Predicting fate and transport of fecal bacteria through soils using an advection-dispersion model

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A Thesis

Predicting Fate and Transport of Fecal Bacteria through Soils Using an Advection-Dispersion Model

by

Ryan Walter Jackwood

Submitted to the Graduate Faculty as partial fulfillment of the requirements for the

Master of Science Degree in Biology

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Bovine liquid manure (BLM) obtained from concentrated animal feeding operations (CAFOs) often is added to agricultural fields as fertilizer. Unfortunately, pathogens inherent to BLM might contaminate surface and ground waters via field runoff and expedited transport via field drain tiles. Buffer strips, placed at the edge of agricultural fields, can intercept pathogens that infiltrate into soil. Buffer strip efficiency to intercept pathogens is dependent on soil type and microbial attributes. Parameters for each have been included in models (e.g. the advection-dispersion model) that describe distance-dependent densities of microorganisms being transported with saturated water flow:

$$\frac{C(t)}{C_0} = \frac{V_0}{A(4\alpha L^*x)^{0.5}} \exp\left[-\left(\frac{x-x'}{2L^*}\right)^2\right] \exp[-k_p x']$$

In this model, $C(t)/C_0$ represents the ratio of bacteria that transport a given distance ($x$) and time ($t$) through soil from the application source. I hypothesized that (1) an
advection-dispersion model could predict distance-dependent densities of fecal indicator bacteria (*i.e.* *Escherichia coli* and *Enterococcus hirae*) commonly present in BLM. (2) Model predictions could be extrapolated to determine the minimum transport distance (depth of proposed buffer strip) required to decrease microbial densities within BLM to the levels suggested by the Ohio Environmental Protection Agency’s Water Quality Standards (WQS), *i.e.* geometric means of 126 and 35 CFUs/100 mL, respectively. The project’s objectives were to:

1. Formulate a semi-pervious soil that could be used to construct buffer strips using components (sand, clay, and plant material) obtained in northwest Ohio.
2. Use formulated soil in microcosms of three lengths (30, 45, and 60 cm) to obtain numerical values for the model parameters: fluid velocity, $v$; dispersivity, $\alpha L$; initial injection volume, $V_o$; and single collector efficiency, $\eta$.
3. Obtain values for distance-dependent densities of fecal indicator bacteria in BLM during transport through the microcosms, and determine whether the advection-dispersion model predicted these values by using a correlation analysis.
4. Use the model to make predictions of distance-dependent densities for bacterial transport through simulated buffer strips and compare the resultant predictions to observed effluent densities from field lysimeters containing formulated soil.
5. Extrapolate the data to predict transport distances that are needed to sufficiently reduce BLM derived microbes, *i.e.* buffer strip widths needed to obtain target densities of bacteria.
Outcomes for each goal included:

(1) A soil was formulated by combining sand (60 % by volume), clay (20 % by volume), and dried plant material (20 % by volume). Formulated soil had an infiltration rate of 123 mm/hr and fluid velocity of 0.015 - 0.025 cm/s;

(2) Model parameters had the following values: single collector efficiency ($\eta = 6.16 \times 10^{-2}$); dispersivity ($\alpha_L$: 30 cm = 2.17 cm; 45 cm = 2.28 cm; 60 cm = 5.41 cm); and initial injection volume ($V_o = 0.014 – 13$ mL).

(3) The maximum ratio ($C/C_o$) of indicator organisms in effluent from the microcosms decreased with soil depth ($E. coli$ – 30 cm: 0.11, 45 cm: 0.061, 60 cm: 0.0265, $E. hirae$ – 30 cm: 0.11, 45 cm: 0.032, 60 cm: 0.016). The precision of model predictions decreased with system size ($R^2$: $E. coli$ – 30 cm: 0.978, 45 cm: 0.987, 60 cm: 0.741; $E. hirae$, 30 cm: 0.852, 45 cm: 0.737, 60 cm: 0.638).

(4) Results from a correlation analysis of model predictions and field lysimeter data yielded a difference in model precision between indicator species ($E. coli$: $R^2 = 0.4828$; $E. hirae$: $R^2 = 0.020$).

(5) Lysimeter data were used to predict minimum soil distances needed to decrease bacterial densities to WQS; $E. coli$ and $E. hirae$ required 254 cm and 282 cm of soil distance respectively.

A buffer strip that contains soil formulated to optimize infiltration rate and provides a minimum of 254 cm of subsurface flow will reduce the threat of contamination from BLM-derived pathogens to surface waters.
I dedicate this thesis to the best siblings in the world:

Niki Fisk
Kristen Caulier
Taylor Jackwood
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List of Abbreviations

ANOVA ..................... Analysis of Variance
APHA ......................... American Public Health Association
ATCC ........................ American Type Culture Collection

BHI ............................. Brain Heart Infusion
BLM ............................. Bovine Liquid Manure
BMP ............................. Best Management Practices

CAFO .......................... Concentrated Animal Feeding Operation
EPA ............................. Environmental Protection Agency
NMP ............................. Nutrient Management Plan
PVC ............................. Polyvinyl Chloride
SWR ............................. Surface Water Regulations
TSA ............................. Tryptic Soy Agar
TSB ............................. Tryptic Soy Broth
List of Symbols

$\alpha$ .........................Collision Efficiency
$\alpha L$ .........................Bromide Curve Distance
$\eta$ ...........................Single Collector Efficiency
$\theta$ ..........................Porosity

A..............................Cross Sectional Surface Area of Flow
$C_o$ ............................Initial density of bacteria
$C_t$ ............................Density of bacteria at time t
K ...............................Degrees Kelvin
$K_p$ .............................Irreversible Adsorption
v ...............................Fluid Velocity
$V_0$ .............................Initial Injection Volume
$x$ ...............................Soil Depth
$x'$ ..............................Spatial Coordinate for Center of Mass
Chapter 1

Introduction

1.1 Background

1.1.1 Concentrated Animal Feeding Operations

Concentrated animal feeding operations (CAFOs) confine livestock for more than 45 days during the growing season, produce no vegetation and meet specific size thresholds determined by the EPA (C.W.A 2008, Todd 1996). CAFOs are designed for mass production and maintenance of livestock while minimizing space and cost and have been in operation in the U.S. since the 1950’s starting with poultry operations; pork and cattle operations came into existence in the 70’s (Gurian-Sherman 2008). By the 1980s, poultry, cattle and swine CAFOs produced the majority of livestock for human consumption and although by 2008 CAFOs represented only 5% of all animal feeding operations, they produced more than 50% of total livestock (Burkholder et al. 2007, Hribar 2010, MacDonald and McBride 2009).

A single CAFO can produce anywhere from 2,800 to 1.6 million tons of waste per year, depending on size (Government Accountability Office [GAO], 2008). The National Association of Local Boards of Health estimates that in one year the production of CAFO waste is between 3 and 20 times greater than the production of human waste (Hribar
Unlike human waste, CAFO waste is not required to undergo wastewater treatment prior to use or disposal, which leads to the persistence of contaminants that might be present (Cole et al. 2000, Gurian-Sherman 2008, MacDonald and McBride 2009).

Cattle waste produced at a CAFO, referred to as bovine liquid manure (BLM), is routinely applied to agricultural fields as fertilizer (Cole et al. 2000, Gerba and Smith 2005, Gurian-Sherman 2008). BLM is a valuable agricultural fertilizer because it contains nutrients and organic matter that stimulate the growth of crops, and is inexpensive relative to artificial fertilizers (Hribar 2010, Walker et al. 1990). This practice leads to the co-application of beneficial nutrients and contaminants, within BLM, to agricultural fields and provides a vector for contaminants (i.e. human pathogens) to reach the environment (Donham et al. 2007, Gilchrist et al. 2007, Thorne 2007). Land application of BLM should follow a nutrient management plan (NMP) – a document dictating the amount of BLM permitted for agricultural fields depending on size, crop requirements and location (Bradford et al. 2008). However, these recommendations are not related to pathogenic content nor do they eliminate the threat of contamination to adjacent surface waters (Sharpley et al. 2011, Thorne 2007).

1.1.2 Environmental and Health Impacts

More than 150 microbial pathogens (including: Cryptosporidium, E. coli O157:H7, Salmonella, and Giardia lamblia) have been identified in samples of BLM (Mosaddeghi et al. 2010, Unc and Goss 2004, USEPA 2003); exposures have resulted in
human illness and occasional death (Cole et al. 2000). Routes of possible exposure include raw consumption of contaminated crops and recreation in or consumption of contaminated surface waters (Hribar 2010, Mawdsley et al. 1995, Thurston-Enriquez et al. 2005). Survival of pathogens, within surface waters, depend on environmental conditions (e.g. sedimentation, UV light, pH and salt content) and can range from several hours to several weeks which allows pathogens to travel through watersheds and impact human health downstream of the actual source (Bitton et al. 1983, Burton et al. 1987, Ferguson et al. 2003). There are many examples of health impacts from manure-derived pathogens and the likely sources of contamination (Table 1.1) (Gerba and Smith 2005).

In addition to impacts on human health, CAFO waste may also contain contaminants that cause deleterious environmental impacts including: eutrophication by excess nutrients and changes in environmental health brought on by heavy metals, hormones, pesticides and antibiotics (Burkholder et al. 2007, Han et al. 2012, Hoorman et al. 2008, MacDonald and McBride 2009, Ribaudo et al. 2003). My research focuses on fecal coliforms because they are intimately linked to the presence of human pathogens and indicate a possible health hazard if they exist in recreational waters (Dufour 1977). High densities of fecal coliforms result in revenue loss from beach closings, human and animal health hazards, and bad publicity (Francy et al. 2006, Murray et al. 2001, Wade et al. 2006).
Table 1.1: Examples of acute health impacts in humans related to manure contamination (Gerba and Smith 2005).

<table>
<thead>
<tr>
<th>Location</th>
<th>Year</th>
<th>Pathogen</th>
<th>Health Impacts</th>
<th>Suspected source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Walkerton, ON, Canada</td>
<td>2000</td>
<td><em>E. coli</em> O157:H7 and <em>Campylobacter</em> spp.</td>
<td>6 deaths, 2,300 cases</td>
<td>runoff from farm fields entering town’s water supply</td>
</tr>
<tr>
<td>Washington County, NY</td>
<td>1999</td>
<td><em>E. coli</em> O157:H7 and <em>Campylobacter</em> spp.</td>
<td>2 deaths, 116 cases</td>
<td>runoff at fairgrounds</td>
</tr>
<tr>
<td>Carrollton, GA</td>
<td>1989</td>
<td><em>Cryptosporidium parvum</em></td>
<td>13,000 cases</td>
<td>manure runoff</td>
</tr>
<tr>
<td>Swindon and Oxfordshire, UK</td>
<td>1989</td>
<td><em>Cryptosporidium parvum</em></td>
<td>516 excess cases</td>
<td>runoff from farm fields</td>
</tr>
<tr>
<td>Bradford, UK</td>
<td>1994</td>
<td><em>Cryptosporidium parvum</em></td>
<td>125 cases</td>
<td>storm runoff from farm fields</td>
</tr>
<tr>
<td>Milwaukee, WI</td>
<td>1993</td>
<td><em>Cryptosporidium parvum</em></td>
<td>400,000 cases, 87 deaths</td>
<td>animal manure and/or human excrement</td>
</tr>
<tr>
<td>Maine and others</td>
<td>1993</td>
<td><em>E. coli</em> O157:H7</td>
<td>several illnesses</td>
<td>animal manure spread in apple orchard</td>
</tr>
<tr>
<td>Sakai City, Japan</td>
<td>1995</td>
<td><em>E. coli</em> O157:H7</td>
<td>12,680 cases, 3 deaths</td>
<td>animal manure used in fields growing alfalfa sprouts</td>
</tr>
</tbody>
</table>

1.1.3 Protection of Proximal Surface Waters

Pathogens co-applied with BLM have two possible water-borne modes of transport to adjacent surface waters (Fig. 1.1) (Reddy *et al.* 1981), percolation through soil and runoff from the surface. Buffer strips, which are portions of permanently vegetated land between agricultural fields and adjacent surface waters (*e.g.* drainage ditches), are commonly utilized to contain pathogens (Fig. 1-2) (Barling and Moore 1994,
Bulc 2006, Rousseau et al. 2004). During subsurface flow, buffer strips reduce densities of fecal coliforms by up to 99% (Table 1.2) (Atwill et al. 2002, Sullivan et al. 2007).

However, surface runoff might not always penetrate into buffer strips that contain soils with poor hydraulic conductivity, are frozen, or experience severe precipitation events, which gives pathogens uninhibited surface flow to adjacent bodies of water (Coyne et al. 1998, Thurston-Enriquez et al. 2005). Thus, it is not surprising that up to 90% of runoff

Figure 1-1: Pathogens experience several routes of transport after they are co-applied with BLM to an agricultural field: (A) Percolation resulting in subsurface flow, (B) Percolation to field drainage tile, and (C) surface runoff. (Image modified from Dekam Construction, LLC, http://www.dekamconstruction.com/).
Figure 1-2: Surface runoff will encounter a buffer strip at the edge of a field and either infiltrate the buffer strip soil (A) or bypass the buffer strip and discharge into surface waters (C) (Image modified from SuDS Wales, http://www.sudswales.com/).

Table 1.2: Subsurface microbial transport through buffer strips.

<table>
<thead>
<tr>
<th>Width</th>
<th>Target Microbe</th>
<th>Effectiveness</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.5 m – 9 m</td>
<td>Fecal Coliform</td>
<td>75% - 90% Reduction</td>
<td>(Coyne et al. 1998)</td>
</tr>
<tr>
<td>30 m</td>
<td>N/A</td>
<td>75% Reduction predicted</td>
<td>(Walker et al. 1990)</td>
</tr>
<tr>
<td>3 m</td>
<td>Cryptosporidium parvum</td>
<td>&gt;99.9% Reduction</td>
<td>(Atwill et al. 2002)</td>
</tr>
<tr>
<td>6.1 m</td>
<td>Fecal Coliforms</td>
<td>&gt;99% Reduction</td>
<td>(Lim et al. 1998)</td>
</tr>
<tr>
<td>0 m - 1.37 m</td>
<td>Fecal Coliforms</td>
<td>83% - 95% Reduction</td>
<td>(Larsen et al. 1994)</td>
</tr>
</tbody>
</table>
samples, collected from a 75-ha watershed in Vermont, exceeded primary contact levels of fecal coliforms (Baxter-Potter and Gilliland 1988, Kunkle 1970a, b).

Pathogens that percolate through field soil and buffer strips might be adsorbed to particles or filtered by small pores within the soil (Gerba et al. 1975, Schaub and Sorber 1977). Pathogens transported to subsurface drainage tiles (Fig. 1-1) often experience less soil contact because they bypass buffer strips, and move readily off-site (Gerba et al. 1975, Gerba and Smith 2005, Lim et al. 1998). Agricultural lands in the Midwestern United States are traditionally tiled to encourage drainage of excess water and are thus a potential source of pathogens for proximal surface waters (Jamieson et al. 2002, Warnemuende and Kanwar 2002), especially when a storm event provides enough hydrostatic pressure to force water through the tiles (Evans and Owens 1972, Joy et al. 1998, Patni et al. 1984). Fecal coliforms can survive for months within drainage tiles, waiting for water to move them forward (Evans and Owens 1972, Geldreich and Litsky 1976, Jamieson et al. 2002).

As part of my hypothesis, I suggest that soil can be engineered to increase interception of surface runoff and tile drainage before it flows into adjacent surface waters. Thus, pathogens that traditionally transport off-site with surface runoff and tile drainage would be filtered within the engineered soil.

Infiltration rate is the major factor that dictates the amount of runoff that can be intercepted by buffer strip soils. An increase in soil particle size will yield increased water infiltration which in turn reduces microbial filtration capacity because of the larger size of soil pores (Huysman and Verstraete 1993). We can compensate for reduced
microbial filtration by increasing transport distance (i.e. the width of buffer strips and interceptors). Microbes that travel farther within a soil matrix have a higher probability of being filtered or adsorbed by the soil than microbes traveling a shorter distance (Abu-Ashour et al. 1994, Gagliardi and Karns 2000).

### 1.1.4 Research Hypothesis and Objectives

Buffer strips are used to decrease fecal coliforms during subsurface flow (Table 1.2). I hypothesized that soil formulated to intercept field runoff and tile drainage could be engineered to remove pathogenic bacteria from the water by a combination of filtration and interception. Furthermore, data obtained in experiments concerning soil and bacterial characteristics would allow one to use an advection-dispersion model to predict the transport distance of bacteria through soil. The predicted transport distance that reduced bacterial densities to below target densities for fecal indicators (i.e. *E. coli* and *Enterococcus* spp.) from Ohio Environmental Protection Agency’s (OEPA) Water Quality Standards (WQS) was used to predict buffer strip size. Specific research goals were to:

1. Formulate a semi-pervious soil from components obtained in northwest Ohio.
2. Use formulated soil in microcosms of three lengths (30, 45, and 60 cm) to obtain numerical values for the model parameters: fluid velocity, $v$; dispersivity, $\alpha L$; initial injection volume, $V_o$; and single collector efficiency, $\eta$. 
(3) Obtain values for distance-dependent densities of fecal indicator bacteria that were transported through microcosms with saturated flow, and determine whether these results were predicted by the advection-dispersion model.

(4) Make predictions of distance-dependent densities for simulated agricultural fields and compare the resultant predictions to observed effluent densities using field lysimeters.

(5) Process the data using a trend analysis to predict transport distances that are needed to obtain target densities of bacteria.

1.2 Objective 1 – Formulation of Semi-Pervious Soil

1.2.1 Agricultural Soil

The rate bacteria travel through soil depends on soil characteristics, such as hydraulic conductivity and soil particle size (Jamieson et al. 2002, Osborne and Kovacic 1993). Buffer strips are traditionally composed of the soil found on-site (Sullivan et al. 2007). Therefore, one needs to define the characteristics of the soil that are used at any site to understand the transportability of bacteria. As a first step in my research, I formulated a soil from components found in northwest Ohio, a farming region composed of tile drained fields and soil exhibiting an infiltration rate between 1 and 10 mm/h (USDA 2009). Therefore, buffer strips in northwest Ohio that are constructed using on-site agricultural soil allow little to no water infiltration. Thus, a soil was formulated with
larger particle sizes and an increased infiltration rate to represent a potential substitute for current northwest Ohio buffer strip soils.

1.2.2 Buffer Strip Soil

Buffer strip soil must be porous enough to allow for the infiltration of water and reduce densities of target bacteria at a reasonable cost. Therefore, the soil was formulated using components readily available in northwest Ohio to exhibit an infiltration rate (between 100 and 150 mm/hr), which is significantly higher than the rate in common northwest Ohio soils. The formulated soil contained (1) clay, which provided a high surface area to volume ratio for the adsorption of bacteria (Huysman and Verstraete 1993); (2) landscaping sand, which allowed water to infiltrate the soil at a faster rate than clay or silt-based soils (Abu-Ashour et al. 1994, Harter et al. 2000); and (3) dried stems and leaves of Phragmites australis an invasive reed collected from the Stranahan Arboretum and Maumee Bay State Park. P. australis was added to increase the organic content in the soil matrix, thereby increasing the adsorption and filtration of microorganisms (Mosaddeghi et al. 2009). Filtration efficiency of the formulated soil was assessed with the use of an advection-dispersion model, which describes microbial transport through soil.
1.3 Objective 2 – Assumptions and Parameters of the Advection-Dispersion Model

1.3.1 Mechanisms of Bacterial Transport

I determined the major factors that influence the transport of microorganisms through soil in order to effectively understand and utilize the advection-dispersion model. All of the parameters associated with the advection-dispersion model fall into at least one of three categories: (1) advection, (2) dispersion, and (3) adsorption (Harvey and Garabedian 1991).

Bacteria, suspended in water, travel through soils by flowing between soil particles. Larger spaces between soil particles provide bacteria with a faster and more direct route through soil. Advection is defined as the movement of bacteria in the direction of fluid flow through the soil (Harvey and Garabedian 1991, McCaulou 1994, Yates and Yates 1991). Dispersion is a secondary movement in which bacteria diffuse from areas of high density to areas of low density (McCaulou 1994). Advection causes bacteria to travel in a single direction while dispersion causes bacteria to diffuse between soil particles. Bacteria that collide with soil particles during transport might stick to those particles and remain attached – i.e. adsorbed (Huysman and Verstraete 1993). Several factors influence adsorption (e.g. collision efficiency, reversible adsorption and irreversible adsorption). The collision efficiency denotes the frequency that a bacterium might come into contact with the surface of a soil particle (Zhang 2002). Reversible and
irreversible adsorptions determine whether or not a bacterium will permanently attach to a soil particle (Liu et al. 2008). The likelihood of a bacterium colliding and adsorbing to a soil particle increases with transport distance (Jamieson et al. 2002).

1.3.2 Overview and Assumptions of the Advection-Dispersion Model

The advection-dispersion model was modified from a colloid-filtration model for the purpose of describing down-gradient transport of bacteria (Harvey and Garabedian 1991). The model is based on first-order kinetics and, upon completion, yields the ratio of microorganisms that would travel a given distance through soil after a specific duration of time. Assumptions for this model include saturated and uniform flow, a pulse-input of bacteria, and consistent hydrostatic pressure on the soil (Harvey and Garabedian 1991). The model requires the input of several experimental and calculated parameters (Harvey and Garabedian 1991, Zhang 2002):

1. Fluid velocity, \( v \), is the speed at which fluid travels through porous media.
2. Dispersivity, \( \alpha L \), is the dispersion associated with a conservative tracer after a pulse-input into soil has occurred.
3. Initial injection volume, \( V_0 \), is the volume of bacteria within the influent when it comes into contact with soil.
4. Single collector efficiency, \( \eta \), describe the physical factors associated with collisions of colloids to a single soil particle.
1.3.3 Microcosm Justification and Requirements

Microcosms, filled with formulated soil, were standardized to meet model assumptions and used to obtain data to calculate model parameters ($v$ and $\alpha L$). Conservative tracers are only affected by advection and dispersion during transport and are used to obtain data to calculate dispersivity (Levy and Chambers 1987). Dispersivity is calculated from $T_{\text{peak}}$ and $\Delta T$ which describes controlled transport through microcosms (Fig. 1-3) (Harvey and Garabedian 1991). Analyses of sodium bromide (NaBr) tracers are often used to determine whether microcosms exhibit uniform flow (Hornberger et al. 1992, McCaulou 1994, Mosaddeghi et al. 2010).

Figure 1-3: Hypothetical results and target parameters from the transport of a conservative tracer through a microcosm.
1.4 Objective 3 – Controlled Transport of Fecal Indicator Organisms

1.4.1 Fecal Indicator Species

Fecal indicator organisms are used to indicate the potential presence of pathogens and fecal coliforms within surface waters (Bitton et al. 1983, Burton et al. 1987, Reddy et al. 1981). The American Public Health Association (APHA) reports both *E. coli* and *E. hirae* as being fecal indicator organisms with a strong correlation to swimming-associated gastroenteritis (Eaton and Franson 2005, Wade et al. 2003). Quantification of *E. coli* and *E. hirae* was used in this study to measure the densities of fecal bacteria within water samples.

1.4.2 Fecal Indicator Contamination Limits

Target densities for *E. coli* and *E. hirae* were used as goals for post-treatment bacterial densities. If post-treatment bacterial densities were below target densities the treatment system was considered a success. The model was used to calculate the distance through a buffer strip that target densities will be achieved. Ideally, zero bacteria would be permitted to reach surface waters. However, post-treatment densities of bacteria might be below detection limits for membrane filtration (20 CFU/mL) resulting in a false-negative indication for the presence of bacteria. This study utilizes Water Quality Standards (WQS) from the Ohio Environmental Protection Agency (OEPA) to establish
target densities of bacteria. OEPA WQS are based on surface water usage and location to determine maximum limits on how much bacteria is allowed to be present within surface water samples. Streams and ditches adjacent to agricultural fields are labeled as either primary contact or secondary contact water bodies in Ohio. Primary contact water bodies are recommended to have lower densities of fecal coliform than secondary contact water bodies (Heitzman 2004). Standards for a primary contact water body should not exceed 126 and 35 CFUs/100 mL of *E. coli* and *E. hirae* respectively (Heitzman 2004, Nappier and Boone 2012). These densities were used as target values to determine if buffer strip treatment was successful.

1.5 **Objective 4 – Transport through a Simulated Buffer Strip**

Environmental conditions not incorporated into the advection-dispersion model can influence the survival, transport rate and transport distance of fecal coliforms (McMurry *et al.* 1998, Natsch *et al.* 1996). Outdoor field lysimeters were used to represent buffer strips under field conditions. The model was used to predict distant-dependent densities of bacteria for field lysimeters to determine whether environmental conditions influenced the model precision. Field lysimeters were susceptible to environmental conditions such as: changing temperature and humidity, preferential flow, and soil clumping. The variability and consistency of model predictions can be determined by adjusting model parameters to lysimeter conditions and comparing the
resulting model predictions with empirical transport results from the lysimeter experiments (Piñeiro et al. 2008).

1.6 Objective 5 – Model Predictions

Dispersion causes bacteria to travel through soil at different rates, resulting in a range of transport distances among bacteria after a given period of time (Gannon et al. 1991). Therefore, a single pulse of bacteria, that experiences unidirectional flow, diffuses forward and backward from the original front during transport (Harvey and Garabedian 1991). The center of the front contains the highest densities of bacteria (Levy and Chambers 1987). The advection-dispersion model was used to predict this maximum density of bacteria for a given transport distance and determine if the treatment was successful according to OEPA WQS.
Chapter 2

Materials and Methods

2.1 Bacteria and Growth Conditions

*Escherichia coli* (ATCC 700891) and *Enterococcus hirae* (ATCC 10541D-5), were obtained as freeze-dried pellets, rehydrated with 0.5 mL of tryptic soy broth (TSB) and grown using, respectively, tryptic soy broth agar (TSA) (Becton, Dickinson and Company, Sparks, MD) and brain heart infusion agar (BHI) (Remel, Lenexa, Kansas) containing defibrinated sheep’s blood (Remel, Lenexa, Kansas), at 37°C for 48h or until colonies formed. A sterile loop was used to select a single colony, which was used to inoculate an Erlenmeyer flask containing either 25 mL of TSB (for *E. coli*) or 25 mL of BHI (for *E. hirae*). A glycerol stock was made for cultures incubated in a shaking water bath at 37.7 °C for 16 - 18h, following which, 25 mL of a 50% glycerol solution was added to each flask, aliquots (1 mL each) were transferred to sterile microcentrifuge tubes and stored at -70°C. Prior to use, cultures were thawed, inoculated in 25 mL of either TSB or BHI, as appropriate and incubated with shaking (New Brunswick Scientific, 12400 Incubator Shaker) at 37.7 °C for 14 hr to achieve mid-log growth.
2.2 Bovine Liquid Manure Collection and Storage

BLM was collected (Sept. 2011 and Oct. 2012) from a storage lagoon at Wezbra Dairy (Continental, Ohio), transported in sealed five-gallon buckets and stored at 4°C. *E. coli* and *Enterococcus* spp. present in BLM were quantified by membrane filtration according to EPA methods 1604 and 1601 (EPA 2001, 2002). BLM obtained in Sept. 2011 was applied to laboratory microcosms (Oct. 2011 – June 2012) and BLM obtained in Oct. 2012 was applied to lysimeters (Oct. 2012).

2.3 Objective 1 – Formulation of a Semi-Pervious Soil

2.3.1 Soil Formulation

Clay, sand and *P. australis* spp. (harvested as dry stems and leaves from the Stranahan Arboretum, Toledo, OH and Maumee Bay State Park, Oregon, OH) were collected, dried and stored at room temperature. The clay and sand were individually sifted through a 2.0-mm sieve and *P. australis* was milled (Hammer mill) and sifted through 0.5-inch mesh to standardize the particle size distribution for each component. The sieved clay, sand and *P. australis* were mixed in a 1:3:1 volumetric ratio respectively, by hand in 68 g increments (56g sand, 10g dredge and 2g *P. australis*) for use in laboratory microcosms and in a wheelbarrow in 3.9 ft$^3$ (2.34 m$^3$ sand, 0.78 m$^3$ clay, 0.78 m$^3$ *P. australis*) increments for the field study.
2.3.2 Soil Characterization

Grain size distribution was determined by sieve analysis (Day 1965); pH was measured in a suspension of soil homogenized in reverse osmosis (RO) treated water (Toledo Mettler, Seven Go Pro) (Page 1982); and bulk density (g/cm$^3$) was calculated as the mass of soil (g) per unit of volume (cm$^3$). Porosity was determined by saturating soil in five gallons of RO water for 24 h. The soil was then removed and the loss of water from the bucket (equal to soil void volume [cm3] and soil volume [cm3]) was used to calculate porosity (Fetter and Fetter 2001). Hydraulic conductivity was calculated from results of a constant-head test requiring measurements for elapsed time, hydraulic head, volumetric flow, cross-sectional surface area, and soil depth (Fetter and Fetter 2001).

2.4 Objective 2 – Identify and Quantify Model Parameters

2.4.1 Advection Dispersion Model for Bacterial Transport

The equation to describe colloid filtration is written as:

$$\theta \frac{\delta c}{\delta t} + \rho_b \frac{\delta s}{\delta t} = D\theta \frac{\delta^2 c}{\delta x^2} - v\theta(\delta c/\delta x + k_p c)$$

(eq. 1)

and was combined with a standard bacterial transport model to create a one dimensional equation for bacterial transport through a saturated medium. The advection-dispersion model contains parameters for storage, reversible and irreversible adsorption, dispersion, and advection (Harvey and Garabedian 1991) and is written as:

$$\frac{C(t)}{C_0} = V_{\phi}/(\Lambda \theta (4\pi \alpha_L^* x)^{0.5}) \exp[-(x-x')^2/(4\alpha L^* x') - k_p x']$$

(eq. 2)
The advection-dispersion model describes bacterial transport through a porous media and predicts the ratio of bacteria ($C/C_o$) that persists given a transport distance ($x$) and time ($t$) after microbes are added to the porous media (Harvey and Garabedian 1991). Each model component partially described the fate of bacteria as it is transported through soil (Table 2.1).

The measured components in the advection-dispersion model required to calculate $C/C_o$ are as follows: $V_o$ is the initial volume of bacteria and is the volume of bacteria in influent that is surface applied to a column or lysimeter. Both $A$ and $\theta$ are physical factors of the soil, cross sectional surface area and porosity respectively.

Table 2.1: Properties influencing bacterial transport and the associated model components

<table>
<thead>
<tr>
<th>Variable</th>
<th>Symbol</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Injection Volume</td>
<td>$V_o$</td>
<td>Volume of Bacteria injected into soil</td>
</tr>
<tr>
<td>Cross sectional surface area</td>
<td>$A$</td>
<td>Cross sectional surface area of soil</td>
</tr>
<tr>
<td>Porosity</td>
<td>$\theta$</td>
<td>Porosity</td>
</tr>
<tr>
<td>Dispersivity</td>
<td>$\alpha L$</td>
<td>Bromide Variable (See Table 2.2)</td>
</tr>
<tr>
<td>Depth (Distance)</td>
<td>$x$</td>
<td>Depth of Soil</td>
</tr>
<tr>
<td>Spatial Coordinate for center of mass</td>
<td>$x'$</td>
<td>Flow Trend (See Table 2.3)</td>
</tr>
<tr>
<td>Irreversible adsorption</td>
<td>$k_p$</td>
<td>Permanent Adsorption (See Table 2.4)</td>
</tr>
</tbody>
</table>

The soil physical factors also included soil depth, $x$, and these factors help determine the efficiency in which liquid will penetrate through the soil.
The dispersivity factor, $\alpha_L$, requires values for the duration of bromide curve (\(\Delta T\)) and elapsed time to bromide peak (\(T_{peak}\)) to determine its solution (eq. 3 and Table 2.2):

$$\alpha_L = x(\Delta t / t_{peak})^2 / 16 \cdot \ln(2)$$  \hspace{1cm} (eq. 3)

**Table 2.2: Parameters used to calculate dispersivity (eq. 3)**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Symbol</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of Bromide Curve</td>
<td>(\Delta T)</td>
<td>Conservative Tracer Experiment</td>
</tr>
<tr>
<td>Elapsed Time to Bromide Peak</td>
<td>(T_{peak})</td>
<td>Conservative Tracer Experiment</td>
</tr>
<tr>
<td>Depth (Distance)</td>
<td>(x)</td>
<td>Depth of Soil</td>
</tr>
</tbody>
</table>

Where \(\Delta T\) is the duration of the bromide peak and \(T_{peak}\) is the time to peak bromide concentration (Harvey and Garabedian 1991). The spatial coordinate for center of mass describes the distance traveled by the center of the bacterial front and is represented by \(X'\) which equals \(vt/R\) where \(R\) is the retardation coefficient, \(t\) is the time.

**Table 2.3: Parameters used to Calculate Spatial Coordinate for Center of Mass (eq. 3)**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Symbol</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluid Velocity</td>
<td>(v)</td>
<td>Velocity of fluid traveling through soil</td>
</tr>
<tr>
<td>Time</td>
<td>(t)</td>
<td>Time after injection of desired effluent densities</td>
</tr>
<tr>
<td>Retardation Coefficient</td>
<td>(R)</td>
<td>Literature Value</td>
</tr>
</tbody>
</table>

after injection and \(v\) is the fluid velocity (Table 2.3). Irreversible adsorption, \(K_P\), describes the efficiency of adsorption for a single bacterium in a packed bed (Yao et al. 1971) (eq. 4 and Table 2.4):

$$K_P = \frac{3}{2}(1 - \theta) \cdot \alpha / \eta / dc$$  \hspace{1cm} (eq. 4)
Where $\alpha$ is the collision efficiency, $\eta$ is the single collector efficiency, and $d_c$ is the diameter of porous media grains. The single collector efficiency, $\eta$, is used to describe all physical factors effecting contact with grain surface (Yao et al. 1971) and is obtained by solving for $\eta$ in the equation:

$$\ln(C/C_o) = -3/2(1-\theta)\alpha\eta(L/d_c)$$

(5)

Where $C/C_o$ is the ratio of bacteria found in effluent, $\theta$ is soil porosity, $\alpha$ is the collision efficiency (equal to 1), $L$ is soil depth and $d$ is grain size diameter.

### 2.4.2 Conservative Tracer Parameters

Parameters used to calculate dispersivity were obtained from data for transport of a conservative tracer through soil. Triplicate microcosms consisted of PVC pipes (3.84 cm in diameter and 70 cm in height) covered on one end with wire mesh, and filled with formulated soil to three heights (30 cm, 45 cm or 60 cm). The wired ends of the nine microcosms were placed in buckets of RO water for 24 h to saturate the soil.
Saturated microcosms were removed from the RO water, placed upright with the wire mesh end at the bottom to allow for draining. At that point, NaBr solution (50 mL: 100 ppb) was added to the top of a column and allowed to infiltrate after which RO water was added to maintain a consistent hydrostatic pressure. Effluent from the bottom of the soil was collected every 2 min for 1 h and the concentration of NaBr was measured (Mettler Toledo Seven Go Pro with bromide probe) within each effluent sample.

2.5 Objective 3 – Fecal Coliform Distance-Dependent Densities

2.5.1 Transport of Bacteria Suspended in Phosphate Buffer

Cells of *E. coli* and *E. hirae* were extracted (1 mL of TSB/BHI) from growth media by centrifugation (4 min at 2000 rpm) and the pellet was washed twice in phosphate buffered saline (PBS [1 mL]), centrifuged (4 min at 2000 rpm), decanted and suspended in PBS (51 mL). A subsample (1 mL) of suspension was used to determine the microbial density; the remainder (50 mL) was added to the surface of the soil in each microcosm (see section 2.3.2), resulting in nine microcosms (triplicate for each height of 30, 45, and 60 cm). An effluent sample was collected immediately after the bacteria were added to the soil and for every 50 mL of drainage a subsequent effluent sample (5 mL) was collected. Time and cumulative effluent volume were recorded. Quantities of *E.*
coli and E. hirae were determined using methods 1601 and 1604 from the EPA (EPA 2001, 2002).

2.5.2 Transport of Bacteria Suspended in BLM

Cells of E. coli and E. hirae were added (1 mL each) to BLM, and the resultant solution diluted with RO water (50% dilution) to simulate the viscosity of BLM after irrigation or precipitation. A subsample (1 mL) was obtained to determine the microbial density and the remainder (50 mL) was surface applied to each soil column as above. An effluent sample (5 mL) was collected immediately after BLM was added to the soil and for every 50, 75 and 100 mL of drainage a subsequent effluent sample (5 mL) was collected for column heights of 30, 45 and 60 cm respectively. Each effluent sample was tested for densities of E. coli and E. hirae by the 1601 and 1604 EPA methods (EPA 2001, 2002).

2.6 Objective 4 – Simulated Buffer Strips

Soil filtration studies in field conditions were conducted at the University of Toledo’s Stranahan Arboretum. Field lysimeters consisted of stainless steel cylinders (123 cm in diameter and 61 cm in length) with fiberglass grating covered with a layer of root liner. The fiber glass grating supported soil above the effluent holding basin while the root liner prevented soil particles from falling through the grate (Fig. 2-1). The formulated soil was mixed (3.9 ft³ increments to a total of 19.4 ft³) in a wheelbarrow and
added to each lysimeter. Lysimeters were saturated with local well water until standing water was observed above the soil surface. Prior to use, water was pumped out of the effluent holding basin under each lysimeter and then BLM (15.1 L) containing *E. coli* and *E. hirae* (3.4 \( \times 10^5 \) and 1.5 \( \times 10^5 \) CFU/mL respectively), was applied to each lysimeter. Well water (56.8 L) was added to the lysimeter after BLM infiltrated the soil surface and additional water was added to maintain a consistent level. Effluent was constantly pumped from the holding basin using a peristaltic pump. Total leachate was measured and a sample (~ 200 mL) was obtained from the pump after each 10L increment, transported in an ice filled cooler and densities of *E. coli* and *E. hirae* were determined (EPA 2001, 2002).

Figure 2-1: Cross sectional view of lysimeter

and *E. hirae* (3.4 \( \times 10^5 \) and 1.5 \( \times 10^5 \) CFU/mL respectively), was applied to each lysimeter. Well water (56.8 L) was added to the lysimeter after BLM infiltrated the soil surface and additional water was added to maintain a consistent level. Effluent was constantly pumped from the holding basin using a peristaltic pump. Total leachate was measured and a sample (~ 200 mL) was obtained from the pump after each 10L increment, transported in an ice filled cooler and densities of *E. coli* and *E. hirae* were determined (EPA 2001, 2002).
2.7 Objective 5 – Data Analysis and Transport Predictions

2.7.1 Statistical Analysis

The data from each microcosm replicate were graphed (density of bacteria in effluent [CFU/mL] versus cumulative effluent volume [mL]). Cumulative CFUs of bacteria in effluent were determined from the integral of the graph (using GraphPad’s Prism 5) for each microcosm. A one-way ANOVA and Tukey test were used to determine the difference between the averages (n = 3) of cumulative colonies of bacteria found in effluent for each transport distance (30, 45, and 60 cm).

2.7.2 Model Predictions

Model predictions of bacteria (C/C₀) found in effluent for every second (approx. 15000 for each microcosm distance) of transport were calculated to determine the maximum C/C₀ (R project software). The model was then used to predict distance-dependent densities of bacteria for the standardized microcosms and field lysimeters by adjusting only those parameters that are inherent to the conditions relevant to different systems, i.e. cross-sectional surface area, initial volume of bacteria and fluid velocity. At a single time point, the corresponding experimental value for ratio of bacteria (C/C₀) and the value from the prediction matrix were paired and graphed (observed effluent ratios of bacteria [C/C₀] vs. predicted effluent ratios of bacteria [C/C₀]). The resulting observed versus predicted graphs were analyzed using a linear best-fit line to determine how well the observed data was duplicated by the model predictions.
The ratios of bacteria \((C/C_0)\) in effluent for the microcosms (30, 45 and 60 cm) and lysimeter (45.7 cm) were analyzed with a trend analysis (exponential decay). Exponential decay equations were determined from the trend analyses and used to calculate the transport distance required to reduce ratios of bacteria to an appropriate \(C/C_0\). The model was used, with lysimeter parameters, to predict the maximum bacterial ratio for each depth (30 cm, 45 cm, and 60 cm). These three values were graphed \((C/C_0\) versus depth) and first and second-order decay analyses were performed using \textit{Prism 5} to create a trend of \(C/C_0\) relating to depth. From these trends I gained the ability to predict minimum depth necessary to achieve WQS depending on the ratio of bacteria that must be removed from BLM and on the initial density of bacteria applied the system.
Chapter 3

Results

3.1 Objective 1 – Soil Characteristics

The following soil characteristics were obtained for the formulated soil: porosity (0.42), grain size (0.04 cm), bulk density (1.2 g/cm$^3$), hydraulic conductivity (0.024 cm/s), and pH (8.1). Based on grain size and soil texture analysis the soil was classified as semi-pervious well-sorted sand with an increased infiltration rate compared to silt loam (Semi-pervious well-sorted sand: 123 mm/hr. Silt loam: 4.00 mm/hr.), which is common agricultural soil in NW Ohio.

3.2 Objective 2 – Model Parameters

3.2.1 Literature and Constant Parameters

Values for two constants were initially established to satisfy all non-experimental values required by the advection-dispersion model: collision efficiency factor ($\alpha = 1.0$) and retardation coefficient ($R = 1.00011$) (Harvey and Garabedian 1991, Yao et al.)
1971). Single collector efficiency ($\eta = 6.16 \times 10^{-2}$ [eq. 5]) and irreversible adsorption ($k_p = 30 \text{ cm} - 1.34, 45 \text{ cm} - 0.89, \text{ and } 60 \text{ cm} - 0.67$ [eq. 4]) were both calculated from experimentally-derived values in this study.

### 3.2.2 Conservative Tracer Parameters

Dispersivity ($\alpha_L$) was calculated from the results ($T_{\text{peak}}$ and $\delta T$) of the conservative tracer study (Figure 3-1). Fluid velocity (0.015 – 0.025 cm/s) was calculated by dividing $T_{\text{peak}}$ from depth of soil. Results (Table 3.1) were incorporated into eq. 3 and dispersivity ($\alpha_L$: 30 cm = 2.17 cm; 45 cm = 2.28 cm; 60 cm = 5.41 cm) was calculated for each soil depth.

#### Conservative Tracer Results

![Figure 3-1: NaBr concentrations (C/C_o) measured in effluent during transport through time for three depths of soil (30, 45 and 60 cm).](image)

Figure 3-1: NaBr concentrations (C/C_o) measured in effluent during transport through time for three depths of soil (30, 45 and 60 cm).
Table 3.1: Model parameters experimentally determined from conservative tracer results.

<table>
<thead>
<tr>
<th>Model Parameter</th>
<th>30 cm Column Value</th>
<th>45 cm Column Value</th>
<th>60 cm Column Value</th>
<th>Lysimeter Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>11.34 cm³</td>
<td>11.34 cm³</td>
<td>11.34 cm³</td>
<td>11674.5 cm³</td>
</tr>
<tr>
<td>x</td>
<td>30 cm</td>
<td>45 cm</td>
<td>60 cm</td>
<td>45 cm</td>
</tr>
<tr>
<td>C/Co</td>
<td>0.58</td>
<td>0.68</td>
<td>0.56</td>
<td>-----</td>
</tr>
<tr>
<td>Delta t</td>
<td>7 min</td>
<td>15 min</td>
<td>20 min</td>
<td>-----</td>
</tr>
<tr>
<td>t Peak</td>
<td>13 min</td>
<td>40 min</td>
<td>60 min</td>
<td>-----</td>
</tr>
<tr>
<td>Vo</td>
<td>0.8</td>
<td>0.4</td>
<td>0.5</td>
<td>1235</td>
</tr>
<tr>
<td>v</td>
<td>0.0225</td>
<td>0.025</td>
<td>0.015</td>
<td>0.014</td>
</tr>
</tbody>
</table>

3.3 Objective 3 – Fecal Coliform Transport

3.3.1 Transport of E. coli and E. hirae

Baseline values for several model parameters (v, V₀, time to maximum C/C₀, and Depth) were determined from the transport of E. coli (Table 3.2) and E. hirae (Table 3.3) suspended in PBS through microcosms. The model was used to predict C/C₀ values over time, which were displayed on a coordinate plane with the observed results (Figure 3-2, only 30 cm and 60 cm columns are shown).
Table 3.2: Results ($n = 3$) from the transport of $E. coli$ through microcosms at each depth.

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>Effluent Volume (mL) to Maximum $C/C_0$</th>
<th>Time (min) to Maximum $C/C_0$</th>
<th>Maximum $C/C_0$</th>
<th>Initial Injection Volume ($V_o$)</th>
<th>Fluid Velocity (v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>257</td>
<td>24</td>
<td>0.0060</td>
<td>0.8</td>
<td>0.0225</td>
</tr>
<tr>
<td>45</td>
<td>265</td>
<td>36</td>
<td>0.0020</td>
<td>0.4</td>
<td>0.025</td>
</tr>
<tr>
<td>60</td>
<td>388</td>
<td>72</td>
<td>0.0020</td>
<td>0.5</td>
<td>0.015</td>
</tr>
</tbody>
</table>

Table 3.3: Results ($n = 3$) from the transport of $E. hirae$ through microcosms at each depth.

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>Effluent Volume (mL) to Maximum $C/C_0$</th>
<th>Time (min) to Maximum $C/C_0$</th>
<th>Maximum $C/C_0$</th>
<th>Initial Injection Volume ($V_o$)</th>
<th>Fluid Velocity (v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>56.0</td>
<td>9.5</td>
<td>0.00035</td>
<td>13</td>
<td>0.0225</td>
</tr>
<tr>
<td>45</td>
<td>223</td>
<td>18</td>
<td>0.0018</td>
<td>10</td>
<td>0.025</td>
</tr>
<tr>
<td>60</td>
<td>538</td>
<td>34</td>
<td>0.00012</td>
<td>8</td>
<td>0.015</td>
</tr>
</tbody>
</table>
Figure 3-2: *E. coli* values ($C/C_0$) for 30 cm (A) and 60 cm (B) microcosms found in effluent (circular points) compared predicted values ($C/C_0$) from the model (black regression line)
3.3.2 Transport of *E. coli* and *E. hirae* suspended in BLM

Effluent samples from microcosms amended with BLM were monitored for *E. coli* and *E. hirae* (Table 3.4). Maximum densities of bacteria were reduced as depth increased for both indicator organisms (*E. coli* – From $5.75 \times 10^5$ CFU/mL for 30cm to $1.35 \times 10^5$ CFU/mL for 60cm; *E. hirae* – $3.93 \times 10^5$ CFU/mL for 30cm to $5.67 \times 10^4$ CFU/mL for 60cm). A one-way ANOVA and Tukey test revealed whether cumulative colonies of bacteria in effluent were significantly different (p-value < 0.05) between soil depths (Table 3.5). Cumulative CFUs of *E. coli* between the 30 cm vs. 60 cm depths and 45 cm vs. 60 cm depths were significant (p-value of 0.0061 and 0.0023 respectively) while cumulative CFUs of *E. hirae* were only significant between the 30 and 60 cm soil depths (p-value of 0.0155).

Table 3.4: Results from the transport of *E. coli* and *E. hirae* co-applied with BLM to microcosms at three soil depths (30, 45 and 60 cm)

**E. coli**

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>Cumulative Effluent Volume (mL)</th>
<th>Mean Time to Maximum C/Co (min)</th>
<th>Cumulative CFUs in Effluent</th>
<th>Cumulative C/Co</th>
<th>Maximum (CFU/mL)</th>
<th>Maximum (C/Co)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>249</td>
<td>167</td>
<td>6.64E+07</td>
<td>0.29</td>
<td>5.75E+05</td>
<td>0.113</td>
</tr>
<tr>
<td>45</td>
<td>366</td>
<td>193</td>
<td>7.34E+07</td>
<td>0.32</td>
<td>4.07E+05</td>
<td>0.080</td>
</tr>
<tr>
<td>60</td>
<td>552</td>
<td>356</td>
<td>1.32E+07</td>
<td>0.06</td>
<td>1.35E+05</td>
<td>0.026</td>
</tr>
</tbody>
</table>

**E. hirae**

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>Cumulative Effluent Volume (mL)</th>
<th>Mean Time to Maximum C/Co (min)</th>
<th>Cumulative CFUs in Effluent</th>
<th>Cumulative C/Co</th>
<th>Maximum (CFU/mL)</th>
<th>Maximum (C/Co)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>249</td>
<td>183</td>
<td>6.15E+07</td>
<td>0.51</td>
<td>3.93E+05</td>
<td>0.146</td>
</tr>
<tr>
<td>45</td>
<td>366</td>
<td>175</td>
<td>2.99E+07</td>
<td>0.25</td>
<td>1.57E+05</td>
<td>0.058</td>
</tr>
<tr>
<td>60</td>
<td>552</td>
<td>400</td>
<td>1.47E+07</td>
<td>0.12</td>
<td>5.67E+04</td>
<td>0.021</td>
</tr>
</tbody>
</table>
Table 3.5: Results from a one-way ANOVA and Tukey comparison for the cumulative CFUs in microcosm effluent.

<table>
<thead>
<tr>
<th>Depths Compared</th>
<th>30 cm vs. 45 cm</th>
<th>30 cm vs. 60 cm</th>
<th>45 cm vs. 60 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Significant? (p-value &lt; 0.05)</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Adjusted p Value</td>
<td>0.9888</td>
<td>0.0061</td>
<td>0.0023</td>
</tr>
<tr>
<td>DF</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>E. hirae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depths Compared</td>
<td>30 cm vs. 45 cm</td>
<td>30 cm vs. 60 cm</td>
<td>45 cm vs. 60 cm</td>
</tr>
<tr>
<td>Significant? (p-value &lt; 0.05)</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Adjusted p Value</td>
<td>0.1392</td>
<td>0.0155</td>
<td>0.7686</td>
</tr>
<tr>
<td>DF</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
</tbody>
</table>

3.3.3 Evaluation of Predictions for Bacterial Transport

Model parameters associated with the transport of bacteria suspended in BLM were used in the advection-dispersion model. The ratio of bacteria (C/C₀) in effluent was compared to the corresponding model prediction (C/C₀). A best-fit line analysis was performed for each bacteria (E. coli and E. hirae) at each depth (30, 45 and 60 cm) and values for R² (variance) were determined to be: R²: E. coli, 30 cm: 0.978, 45 cm: 0.987, 60 cm: 0.741; E. hirae, 30 cm: 0.852, 45 cm: 0.737, 60 cm: 0.638 (Fig. 3-3, Only 30 cm depth is shown, best-fit line analysis for 45 and 60 cm can be found in Appendix A).
Figure 3-3: Best-fit line analyses were used to compare observed values \((C/C_0)\) in effluent to model predictions \((C/C_0)\) for *E.coli* and *E. hirae* in 30 cm of soil depth.
3.4 Objective 4 – Model Verification Using Simulated Agricultural Field Conditions

3.4.1 Lysimeter Transport Results

Effluent samples from field lysimeters were monitored for the presence of *E. coli* and *E. hirae* (Table 3.6). The maximum density of *E. coli* and *E. hirae* was present in effluent after 98.3 min and 102.3 min respectively. After pulse application of BLM, 172 L of water were required for *E. coli* to reach a maximum C/C$_0$ (0.0082) whereas *E. hirae* required 203 L of water to reach a maximum C/C$_0$ (0.0519).

Table 3.6: Transport results from lysimeter experiments

<table>
<thead>
<tr>
<th></th>
<th><em>E. coli</em></th>
<th><em>E. hirae</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth (cm)</td>
<td>45.7</td>
<td>45.7</td>
</tr>
<tr>
<td>Mean Leachate to Peak (L)</td>
<td>171.67</td>
<td>203.33</td>
</tr>
<tr>
<td>Time to Peak (min)</td>
<td>98.333</td>
<td>102.33</td>
</tr>
<tr>
<td>Peak (col/mL)</td>
<td>2783</td>
<td>7850</td>
</tr>
<tr>
<td>Peak C/C$_0$</td>
<td>0.0082</td>
<td>0.0519</td>
</tr>
<tr>
<td>Area (cm$^2$)</td>
<td>11675</td>
<td>11675</td>
</tr>
</tbody>
</table>

3.4.2 Evaluation of Predictions for Lysimeter Transport

The model was used to predict densities of bacteria in lysimeter effluent (A, v, V$_o$ and ρ were adjusted to lysimeter measurements) and these results were compared to the experimentally determined densities of bacteria in lysimeter effluent (Figure 3-4 and 3-5).
Dispersivity associated with the 45 cm laboratory microcosm ($\alpha_L: 45\text{ cm} = 2.28 \text{ cm}$) was used for lysimeter predictions (lysimeter depth = 45.7 cm). Results from a linear correlation for observed and predicted $E.\ coli C/C_o$ values yielded consistency (linear slope = 1.0941) and variance ($R^2 = 0.4828$). In contrast, the linear correlation of $E.\ hirae$ results was less consistent and had a higher variance than $E.\ coli$ (linear slope = 0.4756, $R^2 = 0.020$).

Figure 3-4: Predicted densities of $E.\ coli$ in effluent ($C/C_o$) versus observed densities of bacteria in effluent ($C/C_o$) through time.
3.5 Objective 5 – Predicting Transport Distance

3.5.1 Predictions Using First-Order Kinetics

The distances that indicator organisms could travel through soils were predicted using first-order kinetics in the form of a one-phase decay equation:

\[ Y = Y_o e^{-KX} \]  

(eq. 6)

with the statistical software Prism 5 (Figure 3.3). Both \( Y_o \) (Y-intercept) and \( K \) (rate constant) were constrained to only allow values greater than zero. The one-phase decay
curve was rearranged to isolate X (soil depth):

\[-\ln\left(\frac{Y}{Y_0}\right)/K = X\]  \hspace{1cm} (eq. 7)

A value for Y ($C/C_0$ found in effluent) was calculated from the density of bacteria present in influent ($C_0$) and the desired density of bacteria in post-treatment effluent (C). An evaluation of eq. 7 yielded a solution for X, the depth of soil necessary to reach the desired Y value. The one-phase decay analysis $R^2$ values were 0.9965 for *E. coli* and 1.000 for *E. hirae*, which indicated a high likelihood of prediction between the X and Y values.

![Predicted Peak C/C_o vs. Depth](image)

*Figure 3-6: Maximum predicted densities of bacteria at each tested depth followed a one-phase decay trend.*
3.4.3 Predictions Using Second-Order Kinetics

A two-phase decay curve allows two different decay rates to help describe a set of data. In this case, I used the existing data for maximum predicted ratios of bacteria at each depth and added a point at (0, 1). Effluent densities of bacteria were equal to the influent densities of bacteria at a y-intercept of \( x = 1 \). **Prism 5** was used to provide a best-fit two-phase decay curve with the same constraints as the one-phase decay curve (\( Y_o \) and \( K \) were constrained to only allow values greater than zero) (Figure 3-8). The resulting \( R^2 \) values (0.9952 and 1.000 for *E. coli* and *E. hirae* respectively) from the two-phase decay analysis indicated a high likelihood of prediction.

A final comparison between the one-phase and two-phase decay curves yielded the distance of microbial transport through soil necessary to reduce densities of bacteria in the starting influent, down to peak bacterial densities that are permitted by EPA SWR (Table 3.7 and 3.8).
Figure 3-7: The plot of $C/C_0$ values versus transport distance followed a two-phase decay trend.

Table 3.7: Minimum soil depth (transport distance) required to reduce initial bacterial densities to below target EPA WQS.

### One-Phase Decay Analysis

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Initial Bacterial Densities (CFU/mL)</th>
<th>Desired $C/C_0$</th>
<th>Depth of Soil (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>5.10E+06</td>
<td>4.61E-07</td>
<td>312</td>
</tr>
<tr>
<td>E. hirae</td>
<td>2.70E+06</td>
<td>1.22E-07</td>
<td>313</td>
</tr>
</tbody>
</table>

### Two-Phase Decay Analysis

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Initial Bacterial Densities (CFU/mL)</th>
<th>Desired $C/C_0$</th>
<th>Depth of Soil (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>5.10E+06</td>
<td>4.61E-07</td>
<td>319</td>
</tr>
<tr>
<td>E. hirae</td>
<td>2.70E+06</td>
<td>1.22E-07</td>
<td>317</td>
</tr>
</tbody>
</table>
Table 3.8: Minimum soil depth (transport distance) required to reduce initial bacterial densities for literature values of *E. coli* and *E. hirae* found in BLM to below EPA WQS (Unc and Goss 2004).

One-Phase Decay Analysis

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Initial Bacterial Densities (CFU/mL)</th>
<th>Desired C/Co</th>
<th>Depth of Soil (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>4.00E+05</td>
<td>5.88E-06</td>
<td>254</td>
</tr>
<tr>
<td><em>E. hirae</em></td>
<td>6.10E+05</td>
<td>5.41E-07</td>
<td>282</td>
</tr>
</tbody>
</table>

Two-Phase Decay

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Initial Bacterial Densities (CFU/mL)</th>
<th>Desired C/Co</th>
<th>Depth of Soil (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>4.00E+05</td>
<td>5.88E-06</td>
<td>259</td>
</tr>
<tr>
<td><em>E. hirae</em></td>
<td>6.10E+05</td>
<td>5.41E-07</td>
<td>285</td>
</tr>
</tbody>
</table>
Chapter 4

Discussion

4.1 Variance in Model Predictions

The variance in model predictions exhibited a trend with distance and target microbe (Fig. 4-1). Variance increased gradually with size. This affect is likely caused by inherent heterogeneities within the system that become intensified with system size. The proposed heterogeneity might not influence microbial transport with any significance on a small scale, but an increase in intensity of heterogeneities with system size could account for the increased variance (Hornberger et al. 1992). Some examples of heterogeneities are identified to be: surface coating, grain size, preferential flow, and adsorption tendencies within microbial populations (Bolster et al. 2000, Hornberger et al. 1992). Unfortunately, these inherent heterogeneities associated with the transport of microbes through soil make it difficult to make predictions on a large scale.

In addition, the differences in variance of model predictions for E. coli and E. hirae were likely attributed to the release kinetics of the individual bacteria. A conservative bromide tracer is a baseline measure of transport used by the advection-dispersion model to predict the transport of colloids, or in this case, bacteria. E. coli
mimics the same release kinetics (first-order release) as bromide. First-order release kinetics described a single rate coefficient which depended only on the concentration of the colloid of interest, in this case either bromide or bacteria. *E. hirae* exhibits significantly different release kinetics and is thought to be attached to the suspended solids in BLM (Guber *et al.* 2006). Thus, advection-dispersion model predictions for *E. coli*, and any other bacterium with first-order release kinetics, are more precise than bacteria that tend to attach to suspended particles. Alternative models should be considered if the target bacterium does not mimic first-order release kinetics.

Figure 4-1: The R-squared values (goodness of fit) describing the relationship between model predictions and experimental results for each microcosm depth and bacteria species. The model replicates observed outcomes more effectively for *E. coli* but for both microorganisms the goodness of fit decreases as system size increases.
### 4.2 Predictions of Minimum Soil Depth

The advection-dispersion model required a unique value for dispersivity at each transport distance. Dispersivity could only be calculated with experimental values from a conservative tracer study, thus predictions of transport distance using the advection-dispersion model were inaccurate. Imagine that the advection-dispersion model was used to make a prediction of $C/C_0$ for distance-dependent densities in a soil 100 cm in depth. The X value would be changed to 100 and the model would yield a prediction of $C/C_0$, however this prediction would be insufficient because dispersivity was not altered. The calculation of dispersivity requires deltaT and Tpeak. These values cannot be determined without performing a conservative tracer study for a soil 100 cm in depth. In addition, I cannot perform a trend analysis on dispersivity to estimate its value for a soil depth of 100 cm because dispersivity does not exhibit any known trends that offer reliable predictions with depth. Therefore, an alternative method (exponential decay analysis) was used to predict soil depths.

Minimum transport distance required to reduce indicator organisms to below WQS were predicted by extrapolating either a one or two-phase exponential decay curve to the appropriate $C/C_0$ value. After the one-phase and two-phase decay curve analyses, both best-fit-lines yielded high $R^2$ values. Thus, little variance influenced the predictions of depth for either decay curve within our data set range (30 cm – 60 cm). However, due to its simplicity the one-phase decay curve was more appropriate when dealing with depths within our experimental range (30 cm – 60 cm) because both decay curves were virtually identical within this range. As depth falls below 30 cm, $C/C_0$ values approached
zero and the one-phase decay curve became less reliable than the two-phase decay curve. However, the two-phase decay curve had a significant threshold between the first and second rate constants (K value). Since confirmed data points from the model were only present on the graph during the second decay rate portion of the trend line it was impossible to know where the threshold of decay rate occurred without additional experiments at smaller depths (< 30 cm). Conceptually the two-phase decay curve was more precise than the one-phase decay curve between depths of 0 and 30 cm of soil if the threshold between the decay rate constants can be determined. As transport distance increased beyond 60 cm there is no difference of predictions between the one and two-phase decay curves. Buffer strips are traditionally more than one meter long, thus the one-phase decay curve was used to predict minimum soil depths.

4.3 Predicting Buffer Strip Size and Feasibility

4.3.1 Infiltration Rate and Transport Distance

Infiltration rate and microbial transport distance have been identified as primary contributing factors for the effective removal of bacteria. Both factors must be appropriate for the location targeted for a buffer strip or drainage interceptor. *E. hirae* predictions of depth (285 cm) were found to be less reliable than *E. coli* predictions of depth (254 cm) associated with first-order kinetics. Thus, a goal of 254 cm of transport distance was used to determine buffer strip and drainage interceptor size. An engineered
soil with an infiltration rate of 123 mm/h was determined to require 254 cm of transport
distance to remove desired amounts of fecal coliforms.

### 4.3.2 Example Buffer Strip

Surface runoff that bypasses a buffer strip is the major problem that the
generated soil will be designed to solve. Therefore, I determined the amount of runoff
that must infiltrate into a buffer strip, and then determine the size (width and length) the
buffer strip must be to intercept all potential runoff. For example, a 12-acre agricultural
field in Lucas County, Ohio experiences substantial precipitation and runoff after
fertilization with BLM (Fig. 4-1). Agricultural soil in Lucas County is classified as
somewhat to very poorly draining and exhibits a hydraulic conductivity of 8.0 x 10^-4
cm/s. Furthermore, the field soil is classified using a runoff curve number (RCN) of 65
indicating it is close-seeded, straight rowed farm field with poor drainage. Surface runoff
will be 0.14 cm deep during a heavy rainfall (5.08 cm or 2 in. of precipitation) according
to RCN estimates. A 0.14-cm deep runoff event from a 12-acre field is equivalent to
67,980 L of water. If field runoff existed for one hour and the maximum width for a
buffer strip could only be 5 m, then the lowest infiltration rate possible for the engineered
soil would be 53.4 mm/h. Table 4.1 displays proposed infiltration rates for decreasing
buffer strip widths. The engineered soil (infiltration rate of 123 mm/h) would require a
surface area of 553 m^2 to intercept the potential surface runoff. The buffer strip must
border one side of the field and depending on that length, the width of the buffer strip can
be calculated \[ i.e. \frac{553 \text{ m}^2}{\text{field length (m)}} = \text{buffer strip width (m)} \].
Figure 4-2: Predicted amount of surface runoff entering a buffer strip from a 12-acre agricultural field after 2 inches of precipitation.

Table 4.1: Buffer strip widths and corresponding infiltration rates appropriate to intercept runoff from a 12-acre field in Lucas County, Ohio after 2 inches of precipitation.

<table>
<thead>
<tr>
<th>Buffer Strip Width (m)</th>
<th>5</th>
<th>4</th>
<th>3</th>
<th>2</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infiltration rate (mm/h)</td>
<td>53.4</td>
<td>66.8</td>
<td>89.0</td>
<td>133.6</td>
<td>267.1</td>
</tr>
</tbody>
</table>
Vertical flow through the buffer strip was the shortest distance that microorganisms could travel through the buffer strip. Therefore, 254 cm was used as the depth of the buffer strip soil. For this example, I ignored microbial dilution from precipitation and the effect that surface vegetation has on rates of surface runoff.

### 4.3.3 Example Drainage Interceptor

Similarly, predictions of size were made for the drainage interceptor. A drainage interceptor is used to intercept field drainage as it flows from a tile drain. Thus, the drainage must flow through soil before it reaches surface waters (Fig. 4-3). The 12-acre field in Lucas County was revisited as a baseline to determine drainage conditions. Precipitation (2-inches of rainfall) is removed from the field by water storage within agricultural soil, evaporation, transpiration and runoff. The remaining precipitation was estimated to become drainage within field tiles (~20 %). Of the 2,467,000 L of precipitation, 493,000 L was estimated to exit the field in the form of field drainage. A 12-acre field was estimated to take 20 h to drain (return to field capacity) and therefore, drain 24,700 L/h, assuming that there is only a single drainage outflow. The engineered soil (123 mm/h) needed 201 m² of contact surface for field drainage to infiltrate the soil. Unfortunately, these dimensions were not practical for a single tile outlet.
Figure 4-3: Side view of a drainage interceptor composed of engineered soil.

However, a hybrid design of the drainage interceptor and buffer strip could offer a practical solution to intercept drainage. The hypothetical design (Fig. 4-4 and Fig. 4-5) would include: two perforated buffer strip tiles which are connected with the field tile that have a 90° turn as they exit the field running parallel to the field in the subsurface of the buffer strip soil. As the hypothetical drainage reaches the end of the buffer strip tiles it would encounter an overflow regulator that would only allow drainage to enter surface water when drainage volume is high (i.e. 100-year rainfall event). The hybrid design provides an increase in contact surface between field drainage and buffer strip soil. The contact surface depends on the diameter of the buffer strip tiles and the length of each tile. A 10-inch diameter buffer strip tile that is half-full of water provides 0.39 m² of contact surface for each meter of tile length. From these assumptions I determined that each buffer strip tile must be a minimum of 258 m in length. The buffer strip tile will
Figure 4-4: Overhead view of buffer strip and drainage interceptor hybrid design to intercept field tile drainage and runoff.

Agricultural Field

Buffer Strip

Tile Drainage: 24,700 L/h or 493,000 L

Subsurface, Perforated Tile through the Buffer Strip Soil

Drainage flows through perforated tile and percolates into the surrounding buffer strip soil

Figure 4-5: Side view of buffer strip and drainage interceptor hybrid design to intercept field tile drainage and runoff.
be placed near the top of the soil to maximize microbial filtration as drainage percolates vertically (254 cm deep, identical to buffer strip depth) through the treatment system. This design is much more reasonable than the drainage interceptor and, with further testing, could prove to be an effective method for intercepting drainage from agricultural fields.

Furthermore, the hybrid design was evaluated with calculated runoff restrictions (553 m$^2$ of surface area) to determine a width and length for a hybrid design capable of intercepting field drainage and runoff. The hybrid buffer strip is required to be 258 m in length to accommodate the length of buffer strip tiles, thus the hybrid buffer strip width must be 2.14 m. The hybrid buffer strip depth remains 2.54 m deep to ensure microorganisms will not contaminate surface waters. The above example of buffer strip development provides a single method to solve several problems facing modern agriculture. A better understanding of field hydrology and microbial transport on a large scale will allow this method of buffer strip development to be practical in a field setting.

4.3.4 Shortcomings

The preceding hypothetical design is a simplified method with the goal of predicting buffer strip sizes. Factors not included in the hypothetical design might influence the performance of the buffer strip system (i.e. buffer slope, flow, and vegetation) (Liu et al. 2008). Vegetation has the ability to influence subsurface hydraulic conductivity and surface runoff rates depending primarily on the root and stem density and size (Archer et al. 2002, Wang et al. 2008). Buffer strip slope influences filtration
effectiveness by significantly reducing the amount of infiltration that would occur if the slope is too shallow or steep (buffers are most effective with a 9% slope) (Liu et al. 2008). Finally, flow refers to the behavior of runoff as it moves across the field and eventually infiltrates the buffer strip soil. Traditionally, runoff is considered to be laminar flow across the surface of a field, however, slopes, berms, and erosion may all cause flow to be directional resulting in concentrated flow in certain areas and no flow in other areas (Lim et al. 1998, Liu et al. 2008, Osborne and Kovacic 1993). Failing to account for concentrated flow situations may result in a reduction of buffer strip filtration efficiency by overloading one section of buffer strip with the majority of field runoff.
Chapter 5

Conclusion

This research offered several important results: (1) Soil was formulated that filtered fecal indicator bacteria at a faster rate than existing agricultural soils. (2) The filtration process was modeled to determine the distance of microbial transport that was necessary to improve water quality. (3) The minimum distance of microbial transport and formulated soil characteristics could be used to design buffer strips to improve water quality of agricultural ditches.
References


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Appendix A

Correlation Analyses for 45 and 60 cm Microcosms

Figure A-1: Best-fit line analysis of *E. coli* (top) and *E. hirae* (bottom) for microcosms 45 cm in depth. R-squared values indicate the models ability to replicate observed C/C₀ values.
Figure A-2: Best-fit line analysis of *E. coli* (top) and *E. hirae* (bottom) for microcosms 60 cm in depth. R-squared values indicate the models ability to replicate observed $C/C_0$ values.