834	0.1688	33	2.27
Treatment*stage	recession	50	rising	50	0.3795	0.2683	33	1.41
Treatment*stage	recession	50	peak	100	0.4520	0.2850	33	1.59
Treatment*stage	recession	50	recession	100	0.2660	0.3490	33	0.76
Treatment*stage	recession	50	rising	100	0.5183	0.2683	33	1.93
Treatment*stage	rising	50	peak	100	0.07255	0.1771	33	0.41
Treatment*stage	rising	50	recession	100	-0.1135	0.2683	33	-0.42
Treatment*stage	rising	50	rising	100	0.1388	0.1488	33	0.93
Treatment*stage	peak	100	recession	100	-0.1860	0.2850	33	-0.65
Treatment*stage	peak	100	rising	100	0.06627	0.1771	33	0.37
Treatment*stage	recession	100	rising	100	0.2523	0.2683	33	0.94

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Pr > |t|	Adjustment	Adj P
Treatment		50		100	0.0997	Tukey-Kramer	0.0997
stage	peak		recession		0.4275	Tukey-Kramer	0.7036
stage	peak		rising		0.2127	Tukey-Kramer	0.4214
stage	recession		rising		0.1054	Tukey-Kramer	0.2337
Treatment*stage	peak	50	recession	50	0.6331	Tukey-Kramer	0.9965
Treatment*stage	peak	50	rising	50	0.1567	Tukey-Kramer	0.6974
Treatment*stage	peak	50	peak	100	0.1119	Tukey-Kramer	0.5834
Treatment*stage	peak	50	recession	100	0.6424	Tukey-Kramer	0.9969
Treatment*stage	peak	50	rising	100	0.0297	Tukey-Kramer	0.2340
Treatment*stage	recession	50	rising	50	0.1666	Tukey-Kramer	0.7183
Treatment*stage	recession	50	peak	100	0.1223	Tukey-Kramer	0.6131
Treatment*stage	recession	50	recession	100	0.4514	Tukey-Kramer	0.9720
Treatment*stage	recession	50	rising	100	0.0620	Tukey-Kramer	0.4018
Treatment*stage	rising	50	peak	100	0.6848	Tukey-Kramer	0.9984
Treatment*stage	rising	50	recession	100	0.6751	Tukey-Kramer	0.9981
Treatment*stage	rising	50	rising	100	0.3577	Tukey-Kramer	0.9350
Treatment*stage	peak	100	recession	100	0.5185	Tukey-Kramer	0.9858
Treatment*stage	peak	100	rising	100	0.7107	Tukey-Kramer	0.9990
Treatment*stage	recession	100	rising	100	0.3539	Tukey-Kramer	0.9329

I.2.14. Suspended Organic Phosphorous Reduced Model

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	1	35	3.71	0.0622
stage	2	35	1.90	0.1645

Least Squares Means

Effect	stage	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment		50	0.7881	0.08765	35	8.99	<.0001
Treatment		100	0.5770	0.08939	35	6.46	<.0001
stage	peak		0.6837	0.09488	35	7.21	<.0001
stage	recession		0.8400	0.1709	35	4.92	<.0001
stage	rising		0.5241	0.07287	35	7.19	<.0001

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment		50		100	0.2111	0.1096	35	1.93	0.0622
stage	peak		recession		-0.1563	0.1955	35	-0.80	0.4292
stage	peak		rising		0.1595	0.1196	35	1.33	0.1910
stage	recession		rising		0.3159	0.1858	35	1.70	0.0980

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Adjustment	Adj P
Treatment		50		100	Tukey-Kramer	0.0622
stage	peak		recession		Tukey-Kramer	0.7056
stage	peak		rising		Tukey-Kramer	0.3866
stage	recession		rising		Tukey-Kramer	0.2194

I.2.15. ln Organic Phosphorous Partitioning Coefficient Full Model

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	1	33	0.15	0.7049
stage	2	33	4.03	0.0271
Treatment*stage	2	33	0.25	**0.7817**

Least Squares Means

Effect	stage	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment		50	-1.1911	0.1931	33	-6.17	<.0001
Treatment		100	-1.2962	0.1962	33	-6.61	<.0001
stage	peak		-1.2865	0.1881	33	-6.84	<.0001
stage	recession		-0.7455	0.3381	33	-2.21	0.0345
stage	rising		-1.6990	0.1441	33	-11.79	<.0001
Treatment*stage	peak	50	-1.1515	0.2555	33	-4.51	<.0001
Treatment*stage	recession	50	-0.8603	0.4781	33	-1.80	0.0811
Treatment*stage	rising	50	-1.5614	0.2039	33	-7.66	<.0001
Treatment*stage	peak	100	-1.4214	0.2760	33	-5.15	<.0001
Treatment*stage	recession	100	-0.6308	0.4781	33	-1.32	0.1961
Treatment*stage	rising	100	-1.8366	0.2039	33	-9.01	<.0001

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Estimate	Standard Error	DF	t Value
Treatment		50		100	0.1051	0.2752	33	0.38
stage	peak		recession		-0.5409	0.3869	33	-1.40
stage	peak		rising		0.4125	0.2370	33	1.74
stage	recession		rising		0.9534	0.3675	33	2.59
Treatment*stage	peak	50	recession	50	-0.2912	0.5421	33	-0.54
Treatment*stage	peak	50	rising	50	0.4099	0.3269	33	1.25
Treatment*stage	peak	50	peak	100	0.2699	0.3762	33	0.72
Treatment*stage	peak	50	recession	100	-0.5208	0.5421	33	-0.96
Treatment*stage	peak	50	rising	100	0.6850	0.3269	33	2.10
Treatment*stage	recession	50	rising	50	0.7011	0.5197	33	1.35
Treatment*stage	recession	50	peak	100	0.5611	0.5520	33	1.02
Treatment*stage	recession	50	recession	100	-0.2296	0.6761	33	-0.34
Treatment*stage	recession	50	rising	100	0.9762	0.5197	33	1.88
Treatment*stage	rising	50	peak	100	-0.1400	0.3431	33	-0.41
Treatment*stage	rising	50	recession	100	-0.9306	0.5197	33	-1.79
Treatment*stage	rising	50	rising	100	0.2752	0.2883	33	0.95
Treatment*stage	peak	100	recession	100	-0.7907	0.5520	33	-1.43
Treatment*stage	peak	100	rising	100	0.4152	0.3431	33	1.21
Treatment*stage	recession	100	rising	100	1.2058	0.5197	33	2.32

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Pr > |t|	Adjustment	Adj P
Treatment		50		100	0.7049	Tukey-Kramer	0.7049
stage	peak		recession		0.1714	Tukey-Kramer	0.3534
stage	peak		rising		0.0910	Tukey-Kramer	0.2055
stage	recession		rising		0.0140	Tukey-Kramer	0.0364
Treatment*stage	peak	50	recession	50	0.5947	Tukey-Kramer	0.9942
Treatment*stage	peak	50	rising	50	0.2187	Tukey-Kramer	0.8071
Treatment*stage	peak	50	peak	100	0.4782	Tukey-Kramer	0.9784
Treatment*stage	peak	50	recession	100	0.3437	Tukey-Kramer	0.9270
Treatment*stage	peak	50	rising	100	0.0439	Tukey-Kramer	0.3142
Treatment*stage	recession	50	rising	50	0.1866	Tukey-Kramer	0.7560
Treatment*stage	recession	50	peak	100	0.3169	Tukey-Kramer	0.9091
Treatment*stage	recession	50	recession	100	0.7363	Tukey-Kramer	0.9993
Treatment*stage	recession	50	rising	100	0.0692	Tukey-Kramer	0.4327
Treatment*stage	rising	50	peak	100	0.6859	Tukey-Kramer	0.9984
Treatment*stage	rising	50	recession	100	0.0825	Tukey-Kramer	0.4853
Treatment*stage	rising	50	rising	100	0.3468	Tukey-Kramer	0.9288
Treatment*stage	peak	100	recession	100	0.1615	Tukey-Kramer	0.7077
Treatment*stage	peak	100	rising	100	0.2349	Tukey-Kramer	0.8289
Treatment*stage	recession	100	rising	100	0.0267	Tukey-Kramer	0.2149

I.2.16. ln Organic Phosphorous Partitioning Coefficient Reduced Model

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	1	35	1.09	0.3033
stage	2	35	4.23	0.0227

Least Squares Means

Effect	stage	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment		50	-1.1323	0.1696	35	-6.68	<.0001
Treatment		100	-1.3538	0.1730	35	-7.83	<.0001
stage	peak		-1.2846	0.1836	35	-7.00	<.0001
stage	recession		-0.7455	0.3307	35	-2.25	0.0305
stage	rising		-1.6990	0.1410	35	-12.05	<.0001

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment		50		100	0.2215	0.2120	35	1.04	0.3033
stage	peak		recession		-0.5391	0.3783	35	-1.43	0.1630
stage	peak		rising		0.4144	0.2315	35	1.79	0.0822
stage	recession		rising		0.9534	0.3595	35	2.65	0.0119

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Adjustment	Adj P
Treatment		50		100	Tukey-Kramer	0.3033
stage	peak		recession		Tukey-Kramer	0.3393
stage	peak		rising		Tukey-Kramer	0.1878
stage	recession		rising		Tukey-Kramer	0.0313

I.2.17. Total Organic Carbon Full Model

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	1	33	9.35	0.0044
stage	2	33	2.22	0.1240
Treatment*stage	2	33	2.06	**0.1438**

Least Squares Means

Effect	stage	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment		50	15.3560	0.3933	33	39.04	<.0001
Treatment		100	17.0708	0.3996	33	42.72	<.0001
stage	peak		15.4605	0.3832	33	40.35	<.0001
stage	recession		16.9495	0.6887	33	24.61	<.0001
stage	rising		16.2303	0.2937	33	55.27	<.0001
Treatment*stage	peak	50	15.2051	0.5206	33	29.21	<.0001
Treatment*stage	recession	50	15.1160	0.9740	33	15.52	<.0001
Treatment*stage	rising	50	15.7470	0.4153	33	37.92	<.0001
Treatment*stage	peak	100	15.7158	0.5623	33	27.95	<.0001
Treatment*stage	recession	100	18.7830	0.9740	33	19.28	<.0001
Treatment*stage	rising	100	16.7136	0.4153	33	40.24	<.0001

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Estimate	Standard Error	DF	t Value
Treatment		50		100	-1.7148	0.5607	33	-3.06
stage	peak		recession		-1.4890	0.7881	33	-1.89
stage	peak		rising		-0.7698	0.4828	33	-1.59
stage	recession		rising		0.7192	0.7487	33	0.96
Treatment*stage	peak	50	recession	50	0.08914	1.1044	33	0.08
Treatment*stage	peak	50	rising	50	-0.5419	0.6660	33	-0.81
Treatment*stage	peak	50	peak	100	-0.5107	0.7663	33	-0.67
Treatment*stage	peak	50	recession	100	-3.5779	1.1044	33	-3.24
Treatment*stage	peak	50	rising	100	-1.5085	0.6660	33	-2.27
Treatment*stage	recession	50	rising	50	-0.6310	1.0588	33	-0.60
Treatment*stage	recession	50	peak	100	-0.5998	1.1247	33	-0.53
Treatment*stage	recession	50	recession	100	-3.6670	1.3774	33	-2.66
Treatment*stage	recession	50	rising	100	-1.5976	1.0588	33	-1.51
Treatment*stage	rising	50	peak	100	0.03117	0.6991	33	0.04
Treatment*stage	rising	50	recession	100	-3.0360	1.0588	33	-2.87
Treatment*stage	rising	50	rising	100	-0.9666	0.5873	33	-1.65
Treatment*stage	peak	100	recession	100	-3.0672	1.1247	33	-2.73
Treatment*stage	peak	100	rising	100	-0.9978	0.6991	33	-1.43
Treatment*stage	recession	100	rising	100	2.0694	1.0588	33	1.95

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Pr > |t|	Adjustment	Adj P
Treatment		50		100	0.0044	Tukey-Kramer	0.0044
stage	peak		recession		0.0677	Tukey-Kramer	0.1577
stage	peak		rising		0.1203	Tukey-Kramer	0.2621
stage	recession		rising		0.3438	Tukey-Kramer	0.6065
Treatment*stage	peak	50	recession	50	0.9362	Tukey-Kramer	1.0000
Treatment*stage	peak	50	rising	50	0.4217	Tukey-Kramer	0.9629
Treatment*stage	peak	50	peak	100	0.5098	Tukey-Kramer	0.9844
Treatment*stage	peak	50	recession	100	0.0027	Tukey-Kramer	0.0299
Treatment*stage	peak	50	rising	100	0.0302	Tukey-Kramer	0.2369
Treatment*stage	recession	50	rising	50	0.5553	Tukey-Kramer	0.9906
Treatment*stage	recession	50	peak	100	0.5974	Tukey-Kramer	0.9944
Treatment*stage	recession	50	recession	100	0.0119	Tukey-Kramer	0.1106
Treatment*stage	recession	50	rising	100	0.1408	Tukey-Kramer	0.6612
Treatment*stage	rising	50	peak	100	0.9647	Tukey-Kramer	1.0000
Treatment*stage	rising	50	recession	100	0.0072	Tukey-Kramer	0.0712
Treatment*stage	rising	50	rising	100	0.1093	Tukey-Kramer	0.5755
Treatment*stage	peak	100	recession	100	0.0102	Tukey-Kramer	0.0965
Treatment*stage	peak	100	rising	100	0.1629	Tukey-Kramer	0.7106
Treatment*stage	recession	100	rising	100	0.0592	Tukey-Kramer	0.3890

I.2.18. Total Organic Carbon Reduced Model

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	1	35	5.78	0.0217
stage	2	35	2.02	0.1480

Least Squares Means

Effect	stage	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment		50	15.6745	0.3638	35	43.09	<.0001
Treatment		100	16.7673	0.3710	35	45.20	<.0001
stage	peak		15.4829	0.3938	35	39.32	<.0001
stage	recession		16.9495	0.7092	35	23.90	<.0001
stage	rising		16.2303	0.3024	35	53.67	<.0001

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment		50		100	-1.0928	0.4547	35	-2.40	0.0217
stage	peak		recession		-1.4666	0.8112	35	-1.81	0.0792
stage	peak		rising		-0.7474	0.4965	35	-1.51	0.1412
stage	recession		rising		0.7192	0.7710	35	0.93	0.3573

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Adjustment	Adj P
Treatment		50		100	Tukey-Kramer	0.0217
stage	peak		recession		Tukey-Kramer	0.1818
stage	peak		rising		Tukey-Kramer	0.3007
stage	recession		rising		Tukey-Kramer	0.6235

I.2.19. Dissolved Organic Carbon Full Model

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	1	33	3.62	0.0658
stage	2	33	0.51	0.6070
Treatment*stage	2	33	3.18	**0.0546**

Least Squares Means

Effect	stage	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment		50	14.4987	0.2668	33	54.33	<.0001
Treatment		100	15.2228	0.2711	33	56.14	<.0001
stage	peak		14.6177	0.2600	33	56.23	<.0001
stage	recession		15.1250	0.4673	33	32.37	<.0001
stage	rising		14.8395	0.1992	33	74.48	<.0001
Treatment*stage	peak	50	14.5871	0.3532	33	41.30	<.0001
Treatment*stage	recession	50	14.9000	0.6608	33	22.55	<.0001
Treatment*stage	rising	50	14.0091	0.2818	33	49.72	<.0001
Treatment*stage	peak	100	14.6483	0.3815	33	38.39	<.0001
Treatment*stage	recession	100	15.3500	0.6608	33	23.23	<.0001
Treatment*stage	rising	100	15.6700	0.2818	33	55.61	<.0001

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Estimate	Standard Error	DF	t Value
Treatment		50		100	-0.7240	0.3804	33	-1.90
stage	peak		recession		-0.5073	0.5347	33	-0.95
stage	peak		rising		-0.2218	0.3275	33	-0.68
stage	recession		rising		0.2855	0.5080	33	0.56
Treatment*stage	peak	50	recession	50	-0.3129	0.7493	33	-0.42
Treatment*stage	peak	50	rising	50	0.5781	0.4518	33	1.28
Treatment*stage	peak	50	peak	100	-0.06119	0.5199	33	-0.12
Treatment*stage	peak	50	recession	100	-0.7629	0.7493	33	-1.02
Treatment*stage	peak	50	rising	100	-1.0829	0.4518	33	-2.40
Treatment*stage	recession	50	rising	50	0.8909	0.7184	33	1.24
Treatment*stage	recession	50	peak	100	0.2517	0.7630	33	0.33
Treatment*stage	recession	50	recession	100	-0.4500	0.9345	33	-0.48
Treatment*stage	recession	50	rising	100	-0.7700	0.7184	33	-1.07
Treatment*stage	rising	50	peak	100	-0.6392	0.4743	33	-1.35
Treatment*stage	rising	50	recession	100	-1.3409	0.7184	33	-1.87
Treatment*stage	rising	50	rising	100	-1.6609	0.3985	33	-4.17
Treatment*stage	peak	100	recession	100	-0.7017	0.7630	33	-0.92
Treatment*stage	peak	100	rising	100	-1.0217	0.4743	33	-2.15
Treatment*stage	recession	100	rising	100	-0.3200	0.7184	33	-0.45

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Pr > |t|	Adjustment	Adj P
Treatment		50		100	0.0658	Tukey-Kramer	0.0658
stage	peak		recession		0.3497	Tukey-Kramer	0.6139
stage	peak		rising		0.5030	Tukey-Kramer	0.7783
stage	recession		rising		0.5779	Tukey-Kramer	0.8411
Treatment*stage	peak	50	recession	50	0.6790	Tukey-Kramer	0.9982
Treatment*stage	peak	50	rising	50	0.2097	Tukey-Kramer	0.7939
Treatment*stage	peak	50	peak	100	0.9070	Tukey-Kramer	1.0000
Treatment*stage	peak	50	recession	100	0.3160	Tukey-Kramer	0.9085
Treatment*stage	peak	50	rising	100	0.0224	Tukey-Kramer	0.1868
Treatment*stage	recession	50	rising	50	0.2237	Tukey-Kramer	0.8140
Treatment*stage	recession	50	peak	100	0.7436	Tukey-Kramer	0.9994
Treatment*stage	recession	50	recession	100	0.6333	Tukey-Kramer	0.9965
Treatment*stage	recession	50	rising	100	0.2916	Tukey-Kramer	0.8890
Treatment*stage	rising	50	peak	100	0.1869	Tukey-Kramer	0.7566
Treatment*stage	rising	50	recession	100	0.0709	Tukey-Kramer	0.4396
Treatment*stage	rising	50	rising	100	0.0002	Tukey-Kramer	0.0026
Treatment*stage	peak	100	recession	100	0.3645	Tukey-Kramer	0.9386
Treatment*stage	peak	100	rising	100	0.0386	Tukey-Kramer	0.2859
Treatment*stage	recession	100	rising	100	0.6589	Tukey-Kramer	0.9976

I.2.20. Dissolved Organic Carbon Reduced Model

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	1	35	10.01	0.0032
stage	2	35	0.37	0.6918

Least Squares Means

Effect	stage	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment		50	14.3702	0.2541	35	56.54	<.0001
Treatment		100	15.3755	0.2592	35	59.32	<.0001
stage	peak		14.6541	0.2751	35	53.26	<.0001
stage	recession		15.1250	0.4955	35	30.52	<.0001
stage	rising		14.8395	0.2113	35	70.24	<.0001

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment		50		100	-1.0053	0.3177	35	-3.16	0.0032
stage	peak		recession		-0.4709	0.5668	35	-0.83	0.4116
stage	peak		rising		-0.1855	0.3469	35	-0.53	0.5962
stage	recession		rising		0.2855	0.5387	35	0.53	0.5995

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Adjustment	Adj P
Treatment		50		100	Tukey-Kramer	0.0032
stage	peak		recession		Tukey-Kramer	0.6866
stage	peak		rising		Tukey-Kramer	0.8548
stage	recession		rising		Tukey-Kramer	0.8572

I.2.21. ln Suspended Organic Carbon Full Model

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	1	33	5.32	0.0275
stage	2	33	0.96	0.3935
Treatment*stage	2	33	3.46	**0.0434**

Least Squares Means

Effect	stage	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment		50	-0.7193	0.2631	33	-2.73	0.0100
Treatment		100	0.1460	0.2674	33	0.55	0.5887
stage	peak		-0.4835	0.2563	33	-1.89	0.0681
stage	recession		-0.3325	0.4608	33	-0.72	0.4756
stage	rising		-0.04398	0.1965	33	-0.22	0.8242
Treatment*stage	peak	50	-0.6827	0.3483	33	-1.96	0.0585
Treatment*stage	recession	50	-1.5352	0.6516	33	-2.36	0.0246
Treatment*stage	rising	50	0.05993	0.2779	33	0.22	0.8306
Treatment*stage	peak	100	-0.2843	0.3762	33	-0.76	0.4552
Treatment*stage	recession	100	0.8702	0.6516	33	1.34	0.1909
Treatment*stage	rising	100	-0.1479	0.2779	33	-0.53	0.5981

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Estimate	Standard Error	DF	t Value
Treatment		50		100	-0.8653	0.3751	33	-2.31
stage	peak		recession		-0.1510	0.5273	33	-0.29
stage	peak		rising		-0.4395	0.3230	33	-1.36
stage	recession		rising		-0.2885	0.5009	33	-0.58
Treatment*stage	peak	50	recession	50	0.8525	0.7389	33	1.15
Treatment*stage	peak	50	rising	50	-0.7426	0.4456	33	-1.67
Treatment*stage	peak	50	peak	100	-0.3984	0.5127	33	-0.78
Treatment*stage	peak	50	recession	100	-1.5529	0.7389	33	-2.10
Treatment*stage	peak	50	rising	100	-0.5348	0.4456	33	-1.20
Treatment*stage	recession	50	rising	50	-1.5952	0.7084	33	-2.25
Treatment*stage	recession	50	peak	100	-1.2509	0.7524	33	-1.66
Treatment*stage	recession	50	recession	100	-2.4054	0.9215	33	-2.61
Treatment*stage	recession	50	rising	100	-1.3873	0.7084	33	-1.96
Treatment*stage	rising	50	peak	100	0.3442	0.4677	33	0.74
Treatment*stage	rising	50	recession	100	-0.8103	0.7084	33	-1.14
Treatment*stage	rising	50	rising	100	0.2078	0.3929	33	0.53
Treatment*stage	peak	100	recession	100	-1.1545	0.7524	33	-1.53
Treatment*stage	peak	100	rising	100	-0.1364	0.4677	33	-0.29
Treatment*stage	recession	100	rising	100	1.0181	0.7084	33	1.44

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Pr > |t|	Adjustment	Adj P
Treatment		50		100	0.0275	Tukey-Kramer	0.0275
stage	peak		recession		0.7764	Tukey-Kramer	0.9559
stage	peak		rising		0.1828	Tukey-Kramer	0.3727
stage	recession		rising		0.5685	Tukey-Kramer	0.8338
Treatment*stage	peak	50	recession	50	0.2569	Tukey-Kramer	0.8550
Treatment*stage	peak	50	rising	50	0.1050	Tukey-Kramer	0.5623
Treatment*stage	peak	50	peak	100	0.4427	Tukey-Kramer	0.9695
Treatment*stage	peak	50	recession	100	0.0433	Tukey-Kramer	0.3112
Treatment*stage	peak	50	rising	100	0.2386	Tukey-Kramer	0.8335
Treatment*stage	recession	50	rising	50	0.0311	Tukey-Kramer	0.2424
Treatment*stage	recession	50	peak	100	0.1059	Tukey-Kramer	0.5650
Treatment*stage	recession	50	recession	100	0.0135	Tukey-Kramer	0.1231
Treatment*stage	recession	50	rising	100	0.0587	Tukey-Kramer	0.3868
Treatment*stage	rising	50	peak	100	0.4669	Tukey-Kramer	0.9759
Treatment*stage	rising	50	recession	100	0.2609	Tukey-Kramer	0.8594
Treatment*stage	rising	50	rising	100	0.6004	Tukey-Kramer	0.9946
Treatment*stage	peak	100	recession	100	0.1345	Tukey-Kramer	0.6454
Treatment*stage	peak	100	rising	100	0.7723	Tukey-Kramer	0.9997
Treatment*stage	recession	100	rising	100	0.1601	Tukey-Kramer	0.7048

I.2.22. ln Organic Carbon Partitioning Coefficient Full Model

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	1	33	4.58	0.0397
stage	2	33	0.89	0.4199
Treatment*stage	2	33	3.63	**0.0376**

Least Squares Means

Effect	stage	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment		50	-3.3907	0.2670	33	-12.70	<.0001
Treatment		100	-2.5757	0.2713	33	-9.49	<.0001
stage	peak		-3.1635	0.2601	33	-12.16	<.0001
stage	recession		-3.0482	0.4675	33	-6.52	<.0001
stage	rising		-2.7380	0.1994	33	-13.73	<.0001
Treatment*stage	peak	50	-3.3600	0.3534	33	-9.51	<.0001
Treatment*stage	recession	50	-4.2355	0.6612	33	-6.41	<.0001
Treatment*stage	rising	50	-2.5768	0.2819	33	-9.14	<.0001
Treatment*stage	peak	100	-2.9670	0.3818	33	-7.77	<.0001
Treatment*stage	recession	100	-1.8609	0.6612	33	-2.81	0.0082
Treatment*stage	rising	100	-2.8991	0.2819	33	-10.28	<.0001

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Estimate	Standard Error	DF	t Value
Treatment		50		100	-0.8151	0.3806	33	-2.14
stage	peak		recession		-0.1153	0.5350	33	-0.22
stage	peak		rising		-0.4255	0.3277	33	-1.30
stage	recession		rising		-0.3102	0.5083	33	-0.61
Treatment*stage	peak	50	recession	50	0.8755	0.7497	33	1.17
Treatment*stage	peak	50	rising	50	-0.7832	0.4521	33	-1.73
Treatment*stage	peak	50	peak	100	-0.3929	0.5202	33	-0.76
Treatment*stage	peak	50	recession	100	-1.4990	0.7497	33	-2.00
Treatment*stage	peak	50	rising	100	-0.4608	0.4521	33	-1.02
Treatment*stage	recession	50	rising	50	-1.6587	0.7188	33	-2.31
Treatment*stage	recession	50	peak	100	-1.2685	0.7635	33	-1.66
Treatment*stage	recession	50	recession	100	-2.3746	0.9351	33	-2.54
Treatment*stage	recession	50	rising	100	-1.3364	0.7188	33	-1.86
Treatment*stage	rising	50	peak	100	0.3902	0.4746	33	0.82
Treatment*stage	rising	50	recession	100	-0.7159	0.7188	33	-1.00
Treatment*stage	rising	50	rising	100	0.3223	0.3987	33	0.81
Treatment*stage	peak	100	recession	100	-1.1061	0.7635	33	-1.45
Treatment*stage	peak	100	rising	100	-0.06789	0.4746	33	-0.14
Treatment*stage	recession	100	rising	100	1.0382	0.7188	33	1.44

Differences of Least Squares Means

Effect	stage	Treatment	stage	Treatment	Pr > |t|	Adjustment	Adj P
Treatment		50		100	0.0397	Tukey-Kramer	0.0397
stage	peak		recession		0.8307	Tukey-Kramer	0.9747
stage	peak		rising		0.2031	Tukey-Kramer	0.4061
stage	recession		rising		0.5458	Tukey-Kramer	0.8155
Treatment*stage	peak	50	recession	50	0.2513	Tukey-Kramer	0.8487
Treatment*stage	peak	50	rising	50	0.0926	Tukey-Kramer	0.5213
Treatment*stage	peak	50	peak	100	0.4554	Tukey-Kramer	0.9730
Treatment*stage	peak	50	recession	100	0.0539	Tukey-Kramer	0.3642
Treatment*stage	peak	50	rising	100	0.3155	Tukey-Kramer	0.9081
Treatment*stage	recession	50	rising	50	0.0274	Tukey-Kramer	0.2198
Treatment*stage	recession	50	peak	100	0.1061	Tukey-Kramer	0.5657
Treatment*stage	recession	50	recession	100	0.0160	Tukey-Kramer	0.1420
Treatment*stage	recession	50	rising	100	0.0719	Tukey-Kramer	0.4440
Treatment*stage	rising	50	peak	100	0.4168	Tukey-Kramer	0.9613
Treatment*stage	rising	50	recession	100	0.3265	Tukey-Kramer	0.9160
Treatment*stage	rising	50	rising	100	0.4246	Tukey-Kramer	0.9639
Treatment*stage	peak	100	recession	100	0.1569	Tukey-Kramer	0.6978
Treatment*stage	peak	100	rising	100	0.8871	Tukey-Kramer	1.0000
Treatment*stage	recession	100	rising	100	0.1581	Tukey-Kramer	0.7005

1.3. Multiple Regression Analysis Details for Transport Plot Study

1.3.1. ln E. coli Partitioning Coefficient

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	131.35607	26.27121	17.18	<.0001
Error	32	48.92658	1.52896		
Corrected Total	37	180.28265			

Root MSE	1.23651	R-Square	0.7286
Dependent Mean	-6.69048	Adj R-Sq	0.6862
Coeff Var	-18.48161		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Variance Inflation
Intercept	Intercept	1	-0.75020	3.13551	-0.24	0.8124	0
TSS	TSS	1	0.00233	0.00044469	5.23	<.0001	1.84077
Diss_Organic_C	Diss_Organic_C	1	-0.47918	0.20490	-2.34	0.0258	1.23733
Total_P_z		1	-9.31584	5.20931	-1.79	0.0832	6112.97036
Diss_P_z		1	1.46206	0.37666	3.88	0.0005	21.18391
Total_Organic_P_z		1	15.83016	10.04681	1.58	0.1249	6143.05110

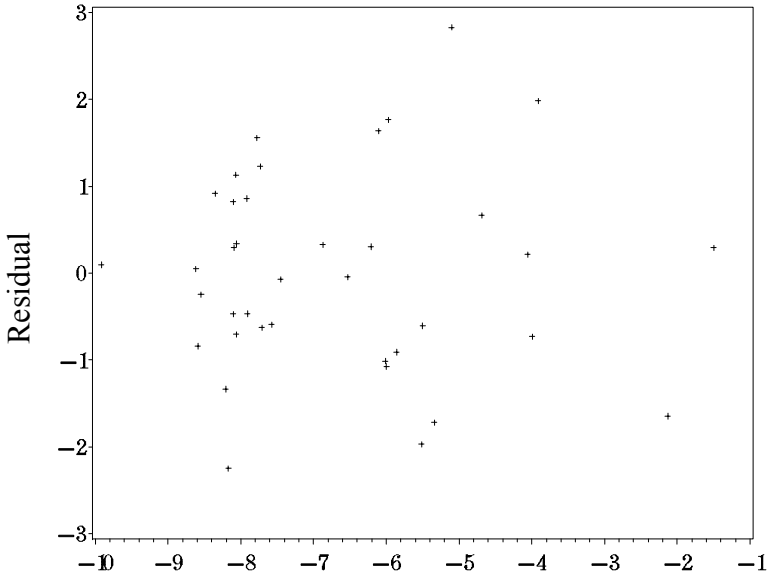


Figure I.1. Residual plots of predicted ln *E. coli* partitioning coefficient in runoff from well managed and poorly managed pastureland.

1.3.2. ln Enterococci Partitioning Coefficient

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	14.63364	7.31682	2.93	0.0687
Error	30	74.86287	2.49543		
Corrected Total	32	89.49651			

Root MSE	1.57969	R-Square	0.1635
Dependent Mean	-5.74089	Adj R-Sq	0.1077
Coeff Var	-27.51651		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Variance Inflation
Intercept	Intercept	1	-6.32960	0.36801	-17.20	<.0001	0
TSS	TSS	1	0.01456	0.00764	1.91	0.0662	320.38353
TSS_z		1	-0.01370	0.00753	-1.82	0.0788	320.38353

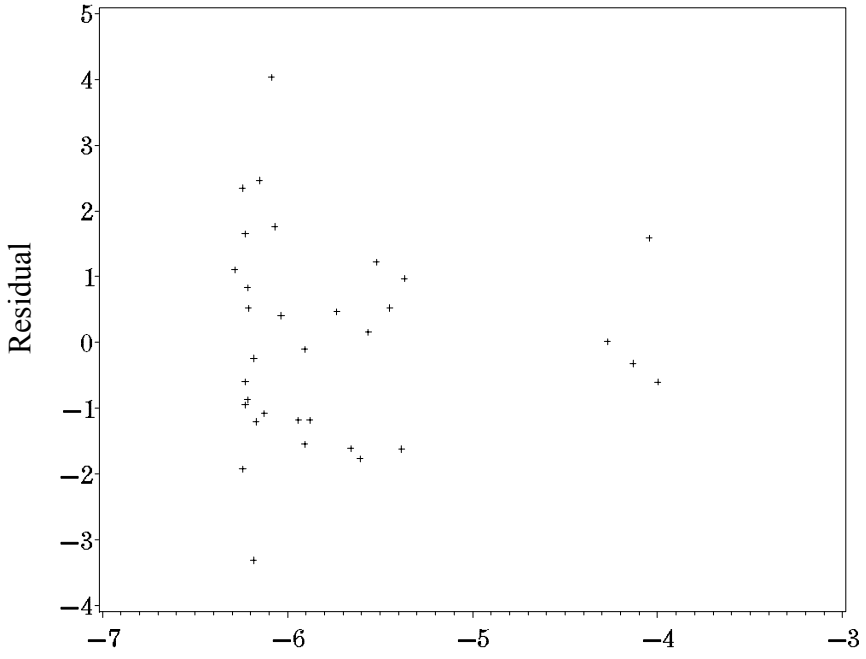


Figure I.2. Residual plots of predicted ln enterococci partitioning coefficient in runoff from well managed and poorly managed pastureland.

1.3.3. E. coli Total Concentration

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	229770805	45954161	7.78	<.0001
Error	33	194865092	5905003		
Corrected Total	38	424635897			

Root MSE	2430.02115	R-Square	0.5411
Dependent Mean	7589.74359	Adj R-Sq	0.4716
Coeff Var	32.01717		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Variance Inflation
Intercept	Intercept	1	957.73302	2764.18578	0.35	0.7312	0
flow	flow	1	-662527	325373	-2.04	0.0498	1.10834
Diss_P	Diss_P	1	7525.91444	2670.12375	2.82	0.0081	49.17136
Diss_Organic_P	Diss_Organic_P	1	-11401	5664.72426	-2.01	0.0524	52.48506
z		1	9449.09132	3868.22848	2.44	0.0201	24.69012
Diss_P_z		1	-2341.46787	821.90835	-2.85	0.0075	26.80878

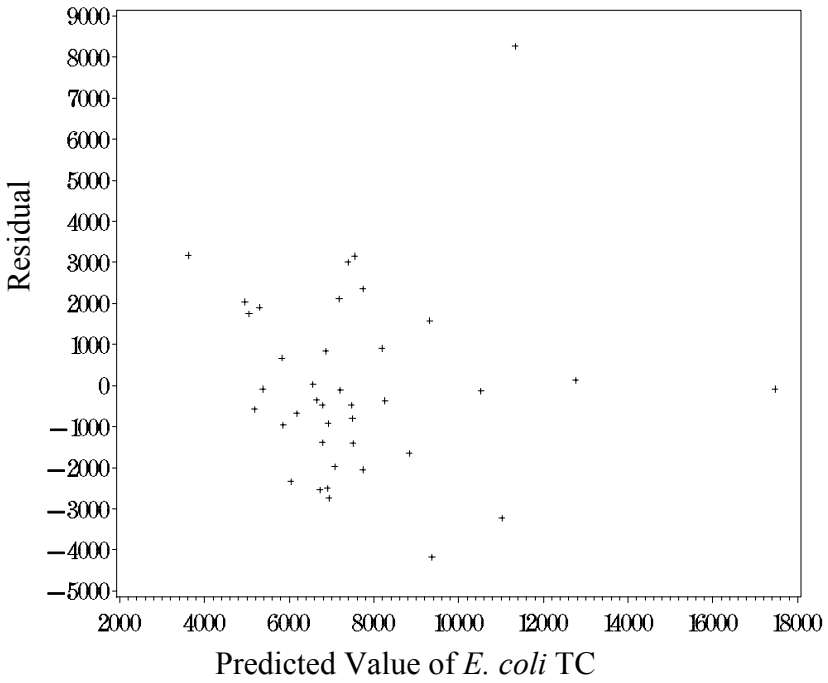


Figure I.3. Residual plots of predicted *E. coli* total concentration in runoff from well managed and poorly managed pastureland.

1.3.4. Enterococci Total Concentration

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	25648402	6412100	7.37	0.0002
Error	34	29570572	869723		
Corrected Total	38	55218974			

Root MSE	932.58926	R-Square	0.4645
Dependent Mean	3471.79487	Adj R-Sq	0.4015
Coeff Var	26.86188		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Variance Inflation
Intercept	Intercept	1	6099.47783	957.55608	6.37	<.0001	0
Total_Organic_P	Total_Organic_P	1	-984.20710	344.87547	-2.85	0.0073	1.28459
flow_z		1	664938	199952	3.33	0.0021	3.39932
TSS_z		1	0.60437	0.35446	1.71	0.0973	2.12888
Total_Organic_C_z		1	-87.72200	29.65804	-2.96	0.0056	2.40696

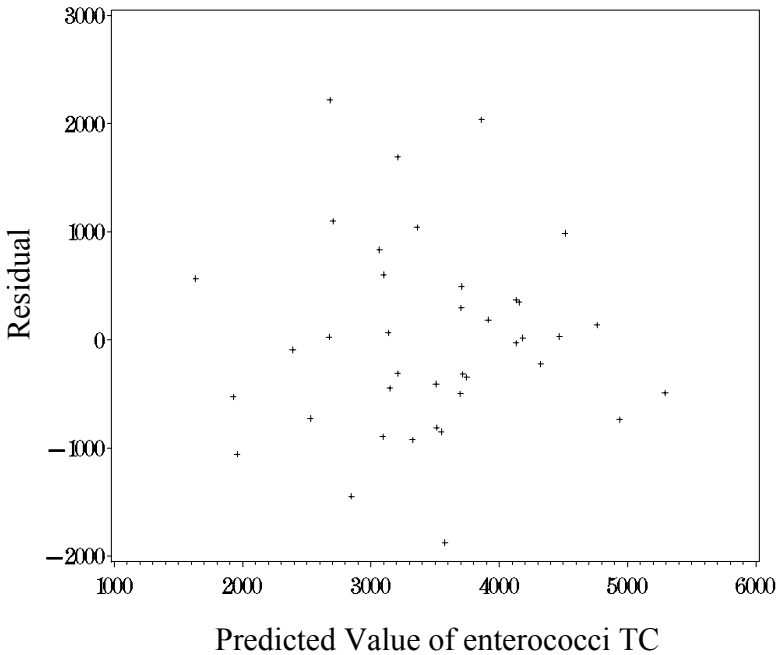


Figure I.4. Residual plots of predicted enterocooci total concentration in runoff from well managed and poorly managed pastureland.

1.3.5. E. coli Unattached Load

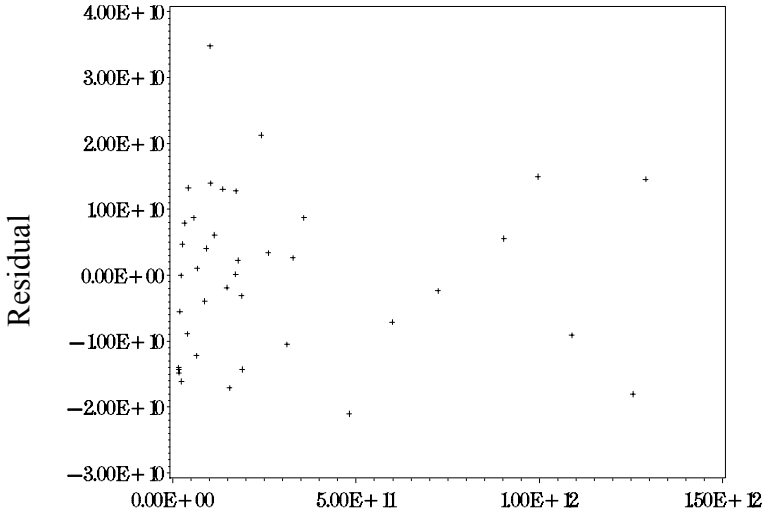
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	10	4.935951E24	4.935951E23	2371.96	<.0001
Error	28	5.826672E21	2.080954E20		
Corrected Total	38	4.941777E24			

Root MSE	14425513143	R-Square	0.9988
Dependent Mean	2.848284E11	Adj R-Sq	0.9984
Coeff Var	5.06463		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Variance Inflation
Intercept	Intercept	1	15180669092	5344897799	2.84	0.0083	0
TSS	TSS	1	-4.6808E10	19484088038	-2.40	0.0232	28083
TDP	TDP	1	1.841513E13	6.513936E12	2.83	0.0086	141994
DOP	DOP	1	-2.96703E13	1.303309E13	-2.28	0.0307	135233
TOC	TOC	1	-3.99426E12	1.017579E12	-3.93	0.0005	39252
DOC	DOC	1	3.944727E12	1.036384E12	3.81	0.0007	35858
TSS_z		1	47739216643	19546259470	2.44	0.0212	28626
TDP_z		1	-4.53297E13	1.754032E13	-2.58	0.0153	591524
DOP_z		1	8.31673E13	3.638527E13	2.29	0.0300	629312
TOC_z		1	4.236025E12	1.272862E12	3.33	0.0025	30242
DOC_z		1	-3.87238E12	1.322519E12	-2.93	0.0067	29297



Predicted Value of *E. coli* Attached Load

Figure I.5. Residual plots of predicted *E. coli* unattached loading rates in runoff from well managed and poorly managed pastureland.

1.3.6. E. coli Attached Load

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1.098996E21	2.747491E20	139.02	<.0001
Error	34	6.719485E19	1.976319E18		
Corrected Total	38	1.166191E21			

Root MSE	1405816187	R-Square	0.9424
Dependent Mean	2569988258	Adj R-Sq	0.9356
Coeff Var	54.70127		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Variance Inflation
Intercept	Intercept	1	-58591770	325214817	-0.18	0.8581	0
TSS	TSS	1	417832268	83865792	4.98	<.0001	54.78487
z		1	1688582913	670975349	2.52	0.0167	2.21960
TP_z		1	-2.45344E12	5.324525E11	-4.61	<.0001	83901
TOP_z		1	4.640915E12	1.031687E12	4.50	<.0001	85800

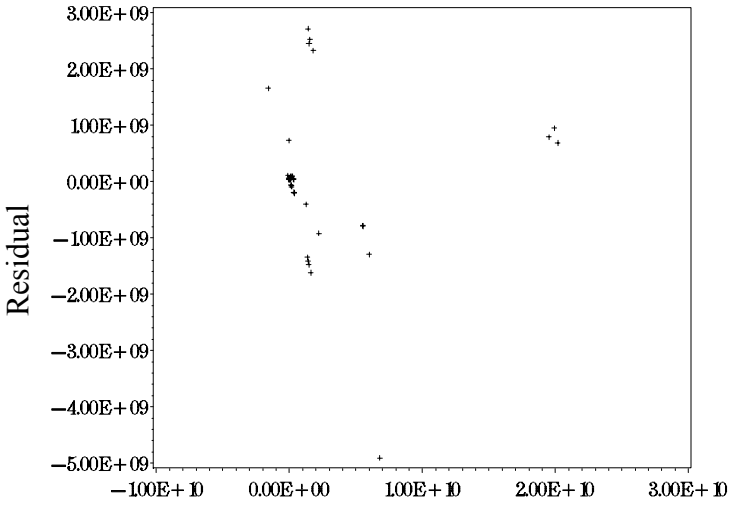


Figure I.6. Residual plots of predicted *E. coli* attached loading rates in runoff from well managed and poorly managed pastureland.

1.3.7. Enterococci Unattached Load

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	1.032744E23	1.475349E22	423.11	<.0001
Error	31	1.080944E21	3.486917E19		
Corrected Total	38	1.043554E23			

Root MSE	5905012693	R-Square	0.9896
Dependent Mean	49709871628	Adj R-Sq	0.9873
Coeff Var	11.87895		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Variance Inflation
Intercept	Intercept	1	-406080451	1778679585	-0.23	0.8209	0
TP	TP	1	-1.29753E13	2.178376E12	-5.96	<.0001	120988
TOP	TOP	1	2.717899E13	4.318781E12	6.29	<.0001	128393
TOC	TOC	1	9.341736E11	3.651235E11	2.56	0.0156	30160
DOC	DOC	1	-1.3063E12	3.752458E11	-3.48	0.0015	28054
TOP_z		1	-2.73445E12	6.085008E11	-4.49	<.0001	1691.72513
TOC_z		1	-7.24508E11	4.091371E11	-1.77	0.0864	18647
DOC_z		1	1.353542E12	4.170759E11	3.25	0.0028	17389

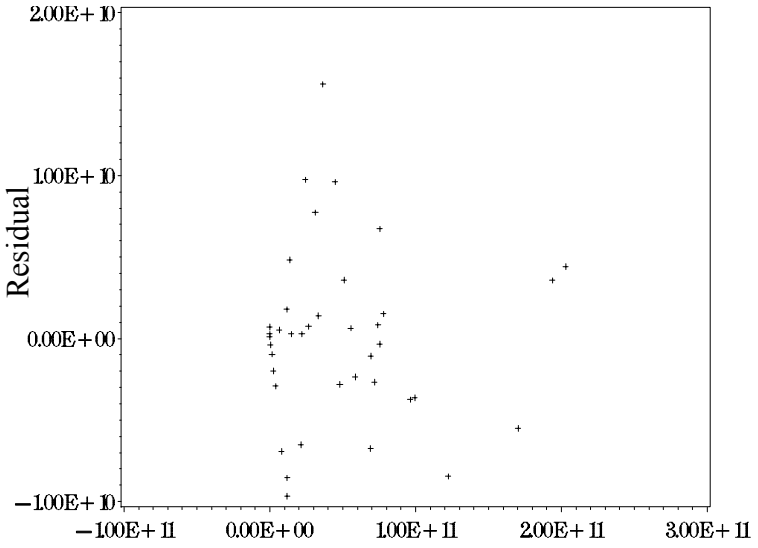


Figure I.7. Residual plots of predicted enterococci unattached loading rates in runoff from well managed and poorly managed pastureland.

1.3.8. Enterococci Attached Load

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	8.850154E18	2.950051E18	57.75	<.0001
Error	35	1.78794E18	5.108401E16		
Corrected Total	38	1.063809E19			

Root MSE	226017725	R-Square	0.8319
Dependent Mean	313438018	Adj R-Sq	0.8175
Coeff Var	72.10922		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Variance Inflation
Intercept	Intercept	1	-34578453	53446765	-0.65	0.5219	0
DOP	DOP	1	1112355521	611253517	1.82	0.0774	1.21174
TP_z		1	19962185115	5396105377	3.70	0.0007	333.37809
TDP_z		1	-1.99183E10	6536277810	-3.05	0.0044	334.60726

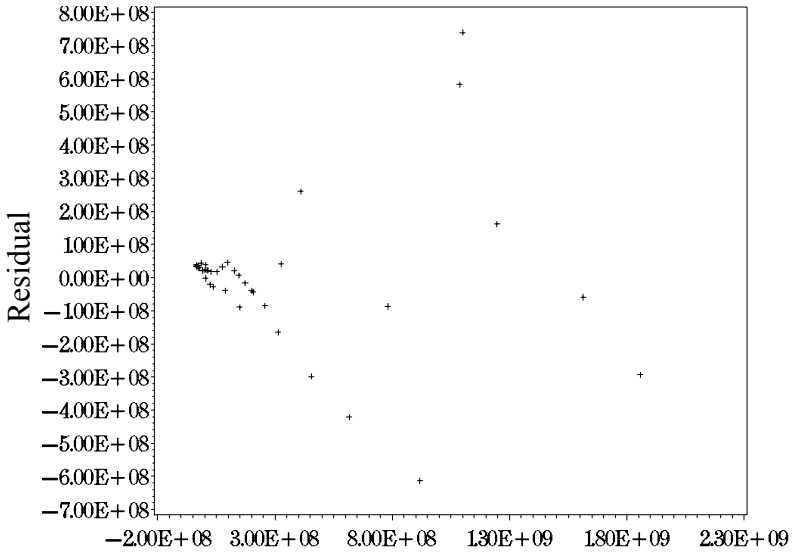


Figure I.8. Residual plots of predicted enterococci attached loading rates in runoff from well managed and poorly managed pastureland.