

## ABSTRACT

Title of Thesis: EFFECT OF INITIAL SOIL MOISTURE  
CONDITIONS ON RUNOFF TRANSPORT OF  
MANURE-BORNE PATHOGENS THROUGH  
VEGETATED FILTER STRIPS

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Pollution of drinking and recreational water supplies with manure-borne pathogenic bacteria through surface runoff from agricultural lands is a public health threat wherever landscapes are exposed to animal manure, but, particularly, where there is concentrated animal production (e.g., Iowa). This study was conducted to investigate the effect of initial soil moisture conditions on the effectiveness of vegetated filters strips (VFS) to mitigate surface runoff transport of two surrogate pathogenic bacteria, *Escherichia coli* and *Salmonella enterica enterica* Typhimurium, from land-applied swine slurry.

A 5% slope lysimeter containing clay loam soil was constructed, partitioned into vegetated and bare plots, and the plots instrumented to collect, measure, and sample runoff at different time intervals and at two distances from the slurry application area during rainfall simulations. Results indicated that the potential of VFS to attenuate runoff transport of pathogens was reduced under increased initial soil moisture conditions, indicating that infiltration is an important factor in the mitigation process.

EFFECT OF INITIAL SOIL MOISTURE CONDITIONS ON RUNOFF  
TRANSPORT OF MANURE-BORNE PATHOGENS THROUGH VEGETATED  
FILTER STRIPS

By

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## Dedication

To my late father, Ercy da Silva Cardoso, with all my love.

You are always on my mind and in my heart. I will always remember you.

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## Chapter 1: Introduction

Pollution of drinking and recreational water supplies with pathogenic bacteria, including manure-borne bacteria, is a public health threat since exposure to these microorganisms can cause serious illnesses to or even the death of humans. One of the possible routes by which manure-borne pathogenic bacteria may enter natural waters is through surface runoff from agricultural fields, where manure is applied as an organic fertilizer (Roodsari et al., 2005).

Several waterborne *E. coli* 0157:H7 (0157) and *Salmonella* outbreaks have been documented in recent years, indicating the need to attenuate microbial pollution to waters. During the winter of 1991, 243 cases of 0157 infection were documented in Cabool, Missouri. The outbreak resulted in 32 hospitalizations and four deaths, and the source of infection was associated with contaminated drinking water (Wang and Doyle, 1998). During the summer of that same year, 21 children were infected with 0157 in Oregon after swimming in a fecally contaminated recreational lake (Wang and Doyle, 1998). Between 1993 and 1998, 454 cases of enteric illness in the United States were attributed to 0157 infections and all such cases were associated with contaminated waters (Johnson et al., 2003).

Also, from 1993 to 1998, water contaminated with *Salmonella* spp., including *Salmonella typhimurium*, resulted in 625 cases of enteric illness, resulting in seven deaths (Johnson et al., 2003). In 1996, for instance, an outbreak of enteric illness was reported in Livingston County, New York, in which approximately 30 individuals became ill with diarrhea and one had to be hospitalized. An investigation of the



outbreak concluded that those individuals had been infected with *Plesiomonas shigelloides* and *Salmonella* Hartford, both of which can be found in poultry manure, and that the possible pathway of contamination was food prepared with contaminated water from an unprotected shallow dug well that may have received surface runoff from surrounding manured farmland following rainfall event(s) (CDCP, 1998; Guan and Holley, 2003).

Agriculture is one of the major industries in the State of Iowa where approximately 89% of the total land area is occupied by farms (IDALS, 2006). Iowa's agriculture industry includes the production of pork, corn and soybean, which made it the leading state nationwide in 2005. According to the latest available National Water Quality Assessment Database, agriculture is also one of the leading possible sources of pollution to rivers, streams, creeks, lakes, ponds and reservoirs in the State of Iowa (USEPA, 2002).

Some of the top agriculture-associated pollutants/stressors identified in these water bodies are nutrients, sedimentation and pathogens, such as viruses, protozoans and bacteria (USEPA, 2002). The presence of *Escherichia coli* and/or fecal coliform (FC) in water bodies is an indication of water contamination with fecal material (Roodsari, 2004). Some of the most common sources of fecal material in water bodies, especially surface waters, are inadequately treated sewage, wildlife and runoff from lands supporting agricultural practices (USEPA, 2000).

Manure has essential nutrients that can enhance soil quality and crop productivity (Roodsari, 2004; Roodsari et al., 2005), but it is also a source of several pathogenic bacteria, such as the *E. coli* 0157:H7 strain and all *Salmonella* spp., that

can deteriorate water quality after being transported from agricultural fields to water supplies (Patni et al., 1985; Collins et al., 2005). *Escherichia coli* 0157:H7 and *Salmonella* spp. can cause enteric diseases such as diarrhea, hemorrhagic colitis and hemolytic uremic syndrome (HUS) (Wang and Doyle, 1998), and are leading causes of gastroenteritis in both the United States and Canada (Johnson et al., 2003).

While bacterial pollution may reach water bodies by leaching to ground water via preferential flow, studies have suggested that surface runoff from agricultural fields is in fact the key process by which manure-borne pathogenic bacteria may enter water bodies, particularly surface waters (Patni et al., 1985; USEPA, 2000; Collins et al., 2005). The risk for surface water contamination is even higher under high rates of land-applied manure such as the ones observed at large, confined animal feeding operations (CAFOs), in which substantial volumes of animal waste are applied to relatively small agricultural areas (Roodsari, 2004). According to Roodsari et al. (2005), CAFOs is a major source of microbial pollution to water bodies.

The process by which fecal bacteria enter runoff flow and are transported within it to surface waters is poorly understood (Khaleel et al., 1980; Muirhead et al., 2005; Roodsari et al., 2005). According to Tyrrel and Quinton (2003), bacteria from a soil-manure mixture may enter runoff flow in at least three ways and may be transported in runoff under two states: either attached to soil or slurry particles, or as free (unattached) cells. Conflicting results have been reported as to the extent of partitioning between these two states (Roodsari, 2004; Muirhead et al., 2005), but some studies suggest that attachment, particularly to soil particles, may be very low

especially if the suspension in which the soil particles are found contains manure (Guber et al., 2005a and 2005b).

Bacteria transported in runoff as free cells are unlikely to settle due to their density, which is similar to that of water (Tyrrel and Quinton, 2003; Roodsari, 2004), but may be intercepted/retained by adsorbing to plant surface, litter or organic matter (Roodsari, 2004). The rate and extent of bacteria transport in runoff, as well as their concentration in it, may be determined by factors such as rainfall intensity/duration, manure application rates, soil characteristics and watershed hydrology (Roodsari, 2004).

Several studies have been conducted to study the effectiveness of vegetated filter strips (VFS) at mitigating the contamination of water bodies by pathogenic microorganisms coming from land-applied manure. This practice consists of directing runoff flow from agricultural fields to VFS where microorganisms (or other pollutants, such as nutrients and sediments) can be removed from incoming runoff by means of infiltration, adsorption to soil and plant surfaces, and/or settlement. The practice has been cited by several authors as one of the best management practices (BMPs) because favorable results may be attained at low costs (Young et al., 1980; Dillaha et al., 1989; Chaubey et al., 1994; Edwards et al., 1996).

Most studies of the effectiveness of VFS have used livestock manure, as opposed to swine manure as the source of microorganisms. In addition, VFS studies using swine manure have reported inconsistent results regarding the degree of efficiency at mitigating microbial runoff transport. For instance, Roodsari (2004) reported that *E. coli* and *S. cholerasuis* from areas treated with liquid swine slurry

were completely removed from runoff after entering a 20%-sloped VFS with sandy loam soil texture. However, Entry et al. (2000) reported that riparian filterstrips consisting of three different types of vegetation (grass, forest and maidencane) did not reduce total or fecal coliform numbers in runoff from areas treated with swine wastewater. According to Roodsari et al. (2005), the inconsistent results may be attributed to the fact that the studies did not take into account infiltration rates within the relevant VFS. Roodsari et al. (2005) studied the effectiveness of VFS at mitigating runoff transport of FC from areas treated with bovine manure and concluded that infiltration was a major mitigating factor.

The objective of this study was to investigate the effects of initial soil moisture conditions within VFS on runoff transport of two surrogate pathogens, *E. coli* and *S. enterica enterica* Typhimurium, from land-applied liquid swine manure (4% solids). This study focused on VFS with loam and clay loam soil texture profiles constructed on 5% slopes and subjected to extreme conditions of rainfall events (80 mm h<sup>-1</sup>). Such soil type and slope specifications were meant to reproduce the characteristics of the soil in the State of Iowa, where agriculture is one of the leading sources of microbial pollution to surface waters.

In addition, this study investigated the potential correlation between runoff transport of each of these two surrogate pathogens and Bromide (Br) through VFS with the purpose of determining whether future similar studies could use relative concentrations of Bromide measured in runoff to infer relative concentrations of pathogens in runoff, thus saving most of the costs associated with pathogens-based studies.

## Chapter 2: Literature Review

### 2.1 Bacteria

#### 2.1.1 Definition, size, shapes, density, categories

Bacteria are the only prokaryote organisms that are ubiquitous inhabitants of moist environments and predominantly unicellular microorganisms (Brock and Madigan, 1988; Holt et al., 1994). They differ from higher forms of life such as animals and plants, and from other microorganisms such as algae, fungi and protozoa, all of which are multicellular eukaryotes.

Bacteria can be single cells or simple associations of similar cells measuring from 0.2 to 10.0  $\mu\text{m}$ , and are grouped based on their cellular instead of organismal properties (Holt et al., 1994). Their cells have several distinct shapes, such as coccus (spherical or egg-shaped), oval, straight or curved rods (cylindrical shape), spiral, spiral helix or filaments (Brock and Madigan, 1988; Holt et al., 1994). Their density is usually in the range of 1.0 to 1.1  $\text{g cm}^{-3}$ , which is very close to the density of water (Tyrrel and Quinton, 2003; Roodsari, 2004).

Most bacteria are beneficial, and in some cases even essential, to the overall health of a person. These bacteria are referred to as an individual's "normal" flora. In some regions of the human body, such as in the gastrointestinal tract, the "normal" flora can appear very early after birth and be well established by the first week of life (Brock and Madigan, 1988). Some bacteria, however, can be harmful to humans and cause several illnesses. Disease-causing bacteria are referred to as pathogenic.

According to Brock and Madigan (1988), bacteria are the most important microbial pathogens.

On a phenotypic basis, bacteria can be divided into four major categories: Gram-negative eubacteria that have cell walls, Gram-positive eubacteria that have cell walls, eubacteria lacking cell walls, and archaeobacteria (Holt et al., 1994). The two bacteria used in this study, *Escherichia coli* and *Salmonella enterica enterica* Typhimurium, belong to the first category.

In addition, each of the four major categories of bacteria can be further divided into family groups. The two bacteria used in this study belong to the *Enterobacteriaceae* family.

#### 2.1.2 *Enterobacteriaceae* family

Bacteria belonging to the *Enterobacteriaceae* family are present worldwide. They can be found in soil, water, fruits, vegetables, grains, flowering plants and trees, and also in animals ranging from worms to humans (Holt et al., 1994). In humans and animals, *Enterobacteriaceae* are present in the intestines, where they are a major component of the normal intestinal flora (Farmer III, 1999). Some strains, however, are associated with several diseases including abscesses, pneumonia, meningitis and septicemia, as well as infections of wounds, the urinary tract and the intestines (Farmer III, 1999). As mentioned earlier, *Escherichia* and *Salmonella* are two of several genera that constitute the *Enterobacteriaceae* family, and are two of four genera in the *Enterobacteriaceae* family that have been clearly documented as enteric pathogens (Farmer III, 1999).

### 2.1.3 Characteristics of genus *Escherichia* and its clinical significance

Species belonging to the genus *Escherichia* are facultatively anaerobic Gram negative straight rods, which can measure  $1.1\text{--}1.5\ \mu\text{m} \times 2.0\text{--}6.0\ \mu\text{m}$  and exist singly or in pairs (Holt et al., 1994; Farmer III, 1999). These species are either motile by peritrichous flagella or nonmotile. Their optimal temperature for growth is  $37^\circ\text{C}$  but they can grow well on MacConkey agar plates at  $44.5^\circ\text{C}$  (Holt et al., 1994; Bopp et al., 1999).

Although extremely variable biochemically, *Escherichia* species generally catabolize D-Glucose and other carbohydrates, producing an acid and gas in the process (Holt et al., 1994; Bopp et al., 1999). In addition, they are oxidase and Voges-Proskauer negative, catalase, indole and methyl red positive, and usually citrate negative. They are negative for  $\text{H}_2\text{S}$ , urea hydrolysis and lipase, and all species reduce nitrates (Holt et al., 1994). Still, in terms of biochemical reactions, all or most strains of *Escherichia* species ferment a variety of sugars, including L-arabinose, maltose, D-mannitol, D-mannose and L-rhamnose (Holt et al., 1994).

Five species of bacteria make up the genus *Escherichia*: *Escherichia blattae*, *Escherichia fergusonii*, *Escherichia hermannii*, *Escherichia vulneris* and *Escherichia coli* (Bopp et al., 1999). All of these species are commonly found in the intestines of warm-blooded animals, where they occur as part of the normal flora in the lower portion of these organs. In particular, *Escherichia coli* is ubiquitous in human and animal feces so much so that its presence in water is an indication of fecal contamination (Brock and Madigan, 1988; Bopp et al., 1999).

Although it occurs as part of the intestinal flora of healthy individuals, certain strains of *E. coli* may cause several infectious illnesses. Along with other species from the *Enterobacteriaceae* family, *E. coli* is the cause of most extraintestinal infections (Holt et al., 1994; Bopp et al., 1999). The most common of these infections is that of the urinary tract (primarily cystitis), followed by those of the respiratory, bloodstream and central nervous systems and wound infections. According to Holt et al. (1994), *E. coli* is the major cause of urinary tract and nosocomial infections, including septicemia (infection of the bloodstream) and meningitis (infection of the central nervous system), which are serious, rapidly progressing and life-threatening infections.

As mentioned above, *Escherichia* is one of four genera of *Enterobacteriaceae* that has been clearly documented as enteric pathogens. This is due to the fact that some strains of *E. coli* are well associated with mild to serious intestinal infections in humans (Bopp et al., 1999). These strains contain enterotoxins associated with diarrheal diseases. They are collectively called diarrheagenic *E. coli* and are separated into at least four categories: Enterohemorrhagic *E. coli* (EHEC), which produce Shiga toxins, enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), and enteroinvasive *E. coli* (EIEC).

At least two strains of EHEC, *E. coli* serotypes 0157:H7 and 0157:nonmotile (NM) (0157 EHEC), have been identified (Bopp et al., 1999). These strains produce one or more Shiga toxins, also called [verocytotoxins], and have the capability to intimately adhere to the intestinal epithelium, generating what is called attaching-and-effacing lesions (A/E lesions) (Bloom et al., 1998; Mainil, 1999). Once adhered to



intestinal epithelium cells, these strains deliver Shiga toxins that are transported into endothelial cells by a transcellular pathway, causing inhibition of protein synthesis and other adverse reactions that in turn result in the inflammation of these cells and their damage (Bloom et al., 1998).

Infections with EHEC strains can result in mild nonbloody diarrhea, severe bloody diarrhea or even a more serious illness called haemolytic-uraemic syndrome (HUS) (O'Brien and Kaper, 1998; Bopp et al., 1999). This syndrome is characterized by microangiopathic hemolytic anemia, thrombocytopenia and acute renal failure. Some other symptoms, particularly from *E. coli* 0157:H7 infections, include abdominal cramps and lack of fever (Bopp et al., 1999).

ETEC strains are also associated with the production of toxins and diarrhea in infected individuals. According to Bopp et al. (1999), these strains produce either heat-labile *E. coli* enterotoxin (LT) or heat-stable *E. coli* enterotoxin (ST), or both, and cause diarrhea (particularly in young children from developing countries), abdominal cramps that could be accompanied by nausea and headache, and little vomiting and fever (Bopp et al., 1999). ETEC strains are frequently the cause of traveler's diarrhea, which is considered mild in intensity but its duration is prolonged.

EPEC strains are epidemiologically associated with infantile diarrhea, but do not produce enterotoxins or Shiga toxins (Bopp et al., 1999). The symptoms associated with infection by these strains include severe chronic nonbloody diarrhea, vomiting and fever that may result in malabsorption, malnutrition, weight loss and growth retardation (Bopp et al., 1999). EIEC strains, on the other hand, are associated

with invasion of the colon cells, producing a generally watery but occasionally bloody diarrhea.

#### 2.1.4 Characteristics of genus *Salmonella* and its clinical significance

Species belonging to the genus *Salmonella* are facultatively anaerobic Gram negative straight rods, measuring  $0.7\text{--}1.5\ \mu\text{m} \times 2.0\text{--}5.0\ \mu\text{m}$  (Holt et al., 1994; Farmer III, 1999). Most of these species are motile by peritrichous flagella, but some are nonmotile, and their optimal temperature for growth is  $37^{\circ}\text{C}$  (Holt et al., 1994; Bopp et al., 1999). According to Farmer III (1999), *Salmonella* species grow on selective or differential media such as Brilliant Green, SS or Rambach agars.

Biochemically, *Salmonella* species catabolize D-Glucose and other carbohydrates, producing an acid and usually gas in the process (Holt et al., 1994; Farmer III, 1999). These species are oxidase, indole and Voges-Proskauer negative, catalase, methyl red and Simmons citrate positive, and reduce nitrates (Holt et al., 1994). In addition, they are lysine and ornithine decarboxylase positive and ferment several sugars, including L-arabinose, maltose, D-mannitol, D-mannose, D-sorbitol and D-xylose (Holt et al., 1994; Bopp et al., 1999).

Species belonging to the genus *Salmonella* are ubiquitous in animal populations and are commonly found in the intestines of humans and other warm blooded animals, but are sometimes found in the intestines of cold blooded animals as well (Holt et al., 1994; Bopp et al., 1999). Exposure to fecally contaminated animal meat or water can be routes for *Salmonella* infections, but such infections can also result from direct contact with animals or exposure to nonanimal foods and

occasionally result from human contact. These species probably do not occur as free-living organisms, but some strains may be able to survive long periods of time (even years) in the environment (Bopp et al., 1999).

Two species, *Salmonella enterica* and *Salmonella bongori*, have been recognized to make up the genus *Salmonella* (Murray et al., 1999). *Salmonella enterica* is separated into six groups of subspecies (subspecies I, II, IIIa, IIIb, IV, and VI), while *S. bongori* is made up of only one subspecies (V) (Murray et al., 1999). The *Salmonella* species used in this study belongs to subspecies I and is scientifically referred to as *Salmonella enterica* subsp. *enterica* Typhimurium, or simply *Salmonella enterica* Typhimurium. Strains from subspecies I are usually isolated from humans and warm-blooded animals, whereas strains belonging to all of the other subspecies are usually isolated from cold-blooded animals (Bopp et al., 1999).

*Salmonella* species are associated with several infectious illnesses including gastroenteritis, enteric (typhoid) fever and septicemia, which are collectively called salmonellosis (Bopp et al., 1999; Bell and Kyriakides, 2002). Infection takes place after the organism grows and multiplies in the small intestines, colonizing the tissues of this organ and producing an enterotoxin that causes an inflammatory reaction and diarrhea (Bell and Kyriakides, 2002). In some cases, *Salmonella* species can overcome the natural defense system of an individual and get into the bloodstream and/or the lymphatic system, thus causing even more severe illnesses.

Nontyphoidal *Salmonella* strains can usually cause intestinal infection presenting symptoms that include diarrhea, vomiting, fever and abdominal cramps (Bopp et al., 1999; Bell and Kyriakides, 2002). One example of such infection is

gastroenteritis, which is usually caused by an infective dose of approximately  $10^4$  cells of *Salmonella* but can also be caused by a smaller number of cells ( $< 100$ ) if organisms are protected, e.g., in high fat foods (Bell and Kyriakides, 2002).

Gastroenteritis has an incubation time of approximately 12-72 h and can last between 2 and 7 days (Bopp et al., 1999; Bell and Kyriakides, 2002). Members of subspecies I have been associated with gastroenteritis, but members of subspecies III have also been associated with this illness.

According to Bopp et al. (1999), and Bell and Kyriakides (2002), typhoid fever and septicemia are serious infections of the bloodstream. Humans have been the only reservoir of typhoid fever, which typically causes a sustained debilitating high fever, headache, malaise, nausea, abdominal pain, anorexia and delirium, as well as constipation during the early stages and diarrhea in the late stages of the illness.

Typically, typhoid fever has a low infectious dose ( $< 10^3$ ), a long incubation period (7-28 days), and can be transmitted through person-to-person contact or exposure to fecally contaminated food or water. *S. Typhi* and *S. Paratyphi*, which belong to subspecies I, are the serotypes associated with typhoid fever. Septicemia, on the other hand, can be caused by several members of subspecies I and is characterized by high fever, malaise, pain in the thorax and abdomen, chills and anorexia (Bell and Kyriakides, 2002).

## 2.2 Factors affecting the growth of *Escherichia* and *Salmonella* spp., and their survival in swine feces, soil, and water

### 2.2.1 Growth

According to Ingraham and Marr (1996), *Escherichia coli* and *Salmonella enterica* Typhimurium are mesophiles with respect to temperature for their growth and neutrophiles with respect to pH. Therefore, these enteric organisms grow over the mid range of temperatures and pH values. The growth rate response of most wild-type strains of *E. coli* and *S. e.* Typhimurium is similar, and the normal temperature range for their growth extends from 21 to 37°C. At higher or lower temperatures, growth rate decreases progressively, and balanced growth may not be sustained at approximately 49°C or higher, or below 7.5°C. In terms of pH values, these organisms grow at maximum rate between pH 6.0 and pH 8.0 (Ingraham and Marr, 1996; Bell and Kyriakides, 2002).

### 2.2.2 Survival in swine feces

Regarding the fate of *Escherichia* and *Salmonella* spp. in animal feces, a variety of physical and chemical characteristics of the manure or manure slurry have been shown to influence their survival, such as temperature, solid content, pH, bacterial concentration, moisture content, and aeration (Kudva et al., 1998). Although the survival of *E. coli* in swine fecal material has not been well documented, several studies suggest that this pathogen can survive well in animal feces (Wang et al., 1996; Kudva et al., 1998; Fukushima et al., 1999).

According to Wang et al. (1996), survival rates of *E.coli* 0157:H7 in bovine feces depend mostly on temperature and moisture content levels in the feces. In their study, this pathogen survived between 63 and 70 days at a low temperature of 5°C and where moisture content levels remained high during the study (74%). They observed that at low temperatures moisture content is retained, which increases the chances for the pathogen survival.

On the other hand, the study by Fukushima et al. (1999) demonstrated that *E. coli* 0157:H7 survived up to 126 days in bovine feces at a temperature of 15°C inoculated with the highest concentration ( $10^5$  CFU /g of feces). They observed a short-term survival of *E. coli* 0157:H7 in the feces at a higher temperature (25°C) even when samples were kept in closed bags, which promoted the retention of moisture. They concluded that survival rates in feces depended on temperature and the initial bacterium inoculum, regardless of dehydration or moisture content levels. Nonetheless, both studies suggested that survival of *E. coli* 0157:H7 in bovine feces is inversely proportional to temperature.

Studies have indicated that *Salmonella* spp. can survive for extended periods of time in swine feces. The study by Gray and Fedorka-Cray (2001) demonstrated that *S. choleraesuis*, shed from infected animals, survived for 3 and 13 months, respectively, in wet and desiccated (dry) form of swine feces while kept at room temperature (26°C).

On the other hand, in the study by Placha et al. (2001), they observed that survival response of *S. typhimurium* in pig slurry was dependent on seasonal variations of atmospheric temperature. They found that survival of this bacterium

during the storage of solid fraction of pig slurry was longer during the winter/spring (85 days) than in the summer season (26 days). Besides other physicochemical factors, they attributed the shorter time survival of *S. typhimurium* to high temperatures in the summer, which increased the levels of dry matter content in the slurry. In another study, conducted by Ajariyakhajorn et al. (1997), it was observed that *S. anatum* can survive as long as 56 days in swine slurry stored at 4°C with pH 7.0.

### 2.2.3 Survival in soil

According to some studies, the survival or inactivation of enteric bacteria in soils depend on several soil physical and chemical properties, such as texture and particle size distribution, moisture content, moisture holding capacity, pH, sunlight, organic matter content, temperature, and microbial interactions (Gerba and Bitton, 1984; Crane and Moore, 1986; Mubiru et al., 2000; Jiang et al., 2002; Tyrrel and Quinton, 2003; Oliver et al., 2005). According to Gerba and Bitton (1984), and Crane and Moore (1986), moisture is the major factor determining the survival of enteric bacteria in soils; whereas Tyrrel and Quinton (2003) suggest that temperature is the most significant environmental factor, and that usually survival times increases with decreasing temperatures

Gerba and Bitton (1984) indicated that enteric bacteria have a greater survival time in moist soils and during times of high rainfall, as well as in soils with high water-holding capacity. Mubiru et al. (2000) pointed out that fine texture soils can support microbial populations three times larger than coarse textured soils, since fine texture soils have higher water-holding capacity and nutrient concentrations.

Survival of enteric bacteria in soils that have been spread with manure may also be affected by atmospheric conditions, such as sunlight and temperature. Survival time may be longer in shaded areas than in areas receiving direct sunlight since ultraviolet light can be lethal to these microorganisms (Gerba and Bitton, 1984; Tyrrel and Quinton, 2003). Lower temperatures may favor the survival of these microorganisms in soils since the processes of soil self-disinfection are slowed-down or suspended in areas of prolonged winters (Gerba and Bitton, 1984; Crane and Moore, 1986). Previous studies have indicated that survival of *E. coli* in exposed soil plots was longer in the autumn than in the summer (Van Donsel et al., 1967).

Organic matter also plays a significant role on bacteria survival in soils. When organic matter content is high survival increases and even regrowth of *E. coli* and *S. e. Typhimurium* may be observed (Gerba and Bitton, 1984; Mubiru et al., 2000). Organic soils, as opposed to mineral soils, are high in organics and in moisture-holding capacity. In terms of soil pH, survival time of enteric bacteria may be shorter in acid soils (pH 3-4) than in alkaline soils (pH 5.8-7.8) (Gerba and Bitton, 1984; Oliver et al., 2005). Low pH may not only adversely affect the availability of the organism but also the availability of nutrients.

Another factor affecting the survival of enteric bacteria in soils is the antagonism from soil microflora (Gerba and Bitton, 1984). In the study by Jiang et al. (2000), in which they investigated the survival of *E. coli* 0157:H7 in manure-amended soils, they observed that the die off of this pathogen was faster in soils containing both plentiful manure nutrients and the highest population of indigenous manure bacteria; and in soils that had not been autoclaved. They suggested that pH



and moisture content levels were favorable for the growth of competitive microorganisms in manure and that antimicrobial activity of microorganisms that were indigenous to both manure and soil contributed to inactivation of *E. coli* 0157:H7.

In addition, Turpin et al. (1993) also found that survival of *S. typhimurium* was greater at 22°C in a sterile than in a non-sterile soil, suggesting that under sterile conditions the salmonella cells had no competition and thus were able to maintain their number, whereas under non-sterile conditions antagonism and/or competition by native microflora for nutrients reduced their survival.

#### 2.2.4 Survival in water

Once *E. coli* and *S. e. Typhimurium* are introduced into natural water bodies by surface runoff, lateral flow, and/or vertical flow from and through agricultural contaminated soils, their survival is dependent on their ability to overcome the physical, chemical, and biological stresses associated with these unfavorable environments (Jones, 1999; and Oliver et al., 2005).

Very often viewed as oligotrophic environments, natural water bodies can have low concentrations of dissolved available nutrients, which decrease the chances of survival of these introduced microorganisms since they have to compete with natural microflora for available nutrients. However, nutrient availability associated with suspended particles (both soil and waste derived) may increase the chances of survival of those microorganism's cells attached to particles since nutrient levels can be 10 to 100 times higher on suspended particle surfaces than in the surrounding aquatic environment (Oliver et al., 2005).

Temperature, UV radiation, and predation are also variables that can affect the survival of *E. coli* and *S. e. Typhimurium* in aquatic systems. It has been reported that decreasing water temperatures often increases the survival of these bacteria in aquatic systems (Jones, 1999; and Oliver et al., 2005). As reported by Jones (1999), *E. coli* 0157 has been shown to survive in river water for up to 90 days at 4°C. On the other hand, Oliver et al. (2005) reported that this bacterium has been shown to survive for even longer periods of time (260 days) at temperatures ranging between 4 and 25°C. In terms of UV radiation, Oliver et al. (2005) pointed out that exposure to solar radiation containing UV-B light is perhaps the most important factor responsible for the decline of bacteria in surface waters since this light can promote DNA damage. Predation by native protozoan populations on enteric bacteria entering aquatic environments is another variable influencing their survival (Oliver et al., 2005).

As pointed out by Wang and Doyle (1998), bacteria, especially the gram-negative ones, can adapt to environmental and nutritional stresses by transforming their physiological state to a viable but nonculturable (VBNC) state as an adopted survival strategy. Several adverse environmental conditions in natural waters, such as those related to temperature, nutrient concentration, salinity, osmotic pressure, and pH, may induce bacteria to the VBNC state, which can be interpreted as one of dormancy. Bacterial cells in this state may maintain viability and metabolic activity, but their pathogenic potential is yet to be determined (Wang and Doyle, 1998).

### 2.3 Processes and factors affecting the transport of bacteria in surface runoff

According to Khaleel et al. (1980), bacterial pollution in runoff waters from agricultural land treated with animal waste is associated with the fact that bacteria are retained at or near the soil surface after manure is applied and may eventually be released from these places and transported in runoff. Nonetheless, the processes by which fecal bacteria enter runoff flow and are transported within it to surface waters are poorly understood (Khaleel et al., 1980; Muirhead et al., 2005; Roodsari et al., 2005).

According to Tyrrel and Quinton (2003), the attempt to describe the processes of pathogen transport in overland flow should first be based on the initial and boundary conditions of the slurry-amended soils. Their study suggests, along with others, that there are three possible states in which bacteria are likely to exist in a soil-slurry mixture: attached to soil particles, attached to waste or slurry particles, and as free cells or clumps (Reddy et al., 1981; Tyrrel and Quinton, 2003). Although understanding of the factors that control the partitioning of bacteria among these three states is limited (Khaleel et al., 1980; Tyrrel and Quinton, 2003), once runoff has initiated there is a great potential that the bacteria present in the soil-slurry mixture will be transported to surface waters.

Tyrrel and Quinton (2003) suggested that there are at least three mechanisms by which bacteria from a soil-slurry mixture can enter overland flow. First, bacteria that exist as free cells or clumps in the mixture, which are likely to be located within soil pore water or water films, are incorporated into the runoff flow as it passes over them. Second, bacteria that are attached to soil or slurry particles are entrained in the

flow after the particles themselves are propelled into it either by the force applied by the flow itself or by the force of rain drops on the particles. Finally, bacteria cells can become detached from soil or slurry particles surfaces by the action of shearing force of raindrops or the flow itself and be transported as free cells in the flow. Therefore, their study suggests that bacteria can be transported in runoff flow in two states: attached to soil or slurry particles, and as free (unattached) cells.

Although partitioning between these two states is not thoroughly understood, some studies have been conducted to determine their proportion. Muirhead et al. (2005) investigated the transport state of *E. coli* cells released from bovine fecal material (fresh and aged cowpats) using either the material alone or mixed with soils. They concluded that the percentage of cells attached to particles in general were very low, with an overall mean of only 8%. They also concluded that the majority of *E. coli* cells found in the large unattached fraction in runoff were individual cells and not clumps. Roodsari (2004), however, reported that bacteria adsorption to soil particles depends on soil texture and that attachment to clay loam soil can be around 25% of the total number of bacteria.

Other studies reinforce the suggestion that attachment of bacteria, in particular to soil particles, is not significant and can be very low, especially if the soil suspension contains manure. According to Guber et al. (2005a and 2005b), the presence of bovine manure in soil suspensions can dramatically reduce the attachment of *E. coli* cells to soil particles, and that, as manure concentration increases in the suspension, bacteria attachment to soil particles decreases. One possible explanation

by the authors for such decrease is that adsorption of bacteria takes place on manure particulates instead of on soil particles.

It is important to note that most studies (if not all) regarding attachment of bacteria to particles used bovine fecal material as the source of bacteria. However, there is a great likelihood that the attached and unattached fraction of bacteria cells in runoff from swine material might be similar to that from bovine manure if bacteria behave similarly in swine fecal material as they do in bovine fecal material.

In addition, studies suggest that deposition/settlement of the unattached fraction of bacteria in runoff is unlikely to occur (Tyrrel and Quinton, 2003; Roodsari, 2004) because the density of bacteria, which range from 1.0 to 1.1 g cm<sup>-3</sup> (Roodsari, 2004), is very close to the density of water, notwithstanding that their size is similar to the size of silt or coarse clay particles (Roodsari et al., 2005). Therefore, once bacteria enter runoff flow, it is unlikely that they will settle by gravity and most likely that they will remain in suspension even if flow conditions are steady and laminar. According to Roodsari et al. (2004), bacteria found in runoff as free cells may be retained during runoff by adsorbing to plant surface, litter or organic matter.

Some factors affecting the rate and extent of bacterial transport as well as their concentration in surface runoff are rain intensity/duration, manure application methods and rates, soil characteristics (slope, texture, and types of vegetation), and the hydrologic characteristics of a watershed (Roodsari, 2004).

#### 2.4 Effectiveness of vegetated filter strips (VFS) at removing pathogens from surface runoff

Several studies have been conducted to investigate the effectiveness of vegetated filter strips (VFS) at improving the quality of runoff from pollutant source areas. Vegetated filter strips are vegetated regions that are designed to receive runoff flow from pollutant source areas with the goal at removing pollutants (e.g., nutrients, sediments and/or microorganisms) from the incoming runoff through infiltration, adsorption to soil and plant surfaces, and/or settlement. This practice has been cited by several authors as one of the best management practices (BMPs) since effectiveness may be attained at low costs (Young et al., 1980; Dillaha et al., 1989; Chaubey et al., 1994; Edwards et al., 1996).

Previous studies have demonstrated that VFS can be very effective at reducing overland flow of nutrients and sediments from agricultural areas. Young et al. (1980) used a 27.4-m VFS to improve the quality of beef feedlot runoff by removing approximately 80% of the incoming masses of solids and nutrients (TN, TP, ammonia nitrogen and orthophosphorus). Schwer and Clausen (1989) found that a 26-m VFS removed 95% and approximately 90%, respectively, of incoming solids and nutrients (TP, and total Kjeldahl nitrogen) from dairy milkhouse wastewater.

Several studies have also been conducted to investigate the effectiveness of VFS at mitigating microbial transport from manure sources. The majority of these studies used bovine or poultry manure as the source of microorganisms (Chaubey et al. 1994) but the degree of effectiveness reported by these studies, particularly the ones with bovine manure, varies considerably. For instance, Lim et al. (1998)

reported that all fecal coliforms (FCs) in runoff from pasture areas receiving cattle manure were removed after entering a 6.1 m length VFS. On the other hand, Schellinger and Clausen (1992) reported that *E. coli* concentrations in runoff from a barnyard were not significantly reduced (only 30%) after flowing through a VFS.

Most studies of the effectiveness of VFS have used livestock manure other than swine manure as the source of microorganisms. In addition, VFS studies using swine manure have reported inconsistent results regarding the degree of efficiency at mitigating microbial runoff transport. For instance, Roodsari (2004) reported that *E. coli* and *S. cholerasuis* from areas treated with liquid swine slurry were completely removed from runoff after entering a 20%-sloped VFS with sandy loam soil texture. However, Entry et al. (2000) reported that riparian filterstrips consisting of three different types of vegetation (grass, forest and maidencane) did not reduce total or fecal coliform numbers in runoff from areas treated with swine wastewater.

Of the numerous studies that have been conducted to evaluate the performance of VFS at improving the quality of runoff from agricultural sources, several of them revolved around the transport of sediment and nutrients. However, such studies may be relevant to understanding the factors that contribute to the effectiveness of VFS at mitigating overland flow transport of microorganisms from manure sources.

Regardless of the type of pollutant studied, there is an indication that the degree of effectiveness of VFS depends on the combination of several factors like environmental conditions (e.g., rain intensity and manure application methods or rates) and VFS characteristics (e.g., slope, size and soil texture), but even studies that

evaluated VFS under similar conditions and/or characteristics reported conflicting results.

According to Edwards and Daniel (1993), the effectiveness of VFS may be negatively impacted by manure application rate and the time between application and the first runoff-producing rainfall event, but not by rainfall intensity. In their study, concentrations of swine slurry constituents (nutrients, chemical oxygen demand (COD) and total suspended solids (TSS)) in runoff from fescue plots generally increased in tandem with the increase in the slurry application rate; and that, by contrast, concentrations decreased with increased rainfall intensity. The authors suggested that the decrease in concentrations under higher rainfall intensity was due to dilution caused by higher runoff volumes.

Some studies suggest that VFS constructed on steep slopes may have their performance compromised if they are subjected to high rainfall intensity. Collins et al. (2005), for instance, used a combination of a steep pastoral land (with approximately 33% slope) and heavy rainfall events ( $35 \text{ mm h}^{-1}$ ) and concluded that, under such conditions, VFS were not particularly effective at mitigating overland flow transport of *E. coli* from a grazed hillside to a headwater pastoral stream. This conclusion differs from that of Roodsari (2004), who subjected 20%-sloped VFS to rainfall intensities at  $61 \text{ mm h}^{-1}$  and concluded that VFS were substantially effective at mitigating overland flow transport of FC from land-applied bovine manure, and of *E. coli* and *S. cholerasuis* from land-applied swine manure.

Other studies suggest that the optimal length of VFS depends on whether they are designed to reduce sediment, nutrients and/or microorganism losses from



agricultural fields. For instance, Chaubey et al. (1994) used 3-, 6-, 9-, 15- and 21-m VFS with 3% slope and found that the 3 m VFS was effective at removing TSS in runoff from an area treated with liquid swine slurry, and that the 9 m VFS was effective at removing most of the nutrients, but that none of these VFS was significantly effective at removing nitrate nitrogen and FC from the incoming runoff.

Roodsari (2004) obtained different results from the ones described above after evaluating VFS that were shorter than 21 m. He used two 6-m VFS with 20% slope and found that both VFS significantly reduced mass transport of *E. coli* and *S. choleraesuis* in runoff from areas treated with liquid swine manure. Bingham et al. (1980) noted that VFS should have the same length as the length of the pollutant source area in order to be effective at treating runoff coming from areas receiving poultry manure.

Some studies suggest that VFS with soil textures that enhance infiltration rates may be highly effective at reducing microorganisms in runoff from areas treated with both bovine and swine manures, even if they are constructed on steep slopes and subjected to high rainfall intensity (Roodsari, 2004; Roodsari et al. 2005). For instance, Roodsari et al. (2005) simulated rainfall at  $61 \text{ mm h}^{-1}$  on 6.4-m  $\times$  6.0-m, 20%-sloped vegetated and bare plots with different soil textures and concluded that not only VFS were significantly more effective than bare surfaces at reducing runoff volumes and the amounts of FC in runoff from land-applied bovine manure, but also that the degree of effectiveness between the two VFS was even higher for the one with the more permeable soil texture.

The authors pointed out that infiltration may be the most important mechanism by which surface runoff of FC from bovine manure may be mitigated, and that conflicting results from previous validation studies of VFS with microorganisms may have resulted from the fact that those studies did not take into account infiltration rates within VFS. Roodsari et al. (2005) also suggested that VFS design should be such that it increases infiltration rates even under extreme environmental conditions, at least for the purpose of controlling surface runoff of microorganisms from manure.

### 2.5 Pork Production in the State of Iowa

According to available information, pork production in the United States is largely concentrated in the State of Iowa (IPPA, 2006). In 2005, Iowa had approximately 8,900 pig farms, which may have raised as much as 16 million pigs. In addition, Iowa accounts for about 25% of the hog production in the country by raising approximately 25 million hogs each year. The ratio between hogs and persons in the State of Iowa is approximately 5 to 1. It is also estimated that the swine industry in the state generated more than 2 million tons of swine manure<sup>1</sup> in 2005 (USDA, 2006).

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<sup>1</sup> Tons of swine manure in dry state (as excreted adjusted for water content).

## Chapter 3: Materials and Methods

This study was conducted to investigate the overland flow transport of two surrogate pathogens, *Escherichia coli* and *Salmonella enterica* Typhimurium, from vegetated and non-vegetated soil strips. In order to conduct the study, a lysimeter containing clay loam soil with a 5% slope was constructed, partitioned and instrumented as the experimental site. The soil type and slope specifications were meant to reproduce the characteristics of the soil in the state of Iowa, where the results of the study were going to be applied. Two sets of experiments were required since the objective was to conduct the investigation under two distinct initial levels of soil moisture: dry conditions and wet conditions. The first set of experiments, on dry initial soil moisture conditions, was performed between June 1 and June 29, 2004, while the second, on rather wet initial soil moisture conditions, was conducted between August 11 and September 21, 2004.

### 3.1 Lysimeter Set Up

#### 3.1.1 Geographic location

The lysimeter was located at the Patuxent Wildlife Research Refuge (U.S. Department of Interior) in Beltsville, MD, which, at the time the study was conducted, was administered by the U.S. Fish and Wildlife Services.

### 3.1.2 Construction

The lysimeter used in the study was set up during the summer of 2002 on a pre existing lysimeter measuring 12.5 m x 18.7 m, complete with a gutter system running along the existing wall located at the bottom of the slope, lined with a heavy-gauge plastic and connected to a working pumping station. In order to conduct the study, however, the surface soil was removed to 1 ft below the gutter and replaced with loam soil, which was packed, and graded in order to create a 5% slope needed for the study. Confinement of the lysimeter was required in order to both isolate and contain the area and prevent potential contamination of local groundwater and other water bodies.

### 3.1.3 Plot partitioning and waste application designation area

The portion along the lysimeter length at the bottom of the slope was further partitioned into four adjacent and equal-sized 3.9 m x 6.5 m sub-plots, hereinafter referred to as plots. Overall schematic of the lysimeter is illustrated in Figure 3.1.

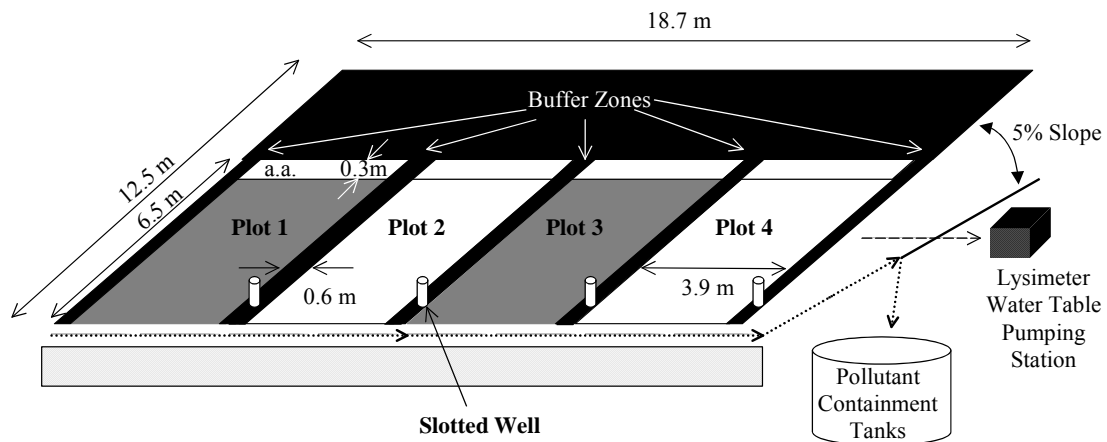


Figure 3.1. Overall schematic of the lysimeter. Plots 1 and 3 were vegetated, while Plots 2 and 4 were bare. Dotted line indicates the direction of flow in the gutter. An application area (a.a.) was located at the top of each plot.

Both sides and the side opposite to the lysimeter's gutter of each plot were encircled by a series of very thin 10 cm x 85 cm metal sheets semi-inserted into the soil, creating a 5 cm wall around each plot. It was important to have each plot confined within these walls in order to prevent runoff loss during the experiments. Plots were set apart approximately 60 cm from each other, creating buffer zones to facilitate plot access without disturbing the adjacent plots.

The first and third plots from the left side of the lysimeter were sowed with fescue grass seeds (hereinafter referred to as vegetated plots) and designated as Plot 1 and Plot 3, respectively. The second and fourth plots from the left side of the lysimeter were kept devoid of vegetation (hereinafter referred to as bare plots) and designated as Plot 2 and Plot 4, respectively. However, a 30 cm strip across the top of all plots, bare and vegetated, was kept bare and designated as the waste application area for the swine slurry containing the pathogens used in the study.

#### 3.1.4 Soil characteristics

The soil used in the repacking of the existing lysimeter was obtained from a construction site at the U.S. Department of Agriculture in Beltsville, MD. The goal was to pack the lysimeter with clay loam soil since this type of soil is the predominant type found in Iowa, where the results of the study will be applied. The soil texture was confirmed by mechanical analysis using the hydrometer method.

In the process of soil texture determination, soil samples from the surface (first 5 cm) and from a depth of 20 cm were collected from each plot using a 2.54 cm ID core sampler. Samples from the soil surface were collected at four different locations (two at the top and two at the bottom) in each plot and combined in order to

obtain the 50 g necessary to conduct the texture analysis. Samples from the soil at a depth of 20 cm were collected directly below from the location where the soil surface samples came from, using the same probe, and were, also, combined in order to obtain the amount of sample necessary to conduct the texture analysis. Therefore, a total of two samples per plot were obtained, one representing the surface and the other the 20 cm down the soil profile.

The texture analysis consisted of first drying (at 104°C overnight) and then sieving (2 mm) each soil sample. Once that step was concluded, 50 g of each sample was weighed out and placed into a mixer cup, making sure that any residual dust from the weighing dish was carefully rinsed into the cup with deionized (DI) water. Subsequently, the cup was 2/3 filled with DI water before 50 mL of a 10% sodium hexametaphosphate solution were added to the soil solution in the mixer cup. Next, the contents were mixed for 3 minutes using an electrical mixer.

Once mixing was completed, the soil suspension was quantitatively poured into a 1000 mL cylinder, making sure that all soil was transferred to the cylinder by rinsing the residuals with DI water. Once all soil suspension was transferred, the mixture was brought to the 1000 mL mark by adding DI water. Next, the contents in the cylinder were vigorously stirred with a plunger for 15 to 20 seconds, and the initial time was recorded as the plunger was removed. Immediately after, the hydrometer (calibrated for 20°C) was carefully placed in the suspension and after 40 seconds since the plunger had been removed, the hydrometer reading was recorded. Between the time that the hydrometer was carefully placed in the suspension and the time that the reading was recorded 40 sec later, 1 drop of 200% ethanol was added to

the cylinder in case that the surface of the suspension was covered with foam in order to obtain an accurate hydrometer reading.

After taking the reading, the hydrometer was carefully removed and rinsed with DI water before going on to the next sample. At 2 hours after the plunger had been removed from each cylinder, the hydrometer was carefully replaced into the suspension and the reading was recorded. During the 2 hours period, the contents in each cylinder were left undisturbed.

After the last hydrometer reading was taken, a control was prepared in order to calibrate the hydrometer readings for the presence of the sodium hexametaphosphate in the soil solutions. In the process, 50 mL of the 10% sodium hexametaphosphate solution was transferred to a cylinder, and the volume brought to the 1000 mL mark by adding DI water. After the contents were vigorously stirred with the plunger for 15 to 20 seconds, the hydrometer was carefully placed into the solution and the reading was recorded. Subsequently, the temperature of the solution was recorded, which was assumed to be the same as the one in the samples since both DI water and the 10% sodium hexametaphosphate solution used for calibration and analysis of the samples came from the same source.

Calculations were performed as follow:

1. Each reading was corrected for temperature deviation from 20°C by adding 0.36 g of soil L<sup>-1</sup> for each degree above 20°C, or subtracting 0.36 g of soil L<sup>-1</sup> for each degree below 20°C.
2. % sand + % silt + % clay = 100%  
% silt + % clay = (corrected 40 sec. reading/dry sample weight)\*100

$$\% \text{ clay} = (\text{corrected 2 hr reading/dry sample weight}) * 100$$

$$\% \text{ silt} = (\% \text{ silt} + \% \text{ clay}) - \% \text{ clay}$$

$$\% \text{ sand} = 100 - (\% \text{ silt} + \% \text{ clay})$$

The texture class of soil samples was determined based on their percent composition of clay, silt, and sand using the soil texture triangle. Table 3.1 shows the results for the soil texture analysis.

Table 3.1. Soil texture classification at the surface and at a depth of 20 cm in each plot.

<b>LOCATION</b>	<b>% CLAY</b>	<b>% SILT</b>	<b>% SAND</b>	<b>SOIL TEXTURE</b>
Plot 1 <sup>†</sup> Surface	17.0	39.0	44.0	<b>loam</b>
Plot 1 20 cm depth	23.0	33.0	44.0	<b>loam</b>
Plot 2 <sup>‡</sup> Surface	24.0	42.0	34.0	<b>loam</b>
Plot 2 20 cm depth	30.0	34.0	36.0	<b>clay loam</b>
Plot 3 <sup>†</sup> Surface	18.5	37.1	44.4	<b>loam</b>
Plot 3 20 cm depth	29.0	35.0	36.0	<b>clay loam</b>
Plot 4 <sup>‡</sup> Surface	22.0	38.0	40.0	<b>loam</b>
Plot 4 20 cm depth	34.0	40.0	26.0	<b>clay loam</b>

<sup>†</sup> Vegetated plots

<sup>‡</sup> Bare plots



Table 3.1 shows that the soil texture on the surface of all four plots was classified as loam. On average, the percent of clay, silt, and sand in the soil surfaces was 20%, 39%, and 41%, respectively. The soil texture below the surface, at a depth of approximately 20 cm, did not have the same classification across all plots. The soil texture in Plot 1 (vegetated) at such depth was classified as loam, with 23% clay, 33% silt, and 44% sand. In Plots 2, 3, and 4 (bare, vegetated, and bare, respectively) the soil texture at such depth was classified as clay loam, with an average of 31% clay, 36% silt, and 33% sand.

#### 3.1.5 Topography of vegetated plots

A topographic map of each vegetated plot (Plots 1 and 3) was constructed in order to provide a better understanding of the micro-relief pattern on these plots. This was not necessary with the bare plots (Plots 2 and 4) since, in the process of maintaining these plots before each set of experiments took place, the surface was constantly graded, which contributed to free the surface of these plots from deep channels and possible different elevations across the plots.

The topographic maps were generated by marking a 50 cm x 50 cm grid in each plot and measuring the elevation at each point in the grid with the help of a transit level. The transit level was first mounted to a tripod, which was then positioned between and away from the top (approximately 3 m) of the two vegetated plots. The transit level itself was then leveled with the help of its built-in spirit level, and elevation was measured and recorded after a cm-calibrated rod was as vertically

as possible placed at each point of the grid. The lowest point measured was used as the base line elevation.

Elevation data were used to generate topographic maps that could show three different terrain characteristics in each of the vegetated plots. The topographic maps, in Figure 3.2, show the average slope in each plot, and were generated using 2D contour maps from SigmaPlot 9.0 software. The topographic maps, in Figure 3.3, show the roughness in each plot, and were generated using 3D mesh plots also from SigmaPlot 9.0. Last, the topographic maps, in Figure 3.4, show the surface area in each plot that contributed to either convergent or divergent flow conditions, and were generated based on the tangential curvature using Surfer<sup>®</sup> 7 software. Tangential curvature is defined as the measurement of “curvature in relation to a vertical plane perpendicular to the gradient direction, or tangential to the contour” (Surfer<sup>®</sup> 7 User’s Guide, 1999). Negative curvature values indicate areas of divergent flow conditions, whereas positive values indicate areas of convergent flow conditions.

#### 3.1.6 Slotted wells

Four slotted wells were installed in the lower portion of the buffer zones next to the gutter, starting in the buffer zone between the first and second plots, to monitor groundwater table levels and the potential subsurface movement of *E. coli* and *S. e.* Typhimurium into the groundwater table. Each well consisted of a slotted PVC pipe measuring approximately 10 cm in diameter and 85 cm long inserted vertically approximately 50 cm into the soil until it touched the vinyl lining at the bottom of the lysimeter. The approximate 30 cm side not inserted into the soil was kept covered all times to avoid incoming direct rainwater, which might compromise the proper

monitoring of the groundwater table. The slotted wells were numbered Well 1, Well 2, Well 3 and Well 4 starting from the left side of the lysimeter in a manner similar to the manner that the plots were numbered.

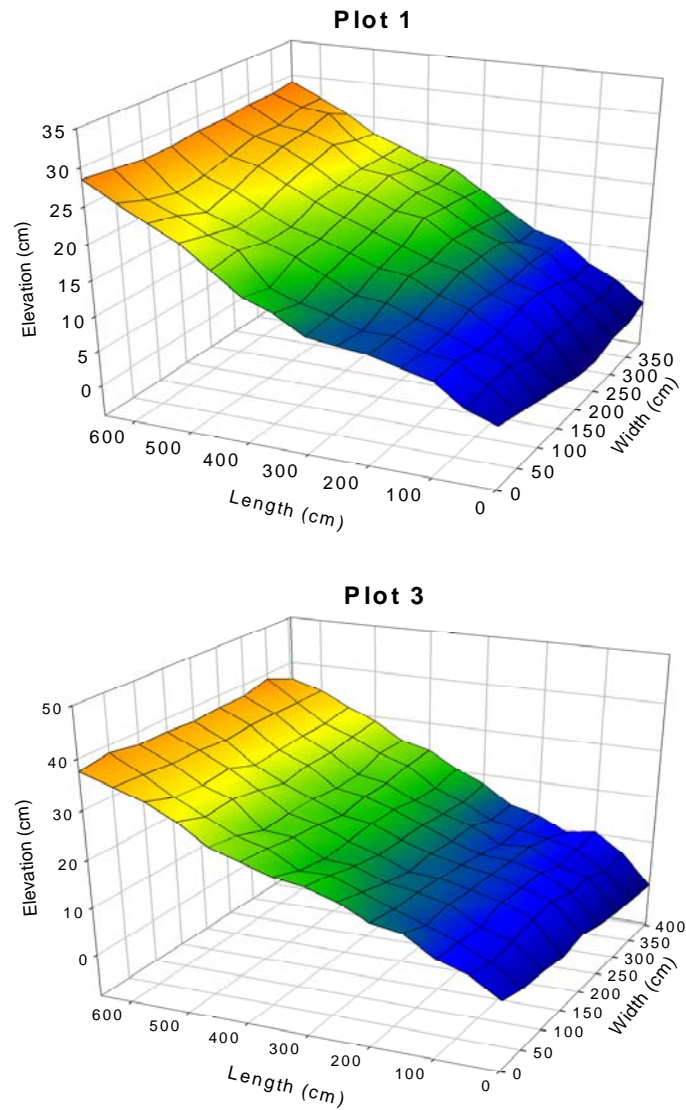


Figure 3.2. Topographic maps showing the different slopes between the two vegetated plots. Average slopes of Plots 1 and 3 were 4.0% and 5.4%, respectively.

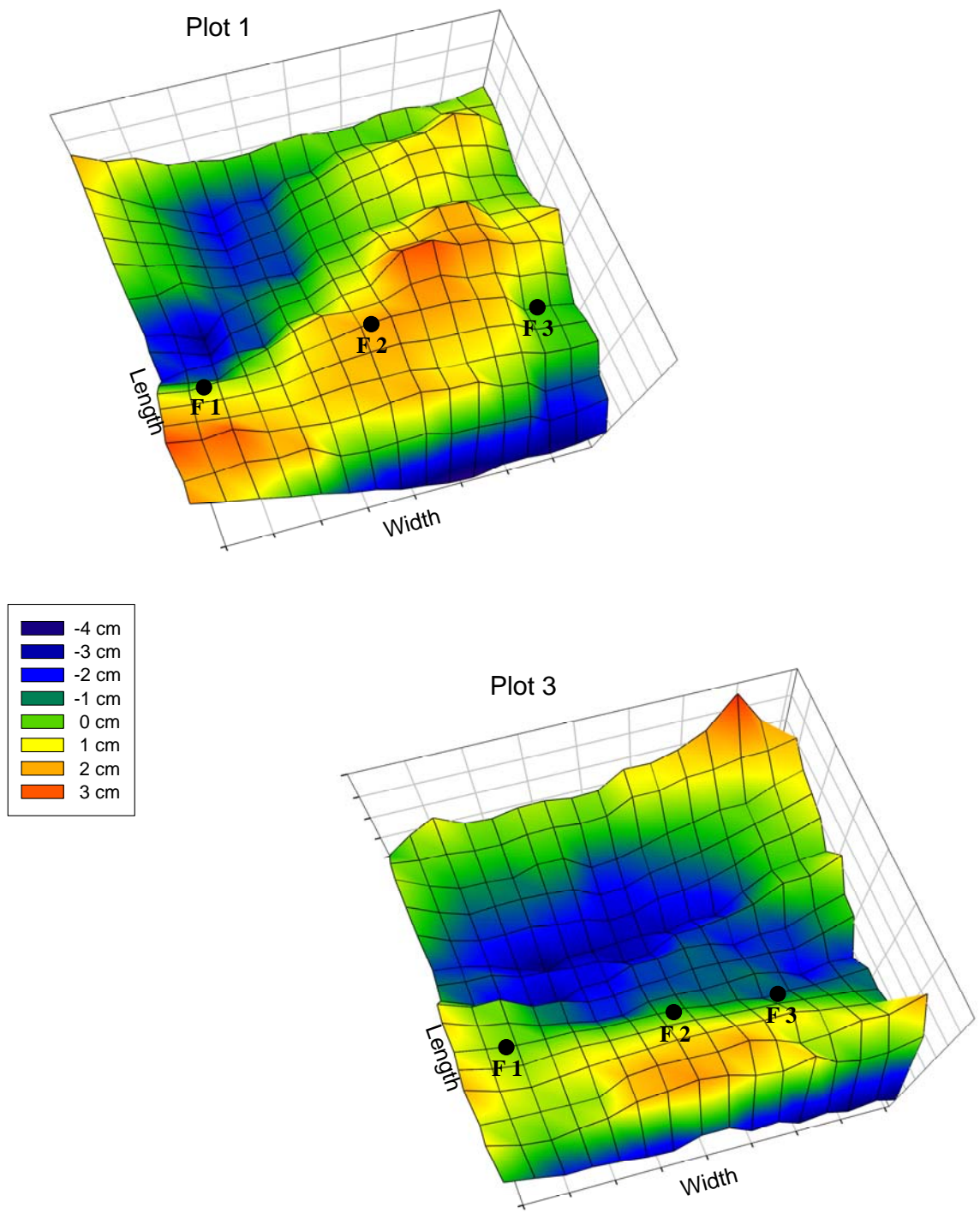


Figure 3.3. Topographic maps showing the surface roughness of Plots 1 and 3 (vegetated plots). Symbol (●) shows the approximate position of each funnel. Legend values appear as residuals, which reflect the elevation deviations from a specific plane, which was calculated using SigmaPlot 9.0.

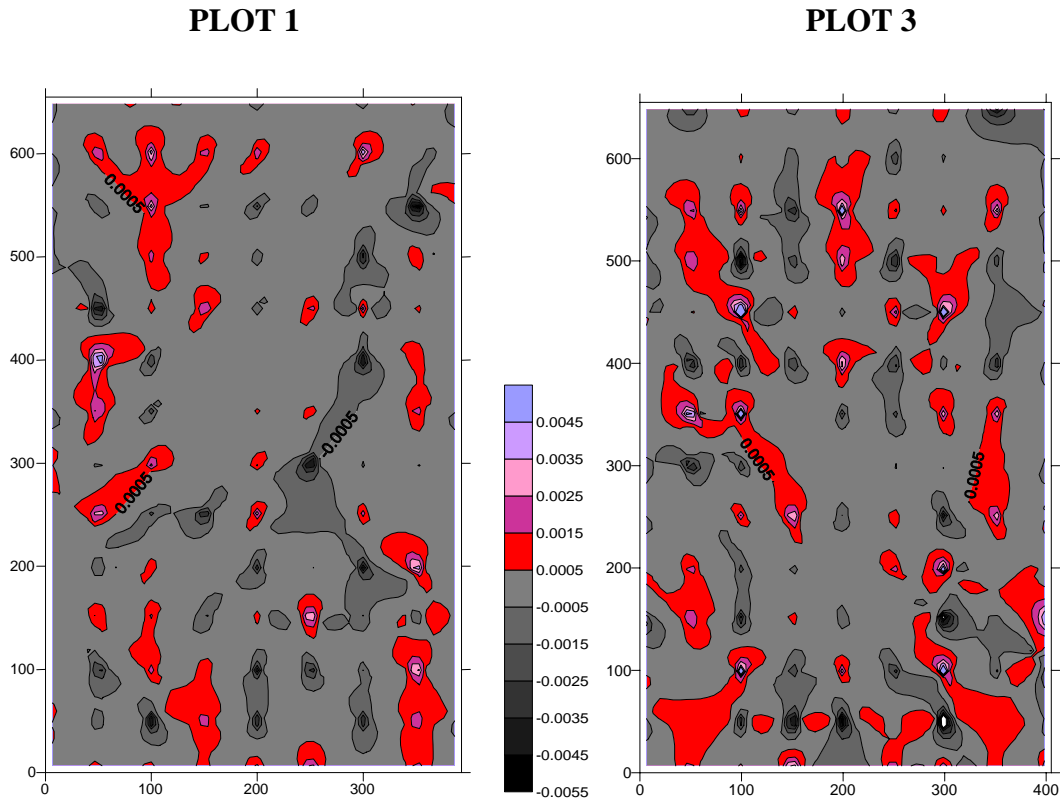


Figure 3.4. Topographic map of vegetated plots (Plots 1 and 3) based on their tangential curvature. Positive values indicate the areas of convergent flow conditions (channels), whereas negative values indicate areas of divergent flow conditions.

### 3.1.7 Surface runoff collectors

Part of surface runoff was collected by three funnels aligned along the width of each plot in a transect located approximately 4.13 m from the bottom edge of the waste application area (at about 2/3 of the plot length down the slope). The funnels were installed to measure temporal and spatial distribution of *E. coli* and *S. e.* Typhimurium in runoff. Additional details on the installation of these funnels are described in section 3.2.4 below.

The remainder of surface runoff was collected by the gutter running along the bottom of the slope located approximately 6.2 m from the bottom edge of the waste application area. As indicated earlier, this gutter system was already installed at the existing lysimeter that was modified for the purposes of this study.

#### 3.1.8 Rainfall source

The rainfall simulator used for this study was built at and provided by the U.S. Department of Agriculture, ARS, in Beltsville, MD. The simulator was a large-scale portable rainfall simulator powered by a gasoline-fired generator, and it contained a boom, four full jet 1/2HH SS30WSQ nozzles (two at the top and two at the bottom of the boom) and four water pressure gauges, all of which were fully adjustable. The boom could be adjusted upwards or downwards to position nozzles at different distances from the soil surface, and each nozzle could be both moved across the boom as well as adjusted at different angles. Each nozzle was connected to a pressure gauge that controlled the intensity of simulated rain. The rain simulator was connected to three 2000 L water tanks, which were supplied with water coming from a well located approximately 60 m from the lysimeter.

#### 3.1.9 Pollutant containment tanks

The lysimeter was connected through a PVC pipe measuring 15 cm in diameter to a runoff collection system comprised of three 2000 L pollutant containment tanks. These tanks were designed to store and treat surface runoff generated during the experiments before it could be released into the environment.

All surface runoff was treated and released together after the final experiment was conducted.

### 3.2 Pre-experiment Field Procedures

#### 3.2.1 Calibration of rainfall simulator

The rainfall simulator was calibrated during the first week prior to the first experiment. Trials were performed early in the morning during non-raining days while the temperature was low and the wind speed was low enough not to affect the uniformity of the synthetic rain. After five 15-minute trials, the rainfall simulator was calibrated to simulate rain at an intensity of approximately  $80 \text{ mm h}^{-1}$  and a uniformity coefficient of 90%, which is above the 80% coefficient recommended by researchers (Roodsari, 2004). Such rain intensity is characteristic of rainfall events having a 75-yr return period in Iowa, where the results of the study were going to be applied. The rainfall uniformity coefficient was determined by applying the Christiansen's uniformity equation,  $C_u = 100(1 - \sum((\text{ABS}(x_i - \text{Mean}))/(\text{Mean} \times n)))$ , where  $C_u$  is the uniformity coefficient,  $x_i$  is the volume collected by each rain gage, and  $n$  is the number of rain gages.

The calibration process involved first adjusting the boom in a manner such that the nozzles were suspended approximately 3.0 m from the ground to ensure that most of the drops attained terminal velocity by the time they hit the ground, thus simulating natural rainfall events (Hirschi et al., 1990). Next, the vegetated plot being used during calibration (Plot 1) was covered with a tarp and a total of 16 rain gages (four rows of four) were symmetrically positioned onto the plot to collect simulated rain. The decision to cover the plot was made in order to avoid excessively

compacting the soil from having to walk over an otherwise wet soil several times during intervals between trials to measure the volume of synthetic rain collected in the rain gages. Once this preparation step was concluded, the rain simulator was turned on and immediately after the water pressure in each gauge was set to 20 PSI. The volume of water collected in each rain gage was then read after 15 minutes of rain simulation.

As the trials took place, the nozzles were rotated in two occasions in order to find an angle that could improve the uniformity of the rain. In one occasion, because there was more rain falling at the bottom than at the top of the plot, the two bottom nozzles were slightly rotated towards the gutter and away from the area receiving too much water. In addition, one of the top nozzles was partially obstructed and had to be unclogged. However, such changes did not solve the uniformity problem at hand since more rain then started falling at the top than at the bottom of the plot.

After a series of trials, bringing the bottom nozzles to their initial position perpendicular to the ground and adjusting the top nozzles at a slight angle towards the top of the plot, while increasing their pressure to 22 PSI, the best rainfall uniformity coefficient was obtained. Unfortunately, leaving the plot covered with the tarp throughout the time of calibration had a negative impact on the surface grass in Plot 1, which had to be the last plot to be tested during the first set of experiments (relatively dry soil conditions) in order for the vegetation to recover.

### 3.2.2 Dry initial soil moisture conditions

In order to achieve dry initial soil moisture conditions required for the first set of experiments, the groundwater table inside the lysimeter was drained by a pump



placed in the pumping station adjacent to the right side of the lysimeter and connected to a gasoline-fired generator.

The draining process was conducted whenever necessary prior to the beginning of any such experiments to lower the groundwater table to levels comparable to those in Iowa, where the results of the study were going to be applied. In addition, the plot to be tested in any given day was covered with a tarp whenever there was an occurrence of natural rainfall the day before. Furthermore, the groundwater table in the lysimeter was drained during the actual experiments whenever the water table levels started to increase. Groundwater levels were monitored before and during the experiments by observing the levels of water as it accumulated in the wells and at the pumping station.

### 3.2.3 Wet initial soil moisture conditions

In order to achieve wet initial soil moisture conditions required for the second set of experiments, the groundwater table inside the lysimeter was never drained and the plots were never covered in order to benefit from the occurrence of natural rainfall events during the two months that separated the first set from the second set of experiments. In addition, in order to rise the groundwater table as much as possible, water was manually applied to the lysimeter with the help of a hose in the following manner: if the plot to be tested was a vegetated plot, water was applied onto the plot with a hose at full flow during the day and into the well adjacent to the plot at low flow during the night before the experiment was to be conducted; if the plot to be tested was a bare plot, water was applied into the well adjacent to the plot at low flow during both the day and the night before the experiment was to be conducted.

#### 3.2.4 Funnels installation

The three funnels, used to partially collect the surface runoff and to measure temporal and spatial distribution of *E. coli* and *S. e. Typhimurium* in runoff, were installed the day before any given experiment at approximately 4.13 m away from the bottom edge of the waste application area. They were strategically positioned onto existing low points along the transect in the plot where they were installed so that the fastest runoff flow could be collected during the experiments. Each funnel was connected to a food-grade Tygon tube long enough to reach over the gutter and out of the plot into buckets where runoff would be collected at different time intervals. Funnels and tubes were fastened onto the ground with the help of oversized u-shaped metal staples.

#### 3.2.5 V-notch weir installation

All surface runoff not collected by the funnels was collected by the gutter located at 6.2 m away from the bottom edge of the waste application area, and its runoff rate (GPD) was measured using a V-notch weir. The V-notch weir not only measured total surface runoff, but, also, helped to create flow hydrographs for each rainfall simulation. It was installed the day before the beginning of each of the two sets of experiments in the gutter near the pumping station. Measurements were taken by reading the graduated scale on the V-notch weir wall, a method demonstrated to be reasonably accurate (Roodsari, 2004).

### 3.2.6 Application area barrier

The slurry used in this study, which was prepared from swine manure, consisted of approximately 4% solids. Details on the manner the slurry was prepared can be read in section 3.3.2. Because of its consistency, the slurry could easily flow down the slope too soon after each application. In order to prevent it from occurring, metal sheets of the type used to encircle the plots were inserted along the bottom of the application area the day before each experiment, to be then removed shortly into the simulation when rain became more uniform.

### 3.2.7 Isolation of plots

Since the area covered by the simulated rain was larger than the area of the plot where any given experiment was conducted, adjacent areas alongside the plot were covered with tarps and the portion of the gutter was covered with metal sheets. This helped to avoid accounting for water falling outside of the plot being tested or flowing directly into the gutter.

## 3.3 Pre-experiment Laboratory Procedures

### 3.3.1 Source of manure

Swine manure used to prepare the slurry used in this study was collected from a swine waste lagoon located in a farm in Germantown, MD. The farm was the property of Dr. Hartsock, a faculty member at the University of Maryland, College Park. Because of the advanced age of the slurry (approximately 2 yr.), it did not have the detectable levels of bacteria necessary for the study (approximately  $10^6$  colonies

of each pathogen mL<sup>-1</sup>), so the slurry had to be inoculated with both *E. coli* and *S. e.* Typhimurium cultures in a process that is described in section 3.3.6.

### 3.3.2 Slurry preparation

The swine waste lagoon had two distinct layers: the top layer was mostly liquid while the bottom layer had most of the solids that settled over time. As neither layer had the desired consistency of 4% solids, samples from each layer had to be collected and mixed in order to achieve such a consistency. Approximately 65 L of swine waste were collected from each layer and analyzed at the laboratory facilities for solid content, which was determined gravimetrically by weighing three replicated samples of each layer of waste before and after drying at 105°C for 24 h. The analysis was repeated three times and the results indicated that the bottom layer had approximately 7% solids and the top layer had approximately 0.3% solids. Each experiment conducted for the purposes of this study required 13 L of slurry (10.94 L m<sup>-2</sup>). In order to obtain the 4% desired consistency and the 13 L required quantity of slurry, the day before each experiment 8 L of the sample containing 7% solids were mixed with 5 L of the sample containing 0.3% solids.

### 3.3.3 *Escherichia coli* culture

*Escherichia coli* culture used in this study was obtained by isolating this bacterium from fresh bovine manure, which was collected at the Dairy Research Unit of the USDA/ARS facility in Beltsville, MD. Two methods to initiate the isolation process were applied and consisted of diluting the bovine manure by a series of increasing folds and streaking it directly onto MacConkey agar plates.

The first method consisted of diluting the manure by a 10-fold in preparation for subsequent 100-fold and 1000-fold dilutions necessary to reduce the initial concentration of *E. coli* colonies found in the fresh manure in order to achieve ideal isolation levels. In order to obtain a 10-fold dilution, 10 grams of manure was added to 90 mL of sterile distilled water and the contents dispersed in a high-speed blender for 2 min. Immediately after the blending process was completed, 1 mL was transferred to 9 mL of sterile distilled water in order to obtain a 100-fold dilution. The 100-fold dilution was then vortexed and 1 mL was immediately transferred to 9 mL of sterile distilled water in order to obtain a 1000-fold dilution.

Subsequently, two 50  $\mu$ L replicates from each of the 100-fold and 1000-fold dilutions were dispensed on MacConkey agar plates with the help of a Spiral BioTech autoplater. Plates were incubated at 44°C for 18 to 20 h to allow colonies to grow before further tests could be performed in order to select one *E. coli* colony.

The second method consisted of streaking fresh manure directly onto MacConkey agar plates, which was performed under a hood to avoid contamination. In the process, two 10  $\mu$ L replicates from fresh manure were first streaked onto the plates and then incubated at 44°C for 18 to 20 h to allow colonies to grow before further tests could be performed in order to select one *E. coli* colony.

After colonies obtained from both methods had grown on the plates overnight, six of them were selected for confirmation based on two factors: colonies that morphologically mostly resembled those of *E. coli* colonies, and colonies that had grown well isolated from other colonies. Presumptive *E. coli* colonies were then

confirmed using the BBL Enterotube II technique, which is used in the rapid identification of *Enterobacteriaceae*.

The application of the technique consisted of first inoculating a microscopic portion from each selected single colony into a different self-contained, compartmented plastic tube with the help of its enclosed inoculating wire. The compartments in each tube consisted of 12 different conventional media that reflected the performance of 15 standard biochemical tests from a single colony. Once each of the six tubes was inoculated with a microscopic portion of a selected colony, it was incubated at 37°C for 18 to 24 h before the code resulting from the combination of reactions of the 15 standard biochemical tests in the relevant tube was read. All six tubes were inoculated and incubated simultaneously. The code obtained was then compared to the coding table provided by the manufacturer in order to identify the species of *Enterobacteriaceae* that had been inoculated.

One of the six colonies that tested positive for *E. coli* was further processed and periodically maintained to remain the pure culture used in the experiments comprising this study. Since the BBL Enterotube II technique applied in the identification of *Enterobacteriaceae* used only a microscopic portion of the selected colony, the remainder of the colony was transferred to 10 mL of Minimal Lactose Broth (MLB) and further incubated at 37°C for 18 to 20 h. Once incubation was completed, three 10 µL replicates of the enriched broth were streaked onto three separate MacConkey agar plates, further incubated at 44°C for 18 to 20 h, and then stored at 4°C. Maintenance of the pure culture of *E. coli* was done weekly by

transferring a colony from the stored plates onto 10 mL of MLB and then performing the incubation and storage steps described above.

MLB was prepared by first adding the following amounts of micro and macro nutrients into 1 L of distilled water: 4.35 g of  $K_2HPO_4$ , 3.4 g of  $KH_2PO_4$ , 2 g of  $(NH_4)_2SO_4$ , 8.5 g of NaCl, 1.5 g of Bile Salts, 1.8 g of Lactose, 1 mL of Trace Metals, 1 mL of Trace Elements, 1 mL of  $MgSO_4$ , and 1 mL of  $FeSO_4$ . The contents were then stirred thoroughly in order to completely dissolve the contents and the pH was adjusted to 7 if necessary. The solution was then filtered through a NALGENE<sup>®</sup> filter unit containing a sterile 0.2  $\mu m$  membrane, and the contents kept in a 37°C incubator to be monitored for possible contamination.

#### 3.3.4 *Salmonella enterica* Typhimurium culture

*Salmonella enterica* subsp. *enterica* Typhimurium (*Salmonella enterica* Typhimurium) culture used in this study was purchased from the American Type Culture Collection<sup>®</sup> in Manassas, VA (ATCC<sup>®</sup> number 53648). The culture arrived as freeze-dried material, which had to be further processed in order to revive the culture.

In the process to revive the culture, Lenox broth and agar plates were used since these are very unselective type of media and suitable for the growth of innumerable types of microorganisms. First, after the ampoule was opened, 1 mL of the Lenox broth was added to and vigorously mixed with the contents in order to get the material in suspension. Subsequently, the contents were transferred to 5 mL of Lenox broth, which was then incubated at 37°C for 15 h. Once incubation was completed, two 10- $\mu L$  replicates of the enriched broth were streaked onto L agar plates, which were further incubated at 37°C for 15 h.

At that point, well-isolated colonies were obtained, which were then further processed using Mannitol Tetrathionate Broth (MTB) and Brilliant Green (BG) agar plates since these media are more selective and suitable for the growth of pure culture of *S. e. Typhimurium*. In the process, one colony, from the L agar plate, was transferred into 10 mL of MTB after 200  $\mu$ L of Iodine (2% v/v) had been added to and vortexed with the broth, and then incubated at 37°C for 18 to 20 h. Once incubation was completed, three 10- $\mu$ L replicates of the enriched broth were streaked onto three separated BG agar plates, which were further incubated at 37°C for 18 to 20 h. The plates containing well-isolated colonies were then stored at 4°C.

Preliminary studies in the experimental site during the summer of 2003 indicated that the Brilliant Green (BG) agar was not very selective for the growth of *S. e. Typhimurium* coming from environmental samples. BG agar worked well for the growth of our pure culture of *S. e. Typhimurium* in the laboratory, but allowed the growth of many other microorganisms that were present at the experimental site. Although some of these microorganisms morphologically dramatically differed from our strain of *Salmonella*, they overtook the plate, suppressing the growth of our strain and making accountability of it practically impossible. To overcome this problem, Nalidixic Acid, an antibiotic, was introduced to the agar to eliminate the growth of unwanted microorganisms.

The first step in the antibiotic introduction to the agar was to expose our *Salmonella* strain to the Nalidixic Acid in order for the organism to mutate and become resistant to the antibiotic. In the process, first a stock solution of the antibiotic was prepared by adding 40 mg of Nalidixic Acid into 2 mL of distilled water (20 mg



mL<sup>-1</sup>) as directed by Maniatis et al. (1982). After the contents had been well vortexed, they were filtered into a sterile test tube using a 25 mm Millex (0.22 µm pore size) syringe-filter unit. As indicated by Maniatis et al. (1982), the concentration of Nalidixic Acid to be used in any growth media should be of 20 µg of the acid mL<sup>-1</sup> of medium. Therefore, in order to grow our strain exposed to working concentrations of the antibiotic, one isolated *S. e. Typhimurium* colony from the BG plates was transferred to 5 mL of L broth after 5 µL of the sterilized stock solution had been added to and vortexed with the broth, which was then incubated at 37°C for 15 h. Once incubation was completed, two 10-µL replicates of the enriched broth were streaked on L agar plates, which were then further incubated at 37°C for 15 h. As mentioned earlier, L broth and agar plates are very unselective type of media that, in this case, could ease the growth of *Salmonella* strain exposed to an antibiotic.

The *S. e. Typhimurium* culture was periodically maintained in order to remain the pure culture to be used in the experiments comprising this study. Accordingly, each week a colony was transferred to 10 mL of Mannitol Tetrathionate Broth (MTB) after 200 µL of Iodine and 10 µL of Nalidixic Acid had been added to and vortexed with the broth. The broth was then incubated at 37°C for 18 to 20 h. Once incubation was completed, three 10 µL replicates of the enriched broth were streaked onto three separate Brilliant Green (BG) agar plates (containing the antibiotic, Section 3.3.5), further incubated at 37°C for 18 to 20 h, and then stored at 4°C.

MTB was prepared by first adding the following amounts of micro and macro nutrients into 1 L of distilled water: 2 g of Mannitol, 2 g of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 1 g of Bile Salts, 30 g of Sodium Thiosulfate, 5 g KHCO<sub>3</sub>, 0.1 g of Yeast Extract, 1 mL of Trace

Metals, 1 mL of Trace Elements, 1 mL of MgSO<sub>4</sub>, and 1 mL of FeSO<sub>4</sub>. The contents were then stirred thoroughly in order to completely dissolve the contents and the pH adjusted to 8 if necessary. The solution was then filtered through a NALGENE® filter unit containing a sterile 0.2 µm membrane, and the contents kept in a 37°C incubator to be monitored for possible contamination.

### 3.3.5 Plate preparation

The MacConkey agar plates used for growing *E. coli* colonies were purchased from Northeast Laboratory Services in Waterville, Maine. All plates were stored at 4°C immediately upon delivery and only those plates needed for any given experiment were removed from storage the day before the experiment.

BG agar plates used for growing *S. e. Typhimurium* colonies were prepared in the laboratory with BG agar purchased from [Difco]™. In the process, 58 g of BG agar were added into a flask containing 1 L of distilled water and the contents thoroughly stirred under high heat. After being brought to a boil for approximately 1 minute, the contents were autoclaved for 15 min at 121°C. Immediately after autoclaving was completed, the flask was placed into a water bath ranging in temperature from 45°C to 55°C in order to cool and maintain its contents within such temperature range. Temperatures outside of this range were not recommended since the contents could easily solidify at temperatures lower than 45°C, while being too hot for pouring at temperatures higher than 55°C.

After the contents had reached the desired temperature range, they were mixed with 1 mL of antibiotic (1 µL of antibiotic stock solution mL<sup>-1</sup> of agar) and thoroughly stirred, and approximately 20 mL of the resulting mix were poured onto

several sterilized petri dishes (plates). Plates were then kept at room temperature for at least 24 hours before being stored at 4°C. Only those plates needed for any given experiment were removed from storage the day before the experiment.

#### 3.3.6 Slurry inoculation

The swine slurry used in this study was inoculated with *E. coli* and *S. e.* Typhimurium cultures prior to each experiment. Samples of the slurry were collected just before inoculation and just before application onto the plot in order to verify the levels of bacteria concentration existing prior to inoculation and at the time of application of the inoculated slurry.

Approximately 48 hours prior to any given experiment, a single *E. coli* colony was transferred to 10 mL of MLB and incubated at 37°C for 18 to 20 h. Once incubation was completed, 1 mL of the enriched broth was transferred to 200 mL of MLB and further incubated at the same temperature and for the same amount of time. Incubation under these conditions usually gave approximately  $10^8$  *E. coli* colonies mL<sup>-1</sup>.

At approximately the same amount of time in advance, 10 µL of antibiotic stock solution and 200 µL of Iodine were added to 10 mL of MTB. The contents were then vortexed and a single *S. e.* Typhimurium colony was transferred to the broth and incubated at 37°C for 18 to 20 h. Once incubation was completed, 1 mL of the enriched broth was transferred to 200 mL of MTB after 200 µL of antibiotic and 4 mL of Iodine had been added to and thoroughly mixed with the broth, and further incubated at the same temperature and for the same amount of time. Incubation under these conditions usually gave approximately  $10^8$  *S. e.* Typhimurium colonies mL<sup>-1</sup>.

Approximately two hours prior to the actual experiment, 130 mL of each enriched broth were thoroughly mixed with the 13 L of slurry, bringing inoculation levels to approximately  $10^6$  colonies of each bacterium  $\text{mL}^{-1}$  required to conduct each experiment.

### 3.3.7 Bromide tracer

Bromide (Br) was added to the swine slurry used for the experiments in order to track the movement of it and, to a certain extent, to determine the transport patterns between this chemical itself and the two surrogate pathogens used in this study. Bromide has been commonly used as a conservative tracer for studying the movement of water in soils (Walton et al., 2000; Roodsari, 2004) because this chemical does not undergo fast microbial transformation or quickly bind to organic materials or soil minerals.

Bromide, in the form of potassium bromide (KBr), was added to each 13 L of slurry such that the final concentration of Br equaled to 2000 ppm. Therefore, 40 g of KBr was added to and thoroughly mixed with the slurry the evening prior to each experiment based on the following calculations:

Molecular Weight (MW), KBr = 119 g; K = 39 g; and Br = 80 g.

For each experiment, 2000 ppm Br or  $2 \text{ g Br/L} * (13 \text{ L}) = 26 \text{ g Br}$  was needed.

And,  $(3 \text{ g KBr}) * (80 \text{ g Br}/119 \text{ g KBr}) = 2 \text{ g Br}$ .

Therefore,  $(26 \text{ g Br}) * (3 \text{ g KBr}/2 \text{ g Br}) = 40 \text{ g KBr}$ .

### 3.4 Field Experimental Procedures

All experiments that comprised this study were conducted early in the morning in order to take advantage of favorable climatic conditions such as low temperature and low wind velocity.

#### 3.4.1 Soil samples and groundwater table

Just before each experiment was conducted, soil samples were collected in order to determine initial soil moisture content within the top 10 cm portion of the relevant plot. To make such a determination, three samples were taken from either side of the plot (upper, middle and lower portions) for a total of six samples.

Samples were collected using a 2.54 cm ID core sampler and placed in pre-weighted aluminum foil sheets to be analyzed after the experiment. After each soil sample was collected, the resulting hole was filled with soil collected from within the upper portion of the lysimeter where experiments were not conducted. Initial soil moisture content was determined gravimetrically by weighing the soil sample before and after drying at 105°C for 24 h. Calculations were performed on a wet basis.

Groundwater table levels in the slotted wells were recorded both before and after any given experiment for most of the experiments. All wells were dry before each experiment under dry initial soil moisture conditions, indicating that the groundwater table was at least 50 cm below the soil surface before each test was conducted. The groundwater table levels for after each experiment under dry conditions were not recorded. Table 3.2 shows the groundwater table levels before and after most of the experiments under wet initial soil moisture conditions.

Table 3.2. Approximate position of groundwater table (cm) below the soil surface recorded in the wells (W) before (BE) and after (AE) the experiments under wet initial soil moisture conditions.

PLOT	W1		W2		W3		W4	
	BE	AE	BE	AE	BE	AE	BE	AE
1 (Vegetated)	NR <sup>†</sup>	NR	19	17	19	18	13	10
2 (Bare)	NR	NR	18	5	NR	NR	NR	NR
3 (Vegetated)	NR	28	24	20	9 <sup>‡</sup>	16	NR	16
4 (Bare)	NR	NR	NR	NR	NR	NR	NR	NR

<sup>†</sup> NR, no record.

<sup>‡</sup> This position may have been underestimated since the hose was placed in this well overnight in order to raise the groundwater table level in Plot 3. Between the times when the hose was removed from this well, which was the time when the reading was taken, and the initiation of the actual experiment (about 1 h), the groundwater table probably leveled off within the lysimeter. Thus, the actual depth of the groundwater table below the surface in Plot 3 right before the experiment was probably greater than 9 cm.

#### 3.4.2 Soil and ambient temperatures

Soil and ambient temperatures were recorded just before each experiment was conducted. Soil temperature within the top 10 cm portion thereof was measured using five soil thermometers, of which two were inserted close to each corner of the upper portion, one in the very center and two close to each corner of the lower portion of the plot, for a total of five locations. Ambient temperature was measured before and after each experiment using a conventional outdoor thermometer.

#### 3.4.3 Slurry application

Thirteen liters (13 L) of liquid swine manure (slurry) was applied uniformly throughout the application area of the plot (approximately 10.94 L m<sup>-2</sup>) where the

experiment was being conducted after all preparatory steps had been concluded, including the collection of soil samples, measurement of soil and ambient temperatures and organization of all materials required for runoff collection and sampling.

Just before application of the slurry, a sample thereof was taken to verify the initial levels of *E. coli* and *S. e. Typhimurium* concentration ( $C_0$ ), which sample was kept in a cooler until further analyses were conducted. Once the slurry was applied, it was let sit for few minutes prior to turning on the rain simulator. As soon as the synthetic rain became fairly uniform, the barrier at the bottom of the application area was removed and the actual 60-minute long experiment began.

The slurry application rate in this study may have been greater than the agronomic rates usually needed for rotating corn after soybean productions in the state of Iowa. Application rates are usually based on two factors: the manure nitrogen (N) availability for meeting the nutrient needs for crop production; and whether application will take place only before crop emergence or before and in-season fertilization. Since all manure N in liquid swine slurry is available to crops the first year (ISUE, 2003), and assuming application only before crop emergence, the rate of N required to supply approximately full corn grain N removal is 168 kg Total (T) N ha<sup>-1</sup> (150 lbs T N acre<sup>-1</sup>) (ISUE, 1997; Sawyer et al., 2001); whereas to supply approximately full soybean grain N removal the rate is 224 kg T N ha<sup>-1</sup> (200 lbs T N acre<sup>-1</sup>) (Sawyer et al., 2001).

According to available information, liquid swine manure consisting of 4% solids (which was the case of the slurry used in this study) contains approximately 4

kg T N per 1000 L of slurry (36 lbs T N per 1000 gal of slurry) (Schmitt and Rehn, 2002; Joern and Brichford, 2006). Therefore, the rate of N applied for this study was approximately 430 kg T N ha<sup>-1</sup> (417 lbs T N acre<sup>-1</sup>), which was computed as:

$$((4 \text{ kg T N}/1000 \text{ L of slurry}) * (13 \text{ L}/1.2 \text{ m}^2) * (1 \text{ m}^2/0.0001 \text{ ha})) = \sim 430 \text{ kg T N ha}^{-1}$$

Therefore, the slurry application rate used in this study was 2.6 times bigger than the rate required for corn, and 1.9 times bigger than the rate required for soybean.

#### 3.4.4 Runoff sampling

Runoff collected by the three funnels and the gutter, which were located at approximately 4.13 m and 6.2 m, respectively, from the bottom edge of the waste application area, was measured and sampled at different time intervals during each experiment. Such time intervals were established in a way to establish a representative profile for discharge. The time intervals at which runoff was measured and sampled during any given simulation are shown in Appendix A.

Runoff from the three funnels at any given time interval was collected and stored in three individual buckets. Upon completion of the experiment, the volume of runoff contained in each bucket was measured and a sub-sample taken using a 250-mL plastic bottle. Sub-samples taken were stored in a cooler until further analyses were conducted.

Runoff collected by the gutter was measured using the V-notch weir installed near the pumping station and sub-sampled using 20-mL vials. Sub-samples taken



were stored in a cooler until further analyses were conducted similarly to runoff collected by the funnels.

### 3.5 Post-experiment Laboratory Analytical Procedures

#### 3.5.1 Processing of slurry samples

In order to verify pre-existing levels of *E. coli* and *S. e. Typhimurium* concentration, the slurry sample collected before each relevant inoculation and application were first diluted. The sample taken before inoculation was not diluted beyond a 10-fold dilution since concentration levels at such stage were expected to be low. However, the sample taken before application was diluted beyond a 10-fold up to a 1000-fold dilution.

The dilution process consisted of thoroughly mixing the contents of any given sample before transferring 1 mL to 9 mL of sterile distilled water in order to obtain a 10-fold dilution. For the sample taken before application, the 10-fold dilution was then vortexed and 1 mL was immediately transferred to 9 mL of sterile distilled water in order to obtain a 100-fold dilution. The resulting 100-fold dilution was then vortexed and 1 mL was immediately transferred to 9 mL of sterile distilled water in order to obtain a 1000-fold dilution.

Subsequently, three 50  $\mu$ L replicates of the 10-fold dilution obtained from the sample taken before inoculation were dispensed onto sets of both MacConkey and BG agar plates using a Spiral BioTech autoplater. In addition, two sets of three 50  $\mu$ L replicates from each of the 100-fold and 1000-fold dilutions obtained from the sample taken before application were dispensed onto sets of both MacConkey and BG agar

plates using a Spiral BioTech autoplater. Further, the MacConkey plates and the BG plates were incubated at 44°C and at 37°C, respectively, for 18 to 20 h prior to *E. coli* and *S. e. Typhimurium* colony counts were performed.

### 3.5.2 Processing of runoff samples from vegetated plots

In order to handle the runoff samples collected from vegetated plots, the samples collected by the funnels, which were contained in 250-mL plastic bottles, were thoroughly mixed and then transferred to 20-mL vials identical to those used to contain the samples collected by the gutter (excess contents were properly discarded of). All samples, which were then contained in 20-mL vials, were either dispensed wholly or diluted prior to be dispensed onto the MacConkey and BG agar plates. The decision on whether to dilute the samples prior to dispensing them onto the plates was based on naked-eye examination of elevated levels of slurry, which were found especially among the samples collected during the initial time intervals.

Samples collected from vegetated plots were diluted by either a 10-fold only or both a 10-fold and a 100-fold dilution depending on how elevated the levels of slurry contained in the sample were. In order to obtain a 10-fold dilution, 1 mL of the relevant sample was transferred to 9 mL of sterile distilled water. In order to obtain a 100-fold dilution, the resulting 10-fold dilution was vortexed and 1 mL was further transferred to 9 mL of sterile distilled water.

Subsequently, two 50 µL replicates of each sample (diluted or not) were dispensed onto sets of both MacConkey and BG agar plates using a Spiral BioTech autoplater. Further, the plates were incubated at 44°C and at 37°C, respectively, for 18 to 20 h prior to *E. coli* and *S. e. Typhimurium* colony counts were performed. All

vials were subsequently stored at 4°C until further analyses for bromide concentration were performed.

### 3.5.3 Processing of runoff samples from bare plots

In order to handle the runoff samples collected from bare plots, the samples collected by both the funnels and the gutter, which were contained in 250-mL plastic bottles and 20-mL vials, respectively, were thoroughly mixed and then transferred to 15-mL conic tubes (excess contents were properly discarded of). All samples were then centrifuged ( $100 \times g$ , 10 min) before dispensing the supernatant either wholly or diluted onto the plates. Similarly to samples collected from vegetated plots, the decision on whether to dilute the supernatant prior to dispensing them onto the plates was based on naked-eye examination of elevated levels of slurry, which were found especially among the samples collected during the initial time intervals.

Supernatant of the samples collected from bare plots were diluted by either a 10-fold only or both a 10-fold and a 100-fold dilution depending on how elevated the levels of slurry contained in the sample were. In order to obtain a 10-fold dilution, 1 mL of the supernatant resulting from centrifugation was transferred to 9 mL of sterile distilled water. In order to obtain a 100-fold dilution, the resulting 10-fold dilution was vortexed and 1 mL was further transferred to 9 mL of sterile distilled water.

Subsequently, two 50  $\mu$ L replicates of the supernatant (diluted or not) were dispensed onto sets of both MacConkey and BG agar plates using a Spiral BioTech autoplater. Further, the plates were incubated at 44°C and at 37°C, respectively, for 18 to 20 h prior to *E. coli* and *S. e.* Typhimurium colony counts were performed. All

tubes were subsequently stored at 4°C until further analyses for bromide concentration were performed.

#### 3.5.4 Colony counting

*Escherichia coli* colonies and *S. e.* Typhimurium colonies from bare plots were counted using a Synoptic Limited Protocol Colony Counter® consisting mainly of a digital high resolution CCD video camera whose imaging is enhanced by a lighting configuration system that provides uniform illumination and enhanced contrast. The video camera is connected to a specifically configured computer system with a series of adjustable settings, including sample amount and dilution factor. Results from the counting of colonies by this system were calculated in Colony Forming Units (CFU) per mL.

Some *E. coli* colonies and all *S. e.* Typhimurium colonies from vegetated plots were counted manually due to some degree of contamination from microorganisms native to the experimental site. In the case of *E. coli*, contamination from microorganisms native to the experimental site was not very high and was mostly due to the fact that *E. coli* can also be found in fecal material of wild animals, such as birds, foxes, etc., that at the time of the experiments inhabited the site. In the case of *S. e.* Typhimurium, contamination was high and was due to the fact that, despite the introduction of the antibiotic in the growth medium, other microorganisms, morphologically identical to our *Salmonella* strain, were present at the site. It is believed that these morphologically identical microorganisms were responsible for decomposition of organic matter in the vegetated plots.

In other to adjust the counts for *E. coli* from the vegetated plots, Bromide (Br) data was used to identify the point in time during the simulations that the manure front really reached the points of surface runoff collection. In other to adjust the counts for *S. e. Typhimurium* from the vegetated plots, *E. coli* and Br data were used to identify the point in time at which the manure front really reached the points of surface runoff collection. In addition, the counts for *S. e. Typhimurium* were farther adjusted using the BBL Enterotube II technique (section 3.3.3). In the process, several plates, that represented different time intervals throughout the simulations, were selected and their colonies were tested in order to give a ratio of counted colonies that were our strain of *Salmonella* to colonies that were not.

#### 3.5.5 Bromide concentration in runoff samples

Bromide (Br) concentration (ppm) in runoff samples was measured based on their electrical chemical potential as related to Bromide content, in millivolts (mV), using an ion-specific electrode Model-525 manufactured by Thermo Orion. In the process, the first step was to prepare, in different volumetric flasks, a stock solution of 10000 ppm Br and 5 standard concentrations of Br that ranged from 1000 to 0.1 ppm Br. The stock solution was prepared by thoroughly mixing 7.45 g of potassium bromide (KBr) to 500 mL of deionized (DI) water. After the stock solution was prepared, the highest concentrated standard (1000 ppm) was obtained by thoroughly mixing 50 mL of the stock solution with 500 mL of DI water. The remaining standard concentrations – 100, 10, 1, and 0.1 ppm Br – were prepared by thoroughly mixing 10, 1, 0.1, and 0.01 mL of the 1000 ppm standard with 100 mL of DI water, respectively.

The electrical chemical potential, in mV, of each standard concentration was then measured with the above instrument in order to generate a standard curve that could be used to estimate the concentration of Br in the runoff samples. In order to accomplish that, 5 mL of each standard was thoroughly mixed with 0.1 mL (50:1) of ionic strength adjuster (ISA) before mV value was measured and recorded. Standard curves were generated by regression of mV values over the standard concentrations values on a semi-log scale. Millivolts of runoff samples were also measured after 5 mL of each sample were thoroughly mixed with 0.1 mL of ISA. Both, standards and runoff samples were brought to the same ambient temperature before any measurement took place; and at every 2 h a new standard curve was generated before going into the next set of samples.

### 3.6 Statistical Analysis

For each set of experiments, an ANOVA for fixed effects was performed to test the effect of cover condition and distance on the reduction rate ( $k$ ) of each pathogen in surface runoff over time and after 15 min of simulation in each plot (P level of significance set at 0.05). This point in time during the simulations was observed to be critical since that was when the trend in relative concentrations with time in the vegetated plots was observed to follow an exponential decrease. An ANOVA for fixed effects was also performed for each set of experiments to test the effect of cover and distance on relative concentrations calculated at  $t = 15$  min ( $C/C_{0(15)}$ , %), and on background concentration levels ( $b$ ) observed in each plot. In

addition, t-test statistics were performed to test whether the mean reduction rate ( $k$ ) observed at both distances were significant different between the two treatments.

# Chapter 4: Results and Discussion

## 4.1 Hydrographs

### 4.1.1 Dry initial soil moisture conditions

Figure 4.1 shows the hydrographs describing the runoff rates obtained with time during each experiment under dry initial soil moisture conditions in which the groundwater table was approximately 50 cm below the surface in each plot (Section 3.4.1). In the bare plots, runoff was first observed 2 min after the initiation of rainfall in Plot 2, and 30 sec in Plot 4. Runoff was first observed in the vegetated plots several minutes later than in the bare plots. Runoff was first observed 13.5 min after the initiation of rainfall in Plot 1, and 10 min in Plot 3.

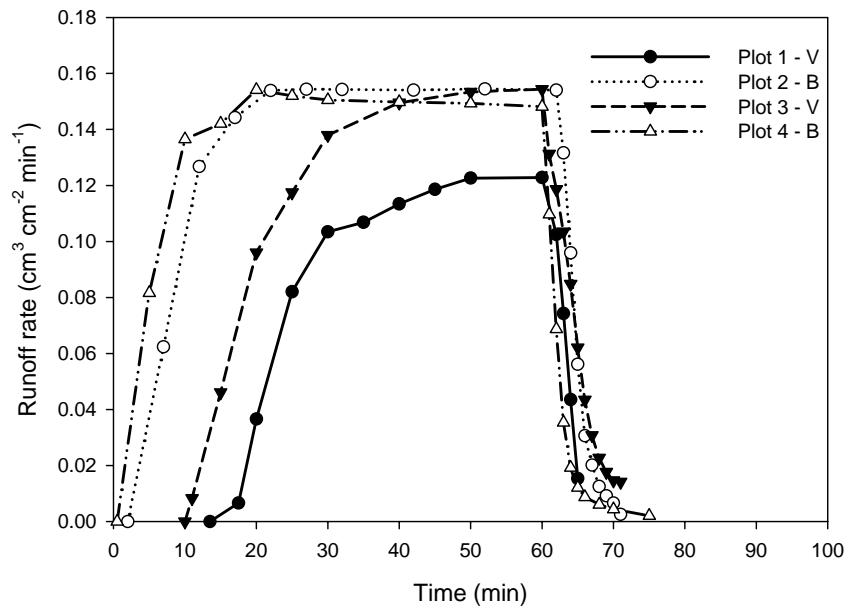


Figure 4.1. Runoff hydrographs of all plots for experiments under dry initial soil moisture conditions. V = Vegetated; B = Bare.



Figure 4.1 indicates that, once runoff initiated in the bare plots (Plots 2 and 4), their rates increased rapidly and sharply during the next 10 min of simulation. This rapid and sharp increase in runoff rates during the early stages of simulation in the bare plots was probably due to surface compaction and sealing resulting from the mechanical action of raindrops on exposed soil surface aggregates (Ward, 1995).

When raindrops strike a bare soil surface, soil aggregates disintegrate, resulting in finer soil particle washing into the soil-surface openings or soil matrix (Gray and Norum, 1967; Beven, 2004). These physical processes result in the formation of a very thin, impervious layer at the surface that rapidly and dramatically decreases the infiltration rate. This process results in a considerable increase of surface runoff (Kirkby, 1969; Ward, 1995; Mualem and Assouline, 1996).

Figure 4.1 also shows that after the initial rapid increase, runoff rates continued to gradually increase for the next 8 to 10 mins, until they reached relatively steady-state conditions (approximately  $0.15 \text{ cm min}^{-1}$ ). The runoff rate remained steady for the duration of the rainfall simulation, which was very close to the applied rainfall rate (approximately  $0.14 \text{ cm min}^{-1}$  in each plot). Therefore, it is very likely that the steady rate of runoff observed in the bare plots was due to the fact that the soil surface layer in these plots eventually became completely saturated, thus causing all of the simulated rain to be discharged as runoff.

As Figure 4.1 indicates, runoff was not observed in the vegetated plots until several minutes after the initiation of rainfall. These lag periods reflect the fact that, at the early stages of simulation, infiltration rates exceeded the simulated rainfall rates. However, as the simulations progressed and the soils became wetter, infiltration rates

decreased such that excess rain either ponded in surface depressions or was discharged as runoff.

Figure 4.1 also indicates that once runoff initiated in the vegetated plots (Plots 1 and 3), rates increased rapidly during the next 10 and 15 min of simulation, but not as sharply as was observed in the bare plots. After this rapid increase, runoff rates continued to gradually increase until near the end of rainfall simulations. Consistent with previous research reported by Adams et al. (2005), runoff rates continued to increase during the rainfall simulations in the vegetated plots due to decreased infiltration rates. This is due to the fact that as soils become wetter, the moisture gradient and available storage in the soil decrease, thus resulting in lower infiltration rates.

Figure 4.1 clearly shows that runoff initiation was delayed and runoff volume substantially diminished in the vegetated plots compared to the bare plots as a consequence of enhanced infiltration. It has long been recognized that vegetation attenuates surface runoff and favors infiltration processes by several distinct mechanisms (Kirkby, 1969; Kilinc and Richardson, 1973; Tromble et al., 1974; Shirmohammadi and Skaggs, 1984; Davies et al., 2004; Roodsari, 2004; Trask et al., 2004).

Mechanisms by which vegetation favors infiltration processes are: (1) vegetation intercepts and dissipates raindrops thereby minimizing the breakdown of soil aggregates, thus minimizing surface sealing; (2) vegetation offers more resistance to overland flow, thus decreasing its velocity and allowing more time for infiltration to occur; (3) plant root systems increase soil hydraulic conductivity by loosening the

soil, resulting in enhanced infiltration; (4) plant roots and invertebrate activity increase soil porosity by opening up channels and macropores, which increases infiltration; and (5) organic matter residues from vegetation aid in the development of more permeable soil surfaces.

As shown in Figure 4.1, there was a considerable difference in runoff volumes between the two vegetated plots (Plots 1 and 3). This was due both to the fact that runoff initiated sooner, and that runoff rates at the end of simulations were substantially higher in Plot 3 than in Plot 1. These differences are most likely due to a combination of three characteristics that were each different between the two plots: slope, topography, and soil texture.

Topographic analysis of Plot 1 and Plot 3 revealed that their slopes were slightly different (Figure 3.2). On average, the slope in Plot 1 was 4.0%, whereas in Plot 3 it was 5.4%. As previously reported by White (1983) and Roodsari (2004), slope can significantly affect total surface runoff: runoff increases as the slope increases.

Topographic analysis (Figure 3.4) also revealed that Plot 1 had less surface area contributing to convergent flow conditions or channelization of flow compared to Plot 3. As some studies have reported (Darboux et al., 2004; Darboux and Huang, 2005; Gomez and Nearing, 2005), these areas of convergent/concentrated flow or surface depressions may delay runoff initiation since water can be stored in puddles, thus decreasing local flow velocity and enhancing infiltration.

However, this ponding effect may be temporary and occur only at the beginning of a rainfall event (Darboux and Huang, 2005; Gomez and Nearing, 2005).

As a rainfall event progresses and (1) depressions become filled and interconnected into a complete drainage network; and (2) the area contributing to runoff expands, flow velocity is higher in these convergent/depression/channel areas, which, in turn, decreases the available time for infiltration to occur and runoff rates increase.

In the studies by Darboux et al. (2004), and Darboux and Huang (2005), they reported similar observations, in which surfaces with initial depressions increased steady state runoff compared to initially smooth surfaces, and they attributed such observations to flow concentration or channelization of flow through surface depressions. According to Dillaha et al. (1986), such concentrated flow conditions, instead of shallow and uniform flow, can substantially decrease the effectiveness of vegetated filter strips (VFS) to attenuate surface runoff as a mean to improve water quality.

Finally, soil texture analysis (Table 3.1) revealed that Plot 1 had a loam soil texture both at the soil surface and at the 20 cm depth, whereas Plot 3 had a loam texture at the surface but a clay loam texture at the 20 cm depth. Soil texture affects infiltration rates; a loam soil is considered to be more permeable than a clay loam soil (Ward and Dorsey, 1995). Therefore, infiltration was higher, and, consequently, less runoff was observed in Plot 1 than in Plot 3.

#### 4.1.2 Wet initial soil moisture conditions

Figure 4.2 shows the hydrographs describing the runoff rates obtained with time during each experiment under wet initial soil moisture conditions in which the groundwater table was just a few centimeters below the surface in each plot (Table

3.2). In the bare plots, runoff was first observed 70 sec after the initiation of rainfall in Plot 2, and 48 sec in Plot 4. Runoff was first observed in the vegetated plots approximately 2 minutes later than in the bare plots. Runoff was first observed 3 min and 50 sec after the initiation of rainfall in Plot 1 and 2 min and 50 sec in Plot 3.

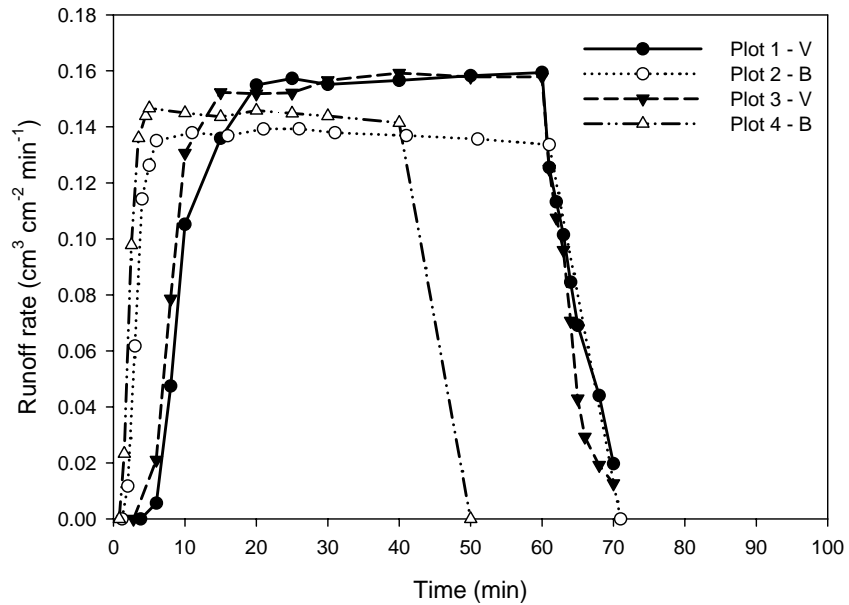


Figure 4.2. Runoff hydrographs of all plots for experiments under wet initial soil moisture conditions. V = Vegetated; B = Bare.

Figure 4.2 indicates that, once runoff initiated in the bare plots (Plots 2 and 4), runoff rates increased rapidly reaching a constant rate (approximately  $0.14 \text{ cm min}^{-1}$ ) within 5 min after the initiation of rainfall. After the first 5 min, the runoff rates and rainfall rates were essentially identical (approximately  $0.14 \text{ cm min}^{-1}$ ). As discussed in the previous section (4.1.1), surface sealing of the bare soil surfaces was probably

the major factor contributing to the rapid increase in runoff rates during the early stages of the simulations.

In addition, the similarity in runoff and rainfall rates after 5 min of simulation was due to the fact that the soil surface layer in the bare plots was completely saturated and possibly sealed. In fact, the simulation in Plot 4 was terminated after 40 min of simulation because the soil was completely saturated and subsurface flow was observed to be overflowing into the gutter.

Figure 4.2 also indicates that, once runoff initiated in the vegetated plots (Plots 1 and 3), rates increased rapidly for the next several minutes, reaching constant rates (approximately  $0.15 \text{ cm min}^{-1}$ ) after 10 and 15 min of simulation in Plots 3 and 1, respectively. Figure 4.2 shows that the increases in runoff rates in vegetated and bare plots were very comparable, and that the constant runoff rates observed in the vegetated plots were actually higher than in the bare plots.

The hydrographs obtained from experiments conducted under wet initial soil moisture conditions (Figure 4.2) were different from those conducted under dry initial soil moisture conditions (Figure 4.1), particularly in vegetated plots. In the bare plots, runoff consistently initiated shortly after rainfall; however, constant runoff rates were observed much sooner under wet conditions than under dry conditions. In vegetated plots, under wet conditions, runoff initiated sooner, runoff rates increased more rapidly and constant runoff rates were higher than under dry conditions.

These differences were due to differences in infiltration capacity. As has been previously reported (Gray and Norum, 1967; Cerda; 1997; Roodsari, 2004), soil moisture content is one of the most important factors controlling infiltration;

infiltration decreases with an increase in the soil moisture content. Therefore, less infiltration and more runoff were observed in the vegetated plots under wet conditions where the water table was much closer to the soil surface.

## 4.2 Bromide

### 4.2.1 Dry initial soil moisture conditions

Figure 4.3 shows Bromide (Br) concentrations measured with time in runoff collected at 413 and 620 cm downslope from the line of slurry application in both bare and vegetated plots.

Br concentrations in runoff decreased as the distance from the source of slurry increased as a result of Br dilution. The dilution effect was due to the fact that the runoff intercepted by three funnels (413 cm downslope), including manure slurry and rainfall, represented only a fraction of the applied rainfall; whereas the gutter (620 cm downslope) included the total amount of rainfall applied to plots. Note that there was substantial variability in Br concentration and in runoff volumes observed at individual funnels (at 413 cm), especially in the vegetated plots (Appendix B), due to the heterogeneity in surface flow pathways arising from non uniform or rough surfaces (Roodsari, 2004).

Figure 4.3 indicates that Br concentrations generally decreased with time, although the kinetics of Br transport were dramatically different between bare and vegetated plots. In bare plots, maximum Br concentrations were observed in the initial runoff samples — approximately 300 ppm in Plot 2 and 418 ppm in Plot 4, at 413 cm — followed by a rapid decline. In contrast, in vegetated plots, maximum Br

concentrations were observed several minutes after runoff initiation — approximately 18 ppm in Plot 1 and 24 ppm in Plot 3, at 413 cm — followed by a more gradual decline. On average, maximum Br concentrations in runoff from vegetated plots were 94% lower than those in runoff from bare plots.

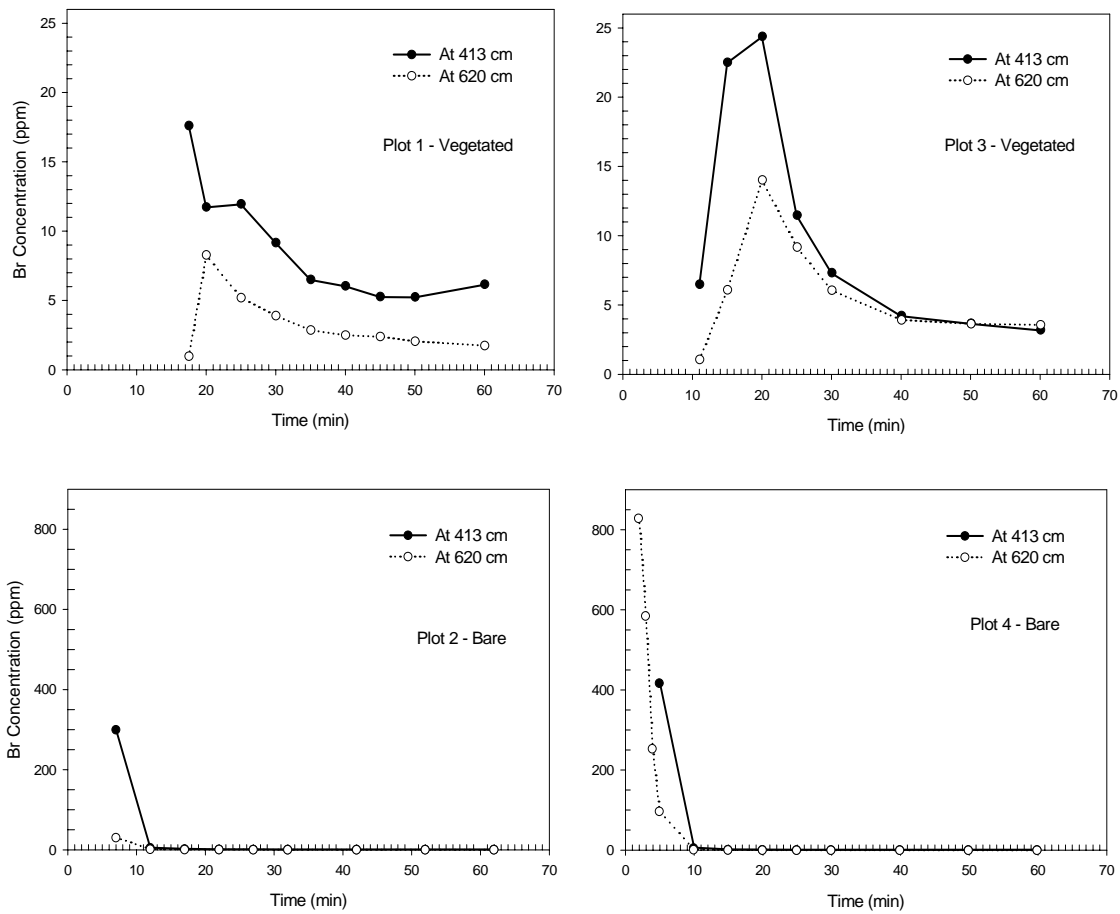


Figure 4.3. Average Bromide (Br) concentrations measured with time in runoff collected at 413 and 620 cm downslope from the line of slurry application in bare and vegetated plots under dry initial soil moisture conditions. The Br concentration in the manure slurry was 2000 ppm.



Overall, there was a substantial reduction in Br recovered in runoff from the vegetated plots compared to the bare plots. These results reflect the fact that substantially greater infiltration occurred in vegetated plots than bare plots, particularly in the initial stages of rainfall simulation when the bulk of manure slurry was transported into the plots (Figure 4.1). Similar findings were also reported by Roodsari (2004), where initial Br concentrations in runoff from a 20% sloped vegetated sandy loam plot were 98% lower than concentrations observed for bare sandy loam plot at a distance of 285 cm from the source of Br application.

#### 4.2.2 Wet initial soil moisture conditions

Figure 4.4 shows Bromide (Br) concentrations measured with time in runoff collected at 413 and 620 cm downslope from the line of slurry application in bare and vegetated plots.

In general, results obtained from wet initial soil moisture conditions were similar to those obtained from dry initial soil moisture conditions (Section 4.2.1). Figure 4.4 indicates that Br concentrations in runoff decreased as the distance from the source of slurry application increased, and that Br concentrations in runoff decreased with time, especially in the bare plots (Plots 2 and 4), at both distances away from the source. Note that there was substantial variability in Br concentration and in runoff volumes observed at individual funnels (at 413 cm), especially in the vegetated plots (Appendix C), due to the heterogeneity in surface flow pathways arising from non uniform or rough surfaces (Roodsari, 2004).

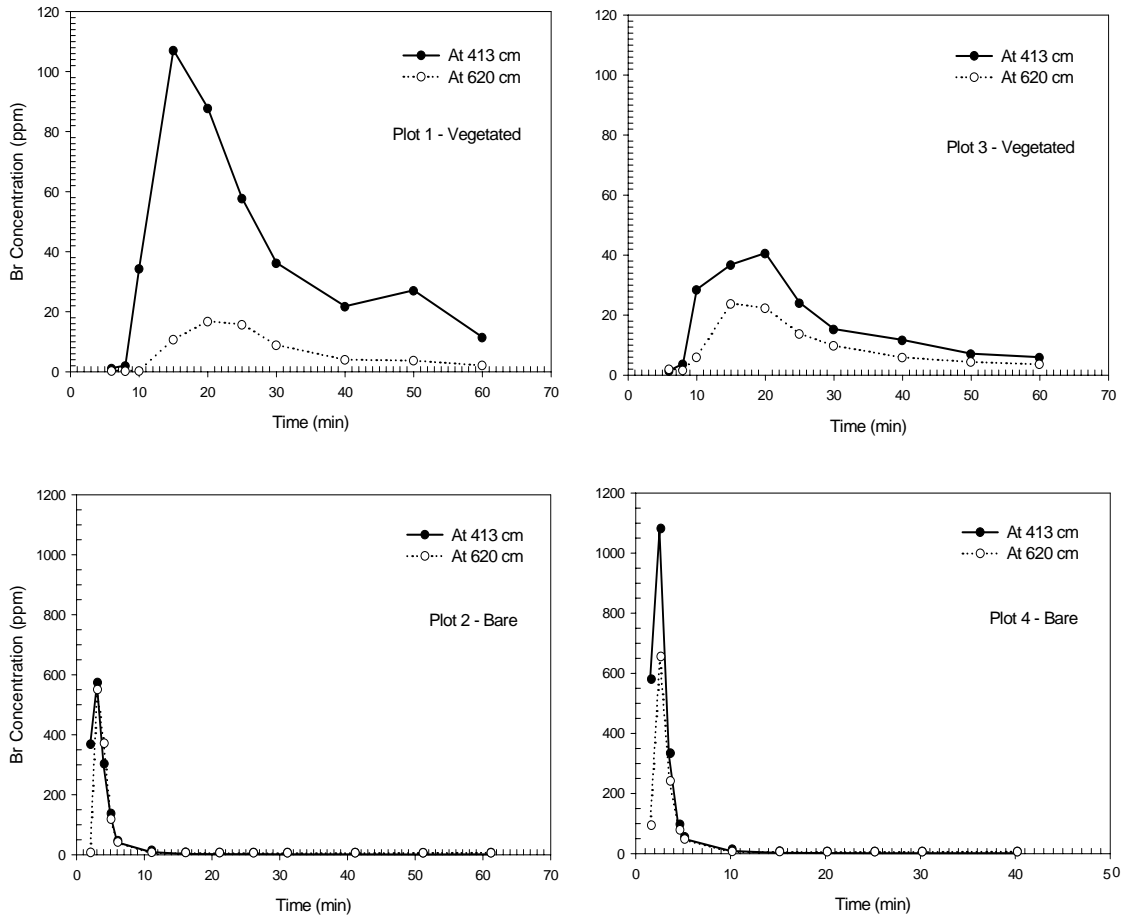


Figure 4.4. Average Bromide (Br) concentrations measured with time in runoff collected at 413 and 620 cm downslope from the line of slurry application in bare and vegetated plots under wet initial soil moisture conditions. The Br concentration in the manure slurry was 2000 ppm.

The maximum Br concentrations in bare plots — approximately 567 ppm in Plot 2 and 1075 ppm in Plot 4, at 413 cm — occurred shortly after the initiation of runoff; while the maximum Br concentrations in vegetated plots — approximately 107 ppm in Plot 1 and 41 ppm in Plot 3, at 413 cm — occurred several minutes after the initiation of runoff.

The primary difference between wet and dry initial soil moisture conditions was observed in vegetated plots, where maximum Br concentrations in runoff were generally higher under wet conditions. On average, the maximum Br concentration at 413 cm in runoff under wet conditions was 252% higher than under dry conditions. These results are consistent with the hydrographs, which show that there was less infiltration in vegetated plots under wet conditions. Less infiltration resulted in reduced amounts of Br transported into the soil profile and increased amounts of Br lost in surface runoff.

### 4.3 Surrogate Pathogens

#### 4.3.1 Dry initial soil moisture conditions

Figures 4.5 and 4.6 show average relative concentrations of *Escherichia coli* and *Salmonella enterica* Typhimurium, respectively, measured with time in surface runoff collected at 413 cm downslope from the source of slurry application in all plots. Figures 4.7 and 4.8 show average relative concentrations of *E. coli* and *S. e.* Typhimurium, respectively, measured with time in surface runoff collected at 620 cm downslope from the source of slurry application in all plots. Relative concentration is defined as  $C/C_0$ , where  $C$  is the concentration of the surrogate pathogens (herein referred to as pathogens) measured in the runoff samples and  $C_0$  is the initial concentration of the pathogens in the slurry.

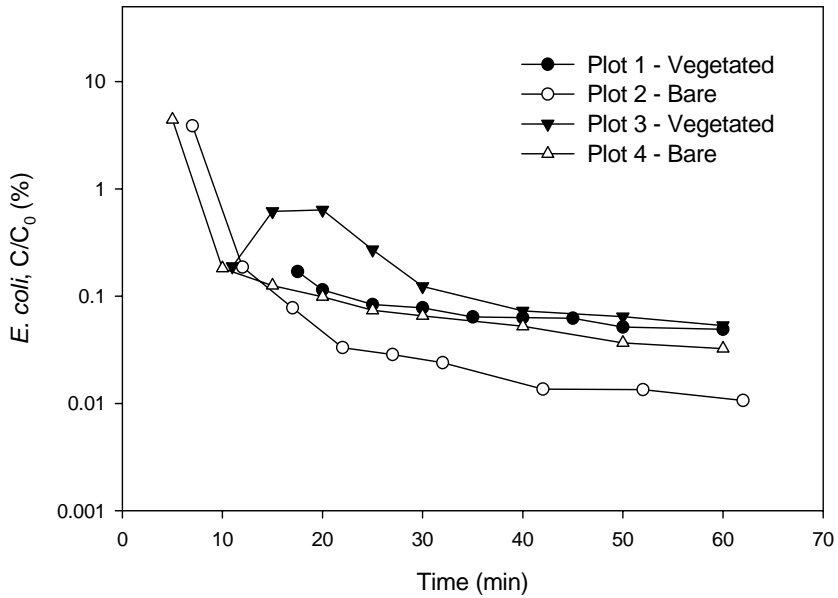


Figure 4.5. Average relative concentrations of *E. coli* measured with time in runoff collected at 413 cm away from the source of slurry in all plots under dry initial soil moisture conditions.

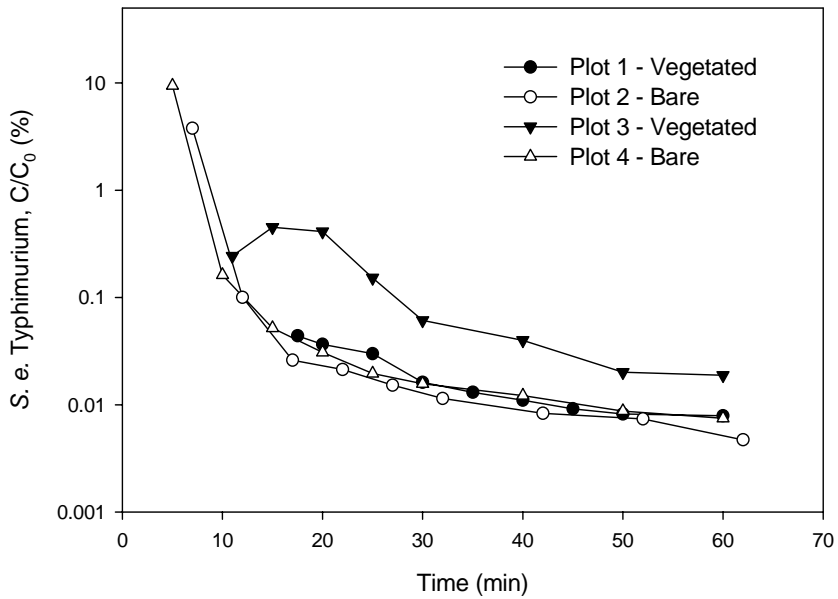


Figure 4.6. Average relative concentrations of *S. e. Typhimurium* measured with time in runoff collected at 413 cm away from the source of slurry in all plots under dry initial soil moisture conditions.

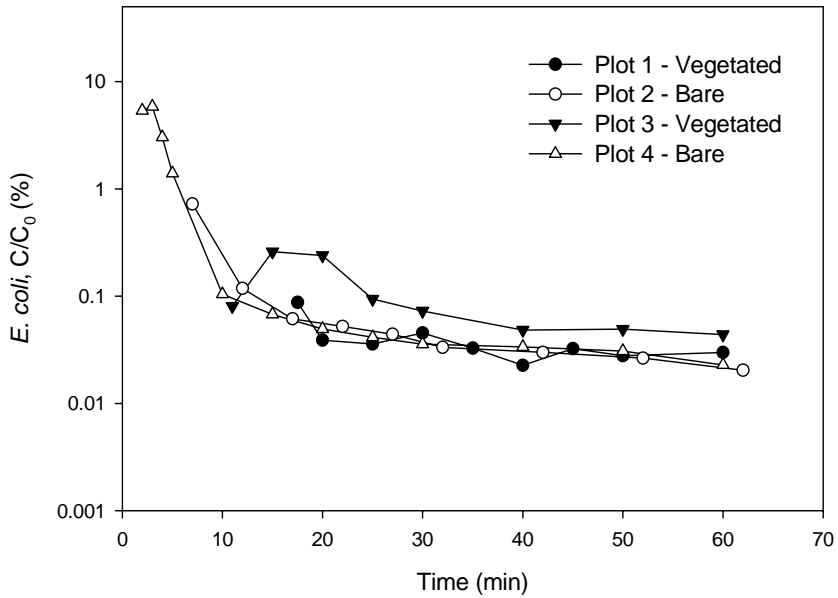


Figure 4.7. Average relative concentrations of *E. coli* measured with time in runoff collected at 620 cm away from the source of slurry in all plots under dry initial soil moisture conditions.

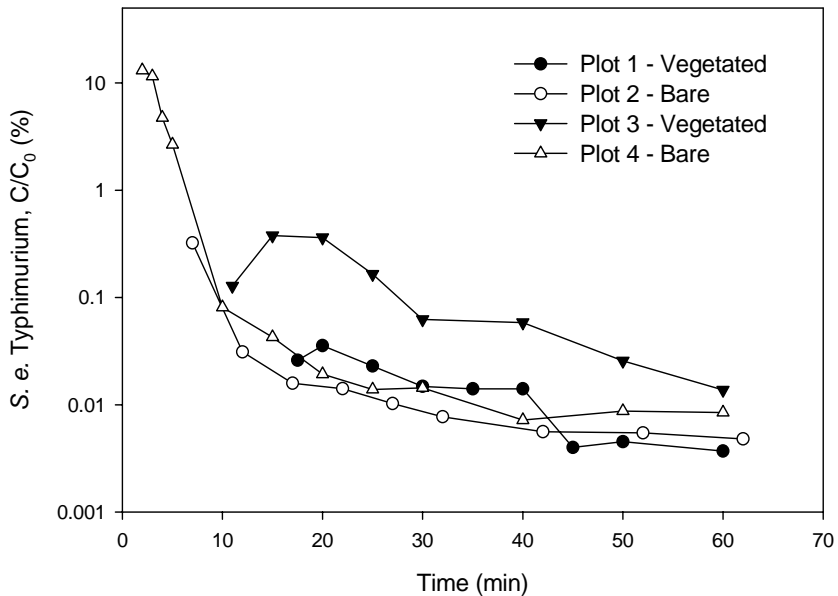


Figure 4.8. Average relative concentrations of *S. e. Typhimurium* measured with time in runoff collected at 620 cm away from the source of slurry in all plots under dry initial soil moisture conditions.

In all plots, relative concentrations of both pathogens in runoff decreased as the distance from the source of slurry increased, except for *E. coli* in Plot 2 (bare) where concentrations became and remained slightly higher at 620 cm than at 413 cm after approximately 20 min of simulation (Figure 4.9). Maximum relative concentrations also decreased with respect to distance in all plots, regardless of cover condition. This decrease in relative concentrations with respect to distance was caused by dilution (Roodsari, 2004; Roodsari et al., 2005), which increased as runoff volumes also increased at distances farther away from the source of slurry.

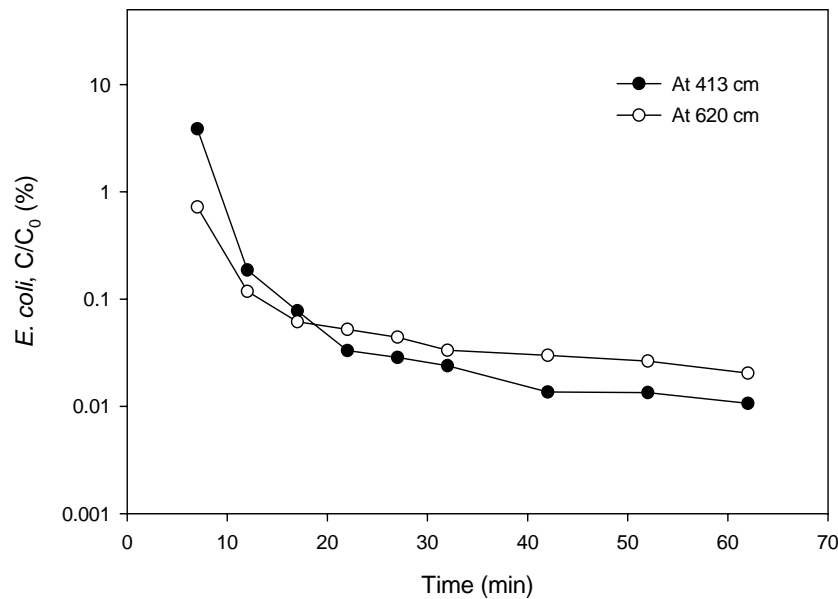


Figure 4.9. Average relative concentrations of *E. coli* measured with time in runoff collected at 413 cm and at 620 cm away from the source of slurry in Plot 2 (bare) under dry initial soil moisture conditions.

Note that there was substantial variability in pathogen concentrations and in runoff volumes observed at individual funnels (at 413 cm), especially in the vegetated plots (Appendix D), due to the heterogeneity in surface flow pathways arising from non uniform or rough surfaces (Roodsari, 2004; Roodsari et al., 2005). For example, no runoff was observed in funnel 1 in the vegetated Plot 3, as it is indicated in Figure 3.3 (Section 3.1.5), until 40 min into the simulation, and no pathogens were ever detected in runoff samples collected from this funnel. This indicates that funnel 1 in Plot 3 was located on a more elevated area of the plot, which collected only locally generated runoff as opposed to runoff originating from the site of manure application.

As Figures 4.5 through 4.8 indicate, relative concentrations of both pathogens in bare plots at both locations from the slurry were very similar, except for *E. coli* measured at 413 cm (Figure 4.5), in which relative concentrations in Plot 4 were substantially higher than in Plot 2 for the period after the initial 15 minutes until the end of simulation. In the vegetated plots, relative concentrations of both pathogens, at both locations from the slurry, were higher in Plot 3 than in Plot 1 throughout the simulations. Note that the two vegetated plots were not identical in terms of their slope, soil texture, and topographic conditions (Section 4.1.1). Runoff rates from Plot 3 were substantially higher than from Plot 1 (Figure 4.1), resulting in higher relative concentrations of both pathogens in Plot 3 than in Plot 1 during the simulations.

As Figures 4.5 to 4.8 indicate, relative concentrations of both pathogens in runoff decreased with respect to time at both distances from the source of slurry, especially in the bare plots. In the bare plots, there was a rapid decrease in relative concentrations within the first 15 min of simulations, while in the vegetated plots,

relative concentrations typically increased to a peak several minutes after the initiation of runoff before decreasing. Relative concentrations in runoff from bare plots were initially substantially higher than from vegetated plots; however, after approximately 15 min of simulations, relative concentrations observed in runoff from vegetated plots were comparable or higher than in bare plots.

Figures 4.10 and 4.11 illustrate the differences in runoff rates between the bare and vegetated plots. Data points situated below the 1:1 line represent samples collected during the first 12 to 15 min of simulations. Both figures show that the pathogens reached both locations in the bare plots earlier than in the vegetated plots, and in higher relative concentrations.

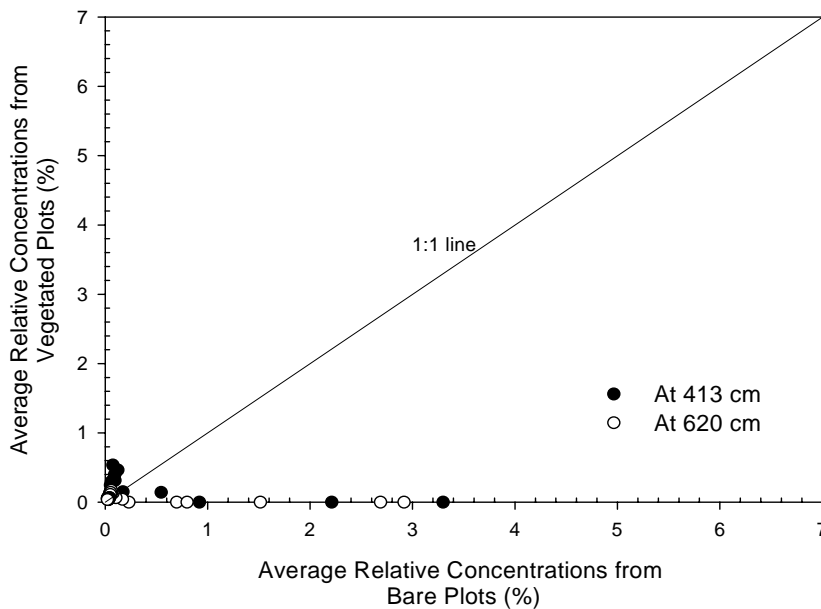


Figure 4.10. Average relative concentrations of *E. coli* measured in runoff at 413 and 620 cm from the source of slurry in vegetated vs. bare plots at identical time intervals during the experiments under dry initial soil moisture conditions.



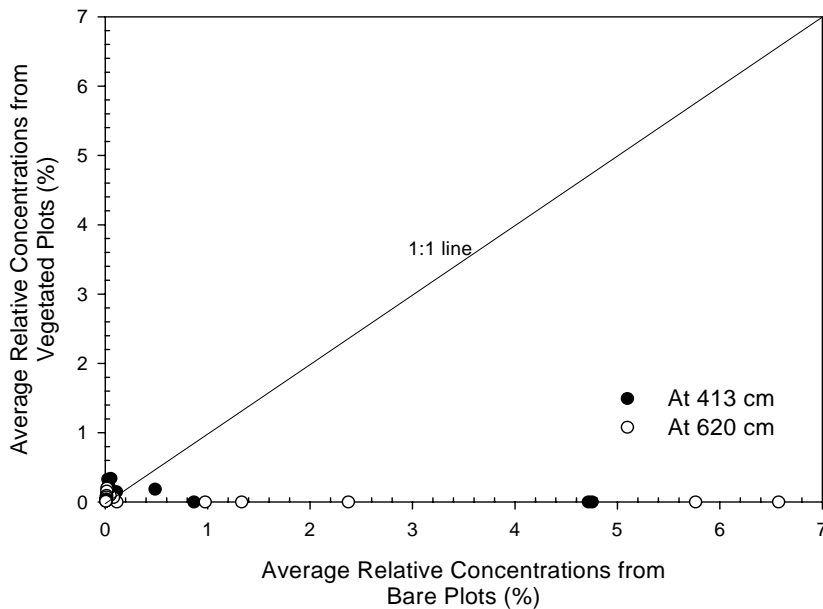


Figure 4.11. Average relative concentrations of *S. e. Typhimurium* measured in runoff at 413 and 620 cm from the source of slurry in vegetated vs. bare plots at identical time intervals during the experiments under dry initial soil moisture conditions.

After the first 15 min of simulations, an exponential decrease in relative pathogen concentrations was observed in both the vegetated and bare plots. This allowed for a comparison of pathogen reduction rates between bare and vegetated plots during this exponential phase. The changes in relative concentrations after 15 min of simulations were modeled as a single exponential with constant background according to Equation 1. A constant background was included in the equation to account for the observation that relative concentrations decreased but leveled off towards the end of each simulation. Equation 1 is:

$$C/C_0 = ae^{(-k*t)} + b \quad (1)$$

where,

$C/C_0$ : percent (%) relative concentration at time  $t$  (in minutes)

$a$ : normalization term

$k$ : the time constant for the decrease or the “rate” of reduction with time

$t$ : time  $t$  (in minutes) during the simulation

$b$ : the background relative concentration (in percent) that does not change with time

Equation 1 was rearranged to incorporate only variables that were meaningful in describing the exponential decrease in relative concentrations with time after 15 min of simulation. In addition, Equation 1 can be rearranged in terms of the relative concentrations at 15 min of simulation. This was done since this time point in the simulations was observed to be critical. It is the starting time when relative concentrations decreased exponentially with time for both bare and vegetated plots. Based on Equation 1, relative concentrations at 15 min of simulation can be described as follow:

$$C/C_{0(15)} = ae^{(-k*15)} + b \quad (2)$$

where,

$C/C_{0(15)}$ : percent (%) relative concentration at 15 min of simulation

$a$ : normalization term

$k$ : the time constant for the decrease or the “rate” of reduction with time

$b$ : background relative concentration (in percent) that does not change with time

Based on the ratio between Equations 1 and 2, exponential decrease of relative concentrations with time after 15 min of simulation in each plot may be described as:

$$\frac{C/C_0 = ae^{(-k*t)} + b}{C/C_{0(15)} = ae^{(-k*15)} + b}$$

Rearranging, the ratio results in,

$$\frac{(C/C_0) - b = ae^{(-k*t)}}{(C/C_{0(15)}) - b = ae^{(-k*15)}}$$

and by cancelling variable “a”, the ratio becomes,

$$\frac{(C/C_0) - b = e^{(-k*t)}}{(C/C_{0(15)}) - b = e^{(-k*15)}}$$

and finally,

$$\frac{(C/C_0) - b}{(C/C_{0(15)}) - b} = e^{-k*(t-15)}$$

Therefore, the exponential decrease in relative concentrations after 15 min of simulation in each plot can be described as:

$$C/C_0 = [(C/C_{0(15)}) - b]e^{-k*(t-15)} + b \quad (3)$$

where,

$C/C_0$ : percent (%) relative concentration at time  $t$  (in minutes)

$C/C_{0(15)}$ : percent (%) relative concentration at  $t = 15$  min

$b$ : the background relative concentration (in percent) that does not change with time

$k$ : the time constant for the decrease or the “rate” of reduction with time

$t$ : point in time (in minutes) during the experiments

Table 4.1 shows the equations describing the exponential decrease in relative concentration after 15 min of simulation in each plot. High  $R^2$  values ( $>0.94$ ) indicate a strong correlation between relative concentrations of both pathogens with time at different distances from the slurry application area, as represented by the exponential functions.

An ANOVA for fixed effects was performed to test whether or not distance (413 cm or 620 cm) from the source of slurry or cover condition (bare or vegetated) had any significant effect on the parameters  $k$ ,  $b$ , and  $C/C_{0(15)}$  of Equation 3. The statistical results, in the form of ANOVA tables, are presented in Tables 4.2 through 4.7.

Table 4.1. Equations relating relative concentrations ( $C/C_0$ ) of each pathogen in runoff to time  $t$  after 15 min of simulation at each distance in each plot under dry initial soil moisture conditions.

<i>E. coli</i>			
PLOT	Distance, cm	Equation, $C/C_0$ , %	$R^2$
Plot 1 (Vegetated)	413	$0.17e^{-0.19(t-15)} + 0.058$	0.995
	620	$0.40e^{-0.80(t-15)} + 0.032$	0.982
Plot 3 (Vegetated)	413	$0.68e^{-0.08(t-15)} + 0.004$	0.948
	620	$0.25e^{-0.10(t-15)} + 0.033$	0.966
Plot 2 (Bare)	413	$0.09e^{-0.18(t-15)} + 0.014$	0.988
	620	$0.05e^{-0.06(t-15)} + 0.019$	0.998
Plot 4 (Bare)	413	$0.10e^{-0.07(t-15)} + 0.029$	0.999
	620	$0.04e^{-0.10(t-15)} + 0.027$	0.997

Table 4.1 (Continued)

S. e. Typhimurium			
PLOT	Distance, cm	Equation, C/C <sub>0</sub> , %	R <sup>2</sup>
Plot 1 (Vegetated)	413	$0.05e^{-0.09(t-15)} + 0.006$	0.995
	620	$0.04e^{-0.05(t-15)} + 5.00E-11$	0.958
Plot 3 (Vegetated)	413	$0.49e^{-0.10(t-15)} + 8.36E-11$	0.956
	620	$0.41e^{-0.08(t-15)} + 7.07E-11$	0.959
Plot 2 (Bare)	413	$0.03e^{-0.07(t-15)} + 0.004$	0.998
	620	$0.01e^{-0.07(t-15)} + 0.004$	0.996
Plot 4 (Bare)	413	$0.04e^{-0.13(t-15)} + 0.009$	0.999
	620	$0.03e^{-0.21(t-15)} + 0.009$	0.992

The ANOVA tables showing the effect of distance and cover on the observed reduction rates (*k*) of *E. coli* and *S. e. Typhimurium* are shown, respectively, in Tables 4.2 and 4.3. According to these data, the rates (*k*) governing the reduction in relative concentrations of both pathogens in runoff, after 15 min of simulations, were not affected by distance (*p* = 0.49 for *E. coli* rates; and *p* = 0.87 for *S. e. Typhimurium* rates) or by cover condition (*p* = 0.34 for *E. coli* rates; and *p* = 0.34 for *S. e. Typhimurium* rates).

Table 4.2. ANOVA table for the effect of distance and cover (at P value set at 0.05) on the observed *E. coli* rates of reduction (*k*) obtained after 15 min of simulations under dry initial soil moisture conditions.

Source of Variation	Df <sup>†</sup>	Sum of Squares	Mean Square	F Value	Pr (F)
Distance	1	0.0353115	0.03531153	0.551087	0.4912543
Cover	1	0.0726758	0.07267578	1.134209	0.3355831
Residuals	5	0.3203809	0.06407619		

<sup>†</sup>Df = degrees of freedom

Table 4.3. ANOVA table for the effect of distance and cover (at P value set at 0.05) on the observed *S. e. Typhimurium* rates of reduction (*k*) obtained after 15 min of simulations under dry initial soil moisture conditions.

Source of Variation	Df <sup>†</sup>	Sum of Squares	Mean Square	F Value	Pr (F)
Distance	1	0.00008845	0.000088445	0.031991	0.8650685
Cover	1	0.00301864	0.003018645	1.091868	0.3439203
Residuals	5	0.01382330	0.002764661		

<sup>†</sup>Df = degrees of freedom

Based on the study by Stout et al. (2005), it was not expected that *k* would be affected between the two locations from the slurry since a short distance separated them from each other. In the above study, although they did not investigate the effect of cover, they found that the rate of fecal coliform (FC) transport in runoff through vegetative filter strips was not affected by distance and the rates were similar at two locations (1 m apart from each other) from the source of dairy manure. However, if *k* observed after 15 min of simulations was to be affected by cover condition, this study could not verify such effect and further studies would have been necessary.

The ANOVA tables showing the effect of distance and cover on the observed background levels (*b*) of *E. coli* and *S. e. Typhimurium* are shown, respectively, in

Tables 4.4 and 4.5. Data indicate that background levels (*b*) of both pathogens were not affected by distance ( $p = 0.91$  for *E. coli* background levels; and  $p = 0.52$  for *S. e. Typhimurium* background levels). Although Figures 4.5 to 4.8 suggest that *b* levels were higher in the vegetated plots than in the bare plots, particularly based on levels observed in Plot 3, Tables 4.4 and 4.5 indicate that *b* of both pathogens were not affected by cover condition ( $p = 0.47$  for *E. coli* levels; and  $p = 0.06$  for *S. e. Typhimurium* levels).

Table 4.4. ANOVA table for the effect of distance and cover (at P value set at 0.05) on the observed *E. coli* background levels (*b*) obtained after 15 min of simulations under dry initial soil moisture conditions.

Source of Variation	Df <sup>†</sup>	Sum of Squares	Mean Square	F Value	Pr (F)
Distance	1	0.000004651	0.0000046513	0.0146380	0.9084125
Cover	1	0.000191101	0.0001911013	0.6014178	0.4731029
Residuals	5	0.001588756	0.0003177512		

<sup>†</sup>Df = degrees of freedom

Table 4.5. ANOVA table for the effect of distance and cover (at P value set at 0.05) on the observed *S. e. Typhimurium* background levels (*b*) obtained after 15 min of simulations under dry initial soil moisture conditions.

Source of Variation	Df <sup>†</sup>	Sum of Squares	Mean Square	F Value	Pr (F)
Distance	1	0.000004205	0.000004205	0.471518	0.5228319
Cover	1	0.000051005	0.000051005	5.719332	0.0622711
Residuals	5	0.000044590	0.000008918		

<sup>†</sup>Df = degrees of freedom

The ANOVA tables showing the effect of distance and cover on observed relative concentrations of *E. coli* and *S. e. Typhimurium* at 15 min of simulations ( $C/C_{0(15)}$ ) are shown, respectively, in Tables 4.6 and 4.7. Data indicate that relative concentrations of both pathogens at 15 min of simulation ( $C/C_{0(15)}$ ) were not affected by distance ( $p = 0.52$  for *E. coli* relative concentrations; and  $p = 0.83$  for *S. e. Typhimurium* relative concentrations). However, Table 4.6 indicates that *E. coli* relative concentrations at 15 min of simulations ( $C/C_{0(15)}$ ) were affected by cover condition ( $p = 0.03$ ). According to a standard Two-Sample t-Test, the mean of *E. coli* relative concentrations at 15 min was significantly higher in the vegetated plots (0.41%) than in the bare plots (0.09%), with  $p = 0.02$ . On the other hand, Table 4.7 indicates that *S. e. Typhimurium* concentrations were not affected by cover condition ( $p = 0.16$ ), despite the fact that Figures 4.6 and 4.8 suggest that concentrations of this pathogen were higher in the vegetated plots than in the bare plots, particularly based on what was observed in Plot 3.

Table 4.6. ANOVA table for the effect of distance and cover (at P value set at 0.05) on relative concentrations of *E. coli* observed at 15 min of simulation ( $C/C_{0(15)}$ ) in the experiments under dry initial soil moisture conditions.

Source of Variation	Df <sup>†</sup>	Sum of Squares	Mean Square	F Value	Pr (F)
Distance	1	0.0114955	0.0114955	0.485467	0.5170126
Cover	1	0.2008928	0.2008928	8.483881	0.0332946
Residuals	5	0.1183968	0.0236794		

<sup>†</sup>Df = degrees of freedom



Table 4.7. ANOVA table for the effect of distance and cover (at P value set at 0.05) on relative concentrations of *S. e. Typhimurium* observed at 15 min of simulation ( $C/C_{0(15)}$ ) in the experiments under dry initial soil moisture conditions.

Source of Variation	Df <sup>†</sup>	Sum of Squares	Mean Square	F Value	Pr (F)
Distance	1	0.0017162	0.00171621	0.051004	0.8302674
Cover	1	0.0911120	0.09111201	2.707726	0.1607822
Residuals	5	0.1682445	0.03364890		

<sup>†</sup>Df = degrees of freedom

In addition to statistical analyses using ANOVA for fixed effects, standard Two-Sample t-Tests were performed on the mean reduction rates ( $k$ ) of both pathogens to test whether or not the means were significantly different between the two treatments (bare and vegetated). The standard Two-Sample t-Test analysis indicated that the mean *E. coli* rate of reduction observed in the bare plots ( $0.10 \text{ min}^{-1}$ ) was not significantly different from the mean rate observed in the vegetated plots ( $0.29 \text{ min}^{-1}$ ) ( $p = 0.31$ ), regardless of distance away from the source of slurry. The same analysis indicated that the mean *S. e. Typhimurium* rate of reduction observed in the bare plots ( $0.12 \text{ min}^{-1}$ ) was not significantly different from the mean rate observed in the vegetated plots ( $0.08 \text{ min}^{-1}$ ) ( $p = 0.30$ ), regardless of distance away from the source of slurry.

Except for relative concentrations of *E. coli* observed at 15 min of simulation ( $C/C_{0(15)}$ ) – which were found to be affected by cover condition and were significantly higher in the vegetated plots than in the bare plots – this study could not verify that cover condition had a significant effect on parameters  $k$  and  $b$  describing the exponential decrease in relative concentrations of *E. coli* in surface runoff; or on

the parameters  $k$ ,  $b$ , and  $C/C_{0(15)}$  describing the exponential decrease in relative concentrations of *S. e. Typhimurium* in surface runoff.

The inability to detect significant effects and/or significant differences between treatments, particularly on parameters  $b$  and  $C/C_{0(15)}$ , however, could be due to the limitations of the experimental design. The lysimeter site where simulations were conducted could accommodate only four plots that were reasonable in size for inclusion of spatial heterogeneity; thereby severely limiting the number of replications per treatment (two vegetated and two bare plots). One should note, however, that decreasing the size of experimental plots in order to obtain statistical replications often hinders the area scale of experiments, thus limiting inclusion of spatial heterogeneity effect in the observations. In addition, as previously described, the two vegetated plots differed in three important characteristics: slope, soil texture at 20 cm depth, and topography. This contributed to large within-treatment variability, hence diminishing the likelihood of detecting statistically significant differences.

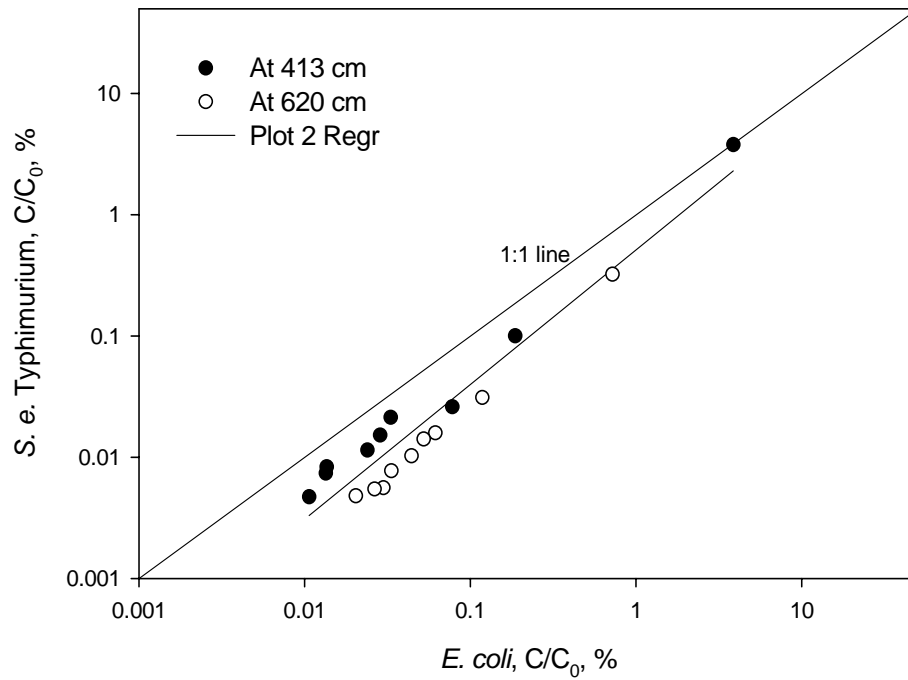
#### 4.3.1.1 Relationship between relative concentrations of *Escherichia coli* and *Salmonella enterica* Typhimurium in surface runoff

Linear regression analysis was performed between relative concentrations of *E. coli* vs. *S. e. Typhimurium* in surface runoff for each plot at each location. Correlation coefficient values (R) were determined based on Pearson correlation analysis, since it is appropriate for variables that are continuous and can be analyzed

statistically at various levels of significance ( $p$ ). In this study, the R values were analyzed against critical values representing three levels of  $p$ : 0.10, 0.05, and 0.01.

Strong correlations were observed between relative concentrations of *E. coli* vs. *S. e. Typhimurium* in runoff from the bare plots (Figures 4.12 and 4.13) at both distances from the manure source ( $p = 0.01$ ), indicating that both pathogens were transported similarly on a bare surface. Similar results have been reported by Roodsari (2004), who examined the surface transport of *E. coli* and *S. choleraesuis* from liquid swine manure on a 20%-sloped plot with a clay loam texture. He also found a strong correlation between the relative concentrations of these two pathogens in runoff from bare plots at various distances from the source of manure.

A strong correlation was observed between relative concentrations of *E. coli* vs. *S. e. Typhimurium* in runoff from both vegetated plots (Figures 4.14 and 4.15) at 413 cm ( $p = 0.01$ ). A strong correlation was also observed between relative concentrations of *E. coli* vs. *S. e. Typhimurium* at 620 cm in Plot 3 ( $p = 0.01$ ); however, this strong correlation was not observed at 620 cm in Plot 1 ( $p > 0.10$ ). It is unclear why the transport of *E. coli* vs. *S. e. Typhimurium* in Plot 1 was not comparable at 620 cm. The pathogens are essentially identical with respect to size, shape, and density. Note, however, that there can be differences among these genera (and between strains within the genera), with respect to the presence of surface appendages (e.g., fimbria, pili) (Ofek et al., 2003).

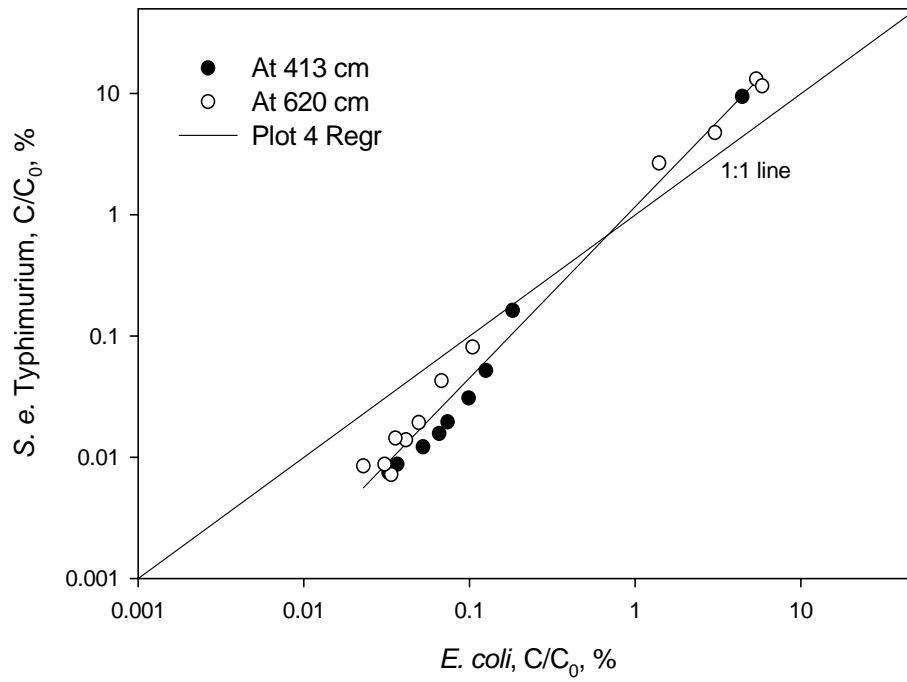


$$S_{413 \text{ cm}} = -0.15 + 1.09E_{413 \text{ cm}} \quad R = 1.000^{***}$$

$$S_{620 \text{ cm}} = -0.34 + 1.21E_{620 \text{ cm}} \quad R = 0.999^{***}$$

$$S_{\text{overall}} = -0.29 + 1.11E_{\text{overall}} \quad R = 0.995^{***}$$

Figure 4.12. Regressions of *S. e. Typhimurium* concentration ratios ( $C/C_0$ ) to *E. coli* concentration ratios ( $C/C_0$ ) in runoff from Plot 2 (bare) from experiment under dry initial soil moisture conditions. \*\*\* Statistically significant at  $p = 0.01$ .

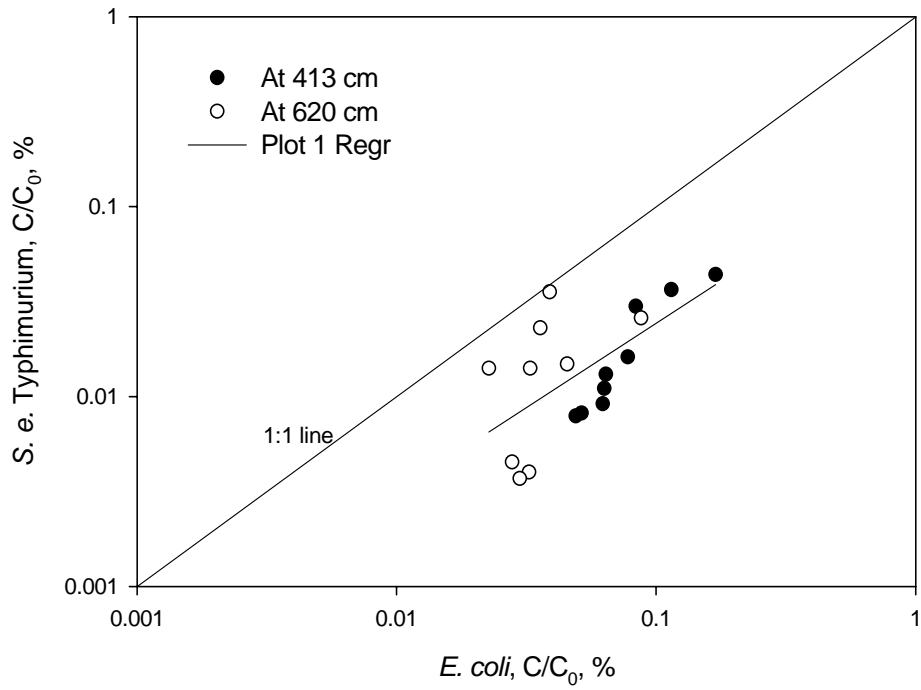


$$S_{413 \text{ cm}} = 0.06 + 1.49E_{413 \text{ cm}} \quad R = 1.000^{***}$$

$$S_{620 \text{ cm}} = 0.10 + 1.37E_{620 \text{ cm}} \quad R = 0.987^{***}$$

$$S_{\text{overall}} = 0.07 + 1.41E_{\text{overall}} \quad R = 0.990^{***}$$

Figure 4.13. Regressions of *S. e. Typhimurium* concentration ratios ( $C/C_0$ ) to *E. coli* concentration ratios ( $C/C_0$ ) in runoff from Plot 4 (bare) for experiment under dry initial soil moisture conditions. \*\*\* Statistically significant at  $p = 0.01$ .

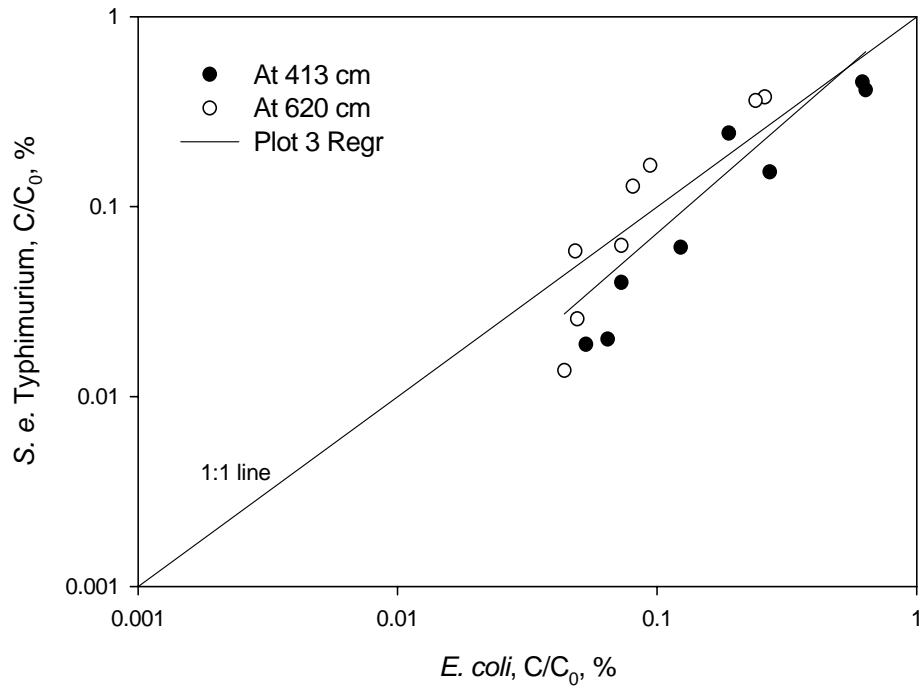


$$S_{413 \text{ cm}} = -0.03 + 1.57E_{413 \text{ cm}} \quad R = 0.936^{***}$$

$$S_{620 \text{ cm}} = -0.33 + 1.11E_{620 \text{ cm}} \quad R = 0.482$$

$$S_{\text{overall}} = -0.73 + 0.89E_{\text{overall}} \quad R = 0.724^{***}$$

Figure 4.14. Regressions of *S. e. Typhimurium* concentration ratios ( $C/C_0$ ) to *E. coli* concentration ratios ( $C/C_0$ ) in runoff from Plot 1 (vegetated) for experiment under dry initial soil moisture conditions. \*\*\* Statistically significant at  $p = 0.01$ .



$$S_{413 \text{ cm}} = -0.04 + 1.26E_{413 \text{ cm}} \quad R = 0.960^{***}$$

$$S_{620 \text{ cm}} = 0.60 + 1.56E_{620 \text{ cm}} \quad R = 0.984^{***}$$

$$S_{\text{overall}} = 0.05 + 1.19E_{\text{overall}} \quad R = 0.869^{***}$$

Figure 4.15. Regressions of *S. e. Typhimurium* concentration ratios ( $C/C_0$ ) to *E. coli* concentration ratios ( $C/C_0$ ) in runoff from Plot 3 (vegetated) for experiment under dry initial soil moisture conditions. \*\*\* Statistically significant at  $p = 0.01$ .

#### 4.3.1.2 Relationship between relative concentrations of each surrogate pathogen and Bromide in surface runoff

Linear regression analysis was performed between relative concentrations of each pathogen and Bromide (Br) in surface runoff for each plot at each location. Correlation coefficient values (R) of the relationships were determined based on Pearson correlation analysis since it is appropriate for variables that are continuous to be analyzed statistically at various levels of significance (p). In this study, the R values were analyzed against critical values representing three levels of p: 0.10, 0.05, and 0.01.

Strong correlations were observed between relative concentrations of each pathogen and Br in runoff from the bare plots (Figures 4.16 to 4.19) at both distances from the source of slurry ( $p = 0.01$ ). Since Br is inert and does not sorb to organic matter or soil particles (Walton et al., 2000), these results indicate that the bare plots offered little resistance to the transport of either pathogen. In addition, these results indicate that pathogens were either predominantly in the liquid phase of the swine slurry or that the manure solids (including pathogens) consisted of suspended colloids, which were all transported at similar rates.

This is consistent with previous studies indicating that bacterial cells attached to particles during runoff can be very low (Davies and Bavor, 2000; Borst and Selvakumar, 2003; Muirhead et al., 2005). These results, however, are very different from those reported for bovine manure (Roodsari, 2004). In contrast to swine slurry (4% solids), bovine manure typically contains a higher solid content (10%), consisting of substantial amounts of residual dietary fiber. Consequently, a fraction of



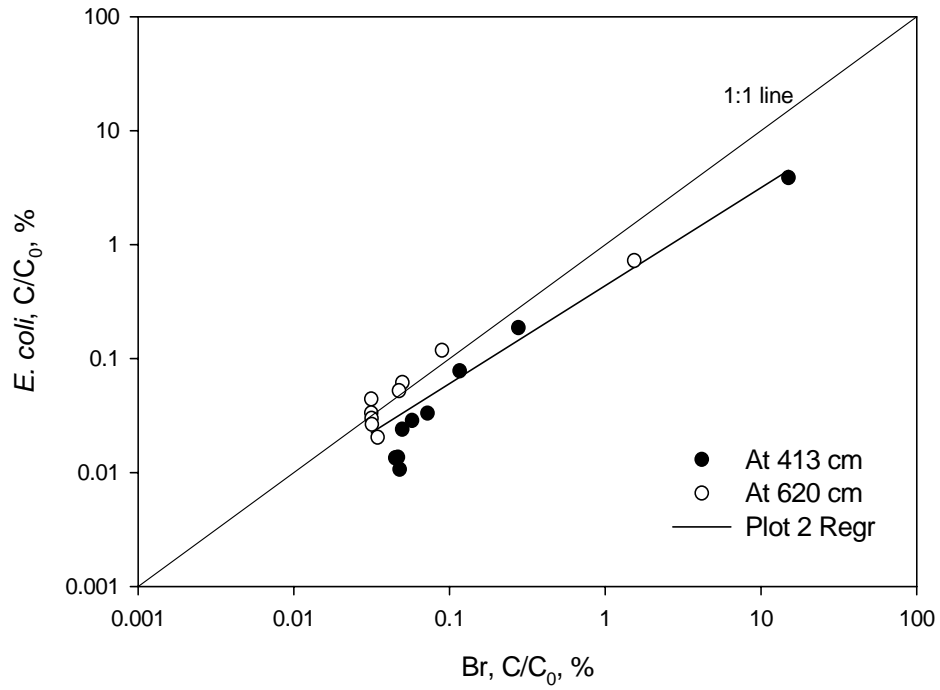
the bacteria must first “release” from the manure solids before they can be transported (Guber et al., 2006).

Figures 4.16 through 4.19 show that, in general, relative concentrations of Br in surface runoff from bare plots were higher than for pathogens (data points appear mostly below the 1:1 line). Note that only pathogens in the aqueous phase of runoff were measured; pathogens attached to soil particles in runoff were not measured. These results may suggest that some fraction of pathogens were attached to sediment in runoff, resulting in higher relative Br concentrations.

Strong correlations were observed between the relative concentrations of each pathogen and Br in runoff from the vegetated plots (Figures 4.20 to 4.23) at 413 cm ( $p = 0.01$ ). However, correlations observed at 620 cm differed between the two pathogens and between the two vegetated plots. In Plot 1, a significant correlation between relative concentrations of *S. e. Typhimurium* and Br was observed ( $p = 0.05$ ), but not between *E. coli* and Br ( $p > 0.10$ ). In Plot 3, the relative concentrations of each pathogen and Br were only weakly correlated ( $p = 0.10$ ). Figures 4.20 through 4.23 also show that the relative concentrations of Br in surface runoff were consistently higher than for pathogens (data points appear mostly below the 1:1 line). This phenomenon was particularly pronounced in Plot 3. Collectively, these results indicate that the mechanisms by which vegetated surfaces affect pathogen transport are different than for Br.

Note that runoff did not initiate in vegetated plots until 10-13 minutes after the start of rainfall simulations and that greater infiltration was subsequently observed in Plot 1 than in Plot 3 (Figure 4.1). Lower cumulative recoveries of Br from Plot 1 than

from Plot 3 (see Table 4.15) are consistent with greater infiltration. However, after the initiation of runoff, substantially higher relative Br concentrations were observed in runoff than relative pathogen concentrations (particularly in Plot 3). Since it is unlikely that infiltration rates for pathogens were higher than for Br, these data suggest that pathogens were selectively retained in the vegetated plots, presumably as a result of sorption to plant litter/organic matter. As previously described in Chapter 3, Section 3.2.1, vegetation/litter densities, as well as decomposing organic matter, were likely higher in Plot 1 than in Plot 3. Consequently, increased sorption of pathogens was likely responsible for lower relative pathogen concentrations in Plot 1, as well as the lower cumulative recovery of pathogens (Table 4.15).

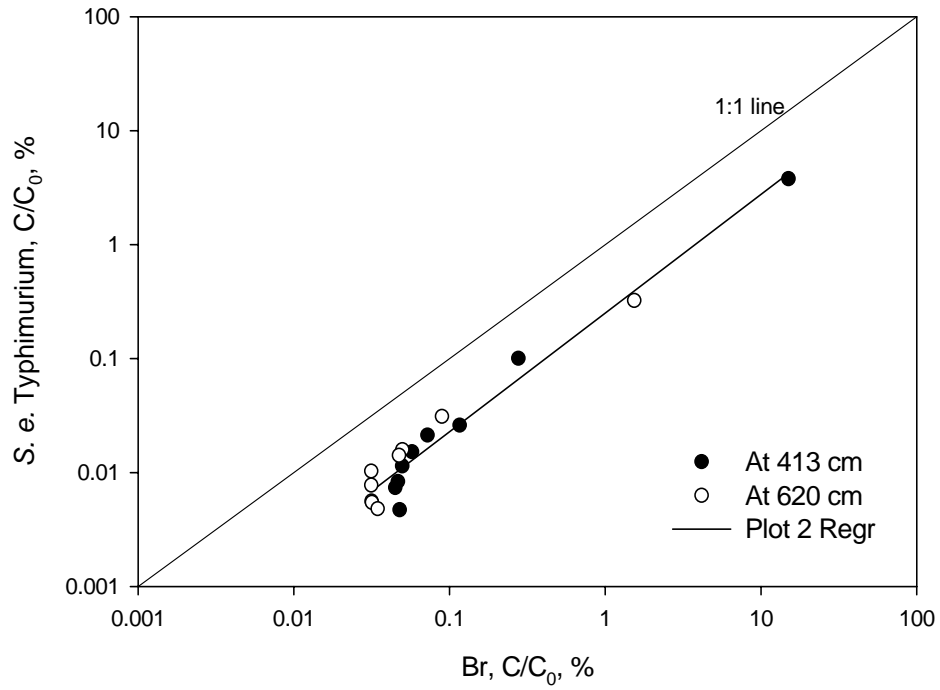


$$E_{413 \text{ cm}} = -0.43 + 0.97Br_{413 \text{ cm}} \quad R = 1.000^{***}$$

$$E_{620 \text{ cm}} = -0.24 + 0.83Br_{620 \text{ cm}} \quad R = 0.996^{***}$$

$$E_{\text{overall}} = -0.36 + 0.86Br_{\text{overall}} \quad R = 0.996^{***}$$

Figure 4.16. Regressions of *E. coli* concentration ratios ( $C/C_0$ ) to Bromide concentration ratios ( $C/C_0$ ) in runoff from Plot 2 (bare) for experiment under dry initial soil moisture conditions. \*\*\* Statistically significant at  $p = 0.01$ .

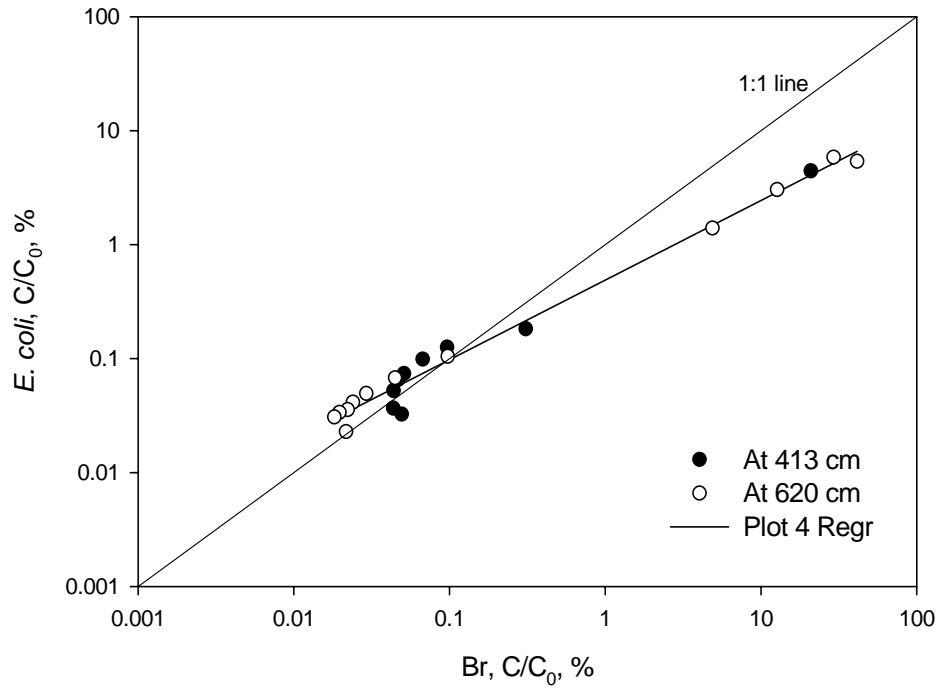


$$S_{413 \text{ cm}} = -0.60 + 1.07Br_{413 \text{ cm}} \quad R = 1.000^{***}$$

$$S_{620 \text{ cm}} = -0.61 + 1.01Br_{620 \text{ cm}} \quad R = 0.999^{***}$$

$$S_{\text{overall}} = -0.60 + 1.04Br_{\text{overall}} \quad R = 1.000^{***}$$

Figure 4.17. Regressions of *S. e. Typhimurium* concentration ratios ( $C/C_0$ ) to Bromide concentration ratios ( $C/C_0$ ) in runoff from Plot 2 (bare) for experiment under dry initial soil moisture conditions. \*\*\* Statistically significant at  $p = 0.01$ .

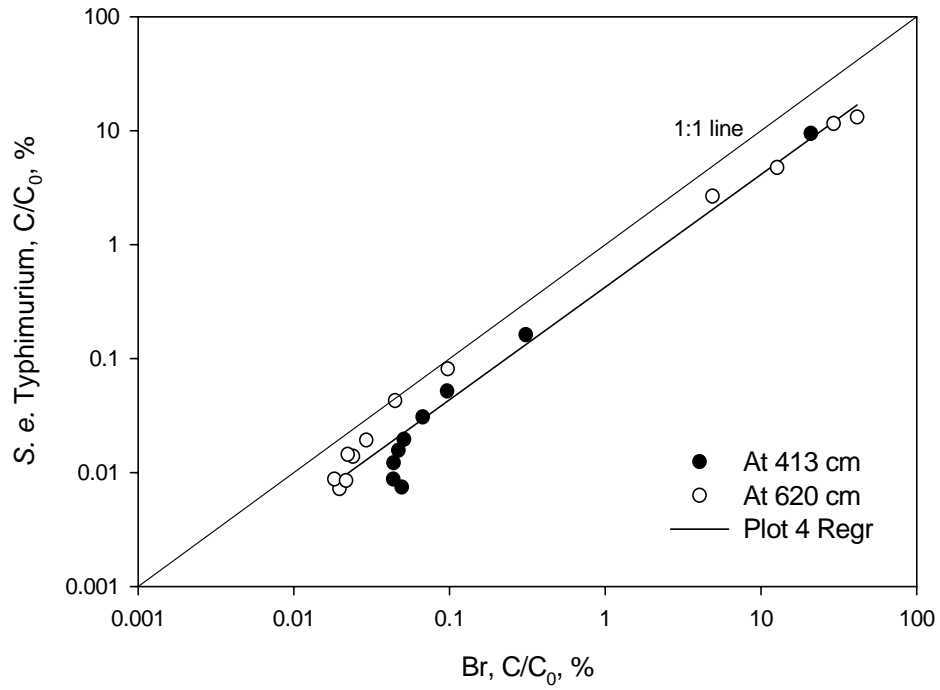


$$E_{413 \text{ cm}} = -0.30 + 0.72Br_{413 \text{ cm}} \quad R = 1.000^{***}$$

$$E_{620 \text{ cm}} = -0.31 + 0.69Br_{620 \text{ cm}} \quad R = 0.962^{***}$$

$$E_{\text{overall}} = -0.31 + 0.70Br_{\text{overall}} \quad R = 0.965^{***}$$

Figure 4.18. Regressions of *E. coli* concentration ratios ( $C/C_0$ ) to Bromide concentration ratios ( $C/C_0$ ) in runoff from Plot 4 (bare) for experiment under dry initial soil moisture conditions. \*\*\* Statistically significant at  $p = 0.01$ .

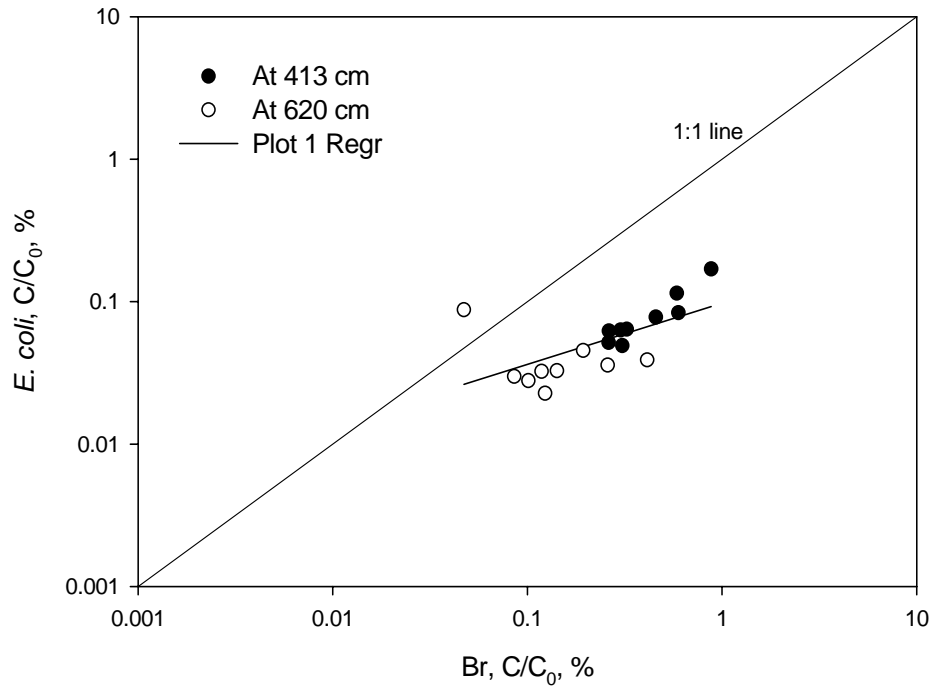


$$S_{413 \text{ cm}} = -0.38 + 1.09Br_{413 \text{ cm}} \quad R = 1.000^{***}$$

$$S_{620 \text{ cm}} = -0.32 + 0.95Br_{620 \text{ cm}} \quad R = 0.992^{***}$$

$$S_{\text{overall}} = -0.37 + 0.99Br_{\text{overall}} \quad R = 0.988^{***}$$

Figure 4.19. Regressions of *S. e. Typhimurium* concentration ratios ( $C/C_0$ ) to Bromide concentration ratios ( $C/C_0$ ) in runoff from Plot 4 (bare) for experiment under dry initial soil moisture conditions. \*\*\* Statistically significant at  $p = 0.01$ .

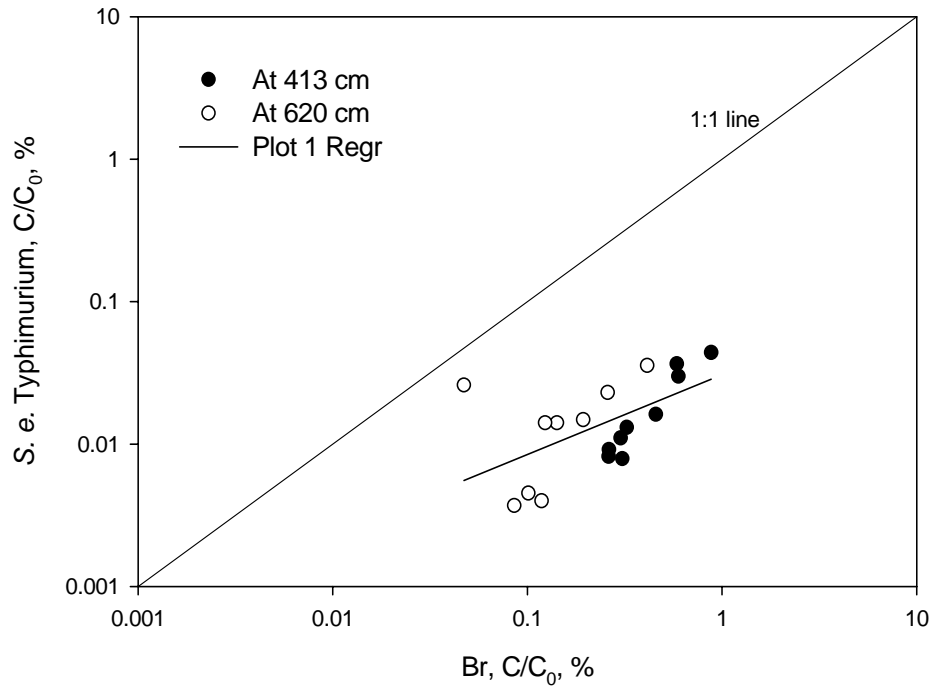


$$E_{413 \text{ cm}} = -0.78 + 0.86Br_{413 \text{ cm}} \quad R = 0.950^{***}$$

$$E_{620 \text{ cm}} = -1.59 - 0.17Br_{620 \text{ cm}} \quad R = -0.191$$

$$E_{\text{overall}} = -1.01 + 0.43Br_{\text{overall}} \quad R = 0.822^{***}$$

Figure 4.20. Regressions of *E. coli* concentration ratios ( $C/C_0$ ) to Bromide concentration ratios ( $C/C_0$ ) in runoff from Plot 1 (vegetated) for experiment under dry initial soil moisture conditions. \*\*\* Statistically significant at  $p = 0.01$ .



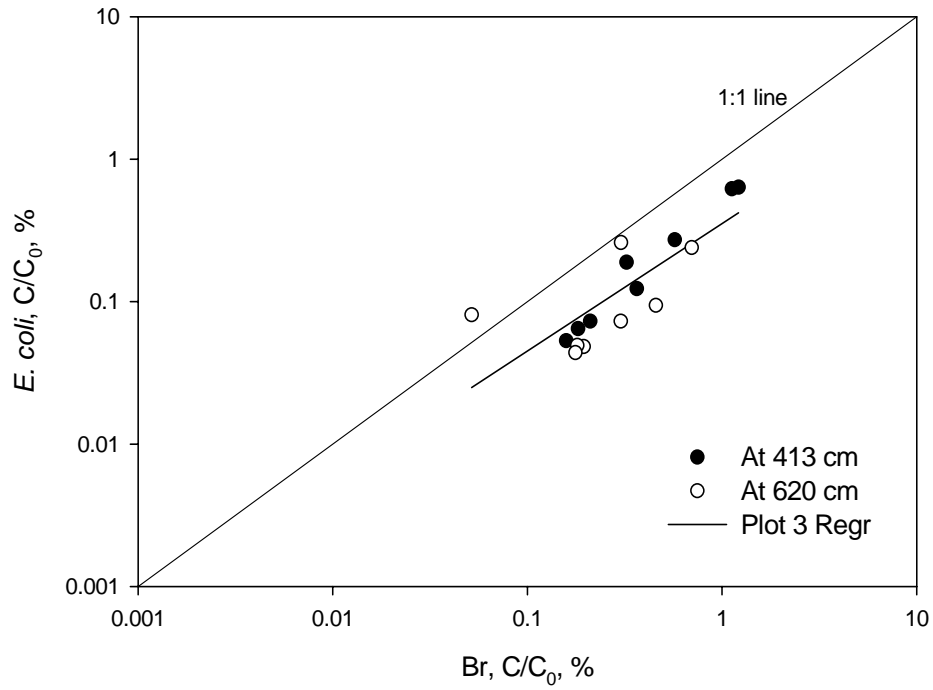
$$S_{413 \text{ cm}} = -1.21 + 1.48Br_{413 \text{ cm}} \quad R = 0.964^{***}$$

$$S_{620 \text{ cm}} = -1.43 + 0.58Br_{620 \text{ cm}} \quad R = 0.682^{**}$$

$$S_{\text{overall}} = -1.51 + 0.56Br_{\text{overall}} \quad R = 0.753^{***}$$

Figure 4.21. Regressions of *S. e. Typhimurium* concentration ratios ( $C/C_0$ ) to Bromide concentration ratios ( $C/C_0$ ) in runoff from Plot 1 (vegetated) for experiment under dry initial soil moisture conditions. \*\*\* Statistically significant at  $p = 0.01$ ; \*\* Statistically significant at  $p = 0.05$ .



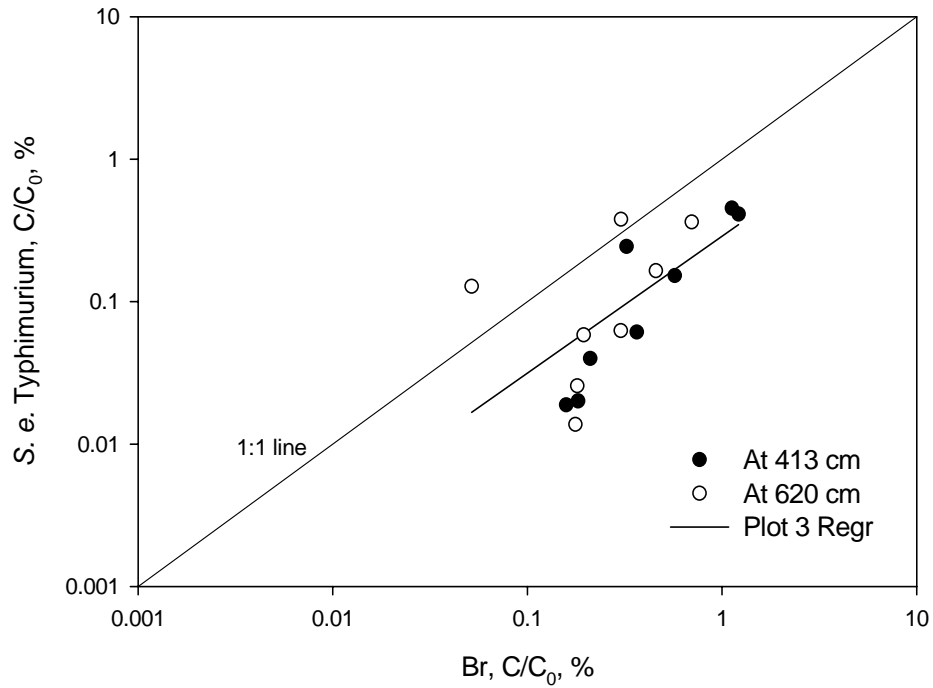


$$E_{413 \text{ cm}} = -0.28 + 1.22Br_{413 \text{ cm}} \quad R = 0.994^{***}$$

$$E_{620 \text{ cm}} = -0.76 + 0.46Br_{620 \text{ cm}} \quad R = 0.637^*$$

$$E_{\text{overall}} = -0.45 + 0.89Br_{\text{overall}} \quad R = 0.944^{***}$$

Figure 4.22. Regressions of *E. coli* concentration ratios ( $C/C_0$ ) to Bromide concentration ratios ( $C/C_0$ ) in runoff from Plot 3 (vegetated) for experiment under dry initial soil moisture conditions. \*\*\* Statistically significant at  $p = 0.01$ ; \* Statistically significant at  $p = 0.10$ .



$$S_{413 \text{ cm}} = -0.42 + 1.49Br_{413 \text{ cm}} \quad R = 0.929^{***}$$

$$S_{620 \text{ cm}} = -0.67 + 0.62Br_{620 \text{ cm}} \quad R = 0.653^*$$

$$S_{\text{overall}} = -0.54 + 0.96Br_{\text{overall}} \quad R = 0.803^{***}$$

Figure 4.23. Regressions of *S. e. Typhimurium* concentration ratios ( $C/C_0$ ) to Bromide concentration ratios ( $C/C_0$ ) in runoff from Plot 3 (vegetated) for experiment under dry initial soil moisture conditions. \*\*\* Statistically significant at  $p = 0.01$ ; \* Statistically significant at  $p = 0.10$ .

#### 4.3.2 Wet initial soil moisture conditions

Figures 4.24 and 4.25 show average relative concentrations of *Escherichia coli* and *Salmonella enterica* Typhimurium, respectively, measured with time in surface runoff collected at 413 cm downslope from the source of slurry application in all plots. Figures 4.26 and 4.27 show average relative concentrations of *E. coli* and *S. e.* Typhimurium, respectively, measured with time in surface runoff collected at 620 cm downslope from the source of slurry application in all plots.

In all plots, relative concentrations of both pathogens in runoff decreased as the distance from the source of slurry increased, particularly in the vegetated plots. This decrease in relative concentrations with respect to distance was caused by dilution (Roodsari, 2004; Roodsari et al., 2005), which increased as runoff volumes also increased at distances farther away from the source of slurry.

Note that there was substantial variability in pathogen concentrations and in runoff volumes observed at individual funnels (at 413 cm), especially in the vegetated plots (Appendix E), due to the heterogeneity in surface flow pathways arising from non uniform or rough surfaces (Roodsari, 2004; Roodsari et al., 2005). For example, no runoff was observed in funnel 2 in the vegetated Plot 1, as it is indicated in Figure 3.3 (Section 3.1.5), until 8 min into the simulation, indicating that this funnel was located on a more elevated area of the plot.

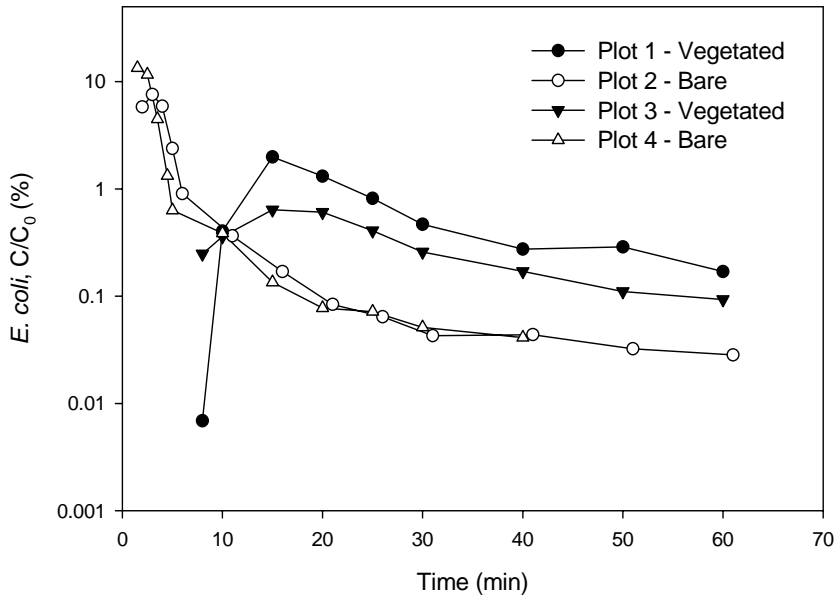


Figure 4.24. Average relative concentrations of *E. coli* measured with time in runoff collected at 413 cm downslope from the source of slurry application in all plots under wet initial soil moisture conditions. *E. coli* was not detected in first runoff samples ( $t = 6$  min) from the vegetated plots.

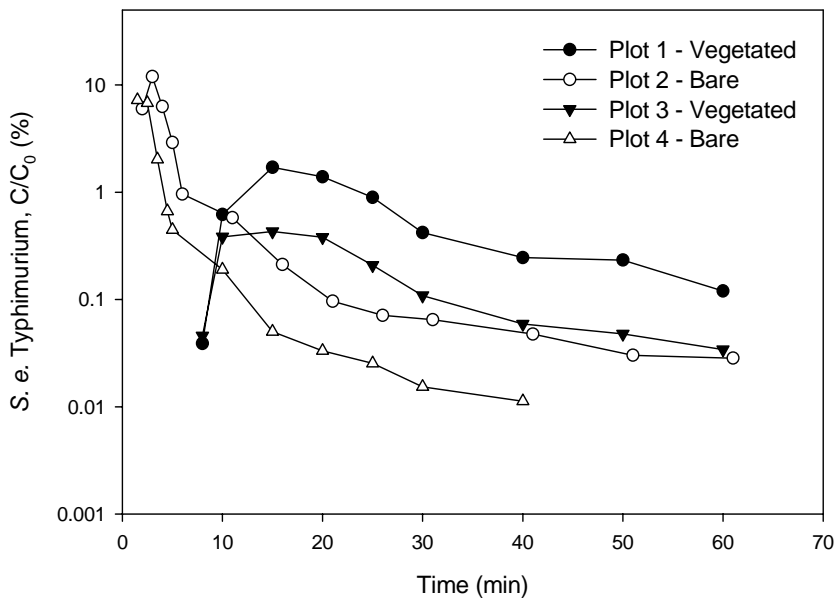


Figure 4.25. Average relative concentrations of *S. e. Typhimurium* measured with time in runoff collected at 413 cm downslope from the source of slurry application in all plots under wet initial soil moisture conditions. *S. e. Typhimurium* was not detected in first runoff samples ( $t = 6$  min) from the vegetated plots.

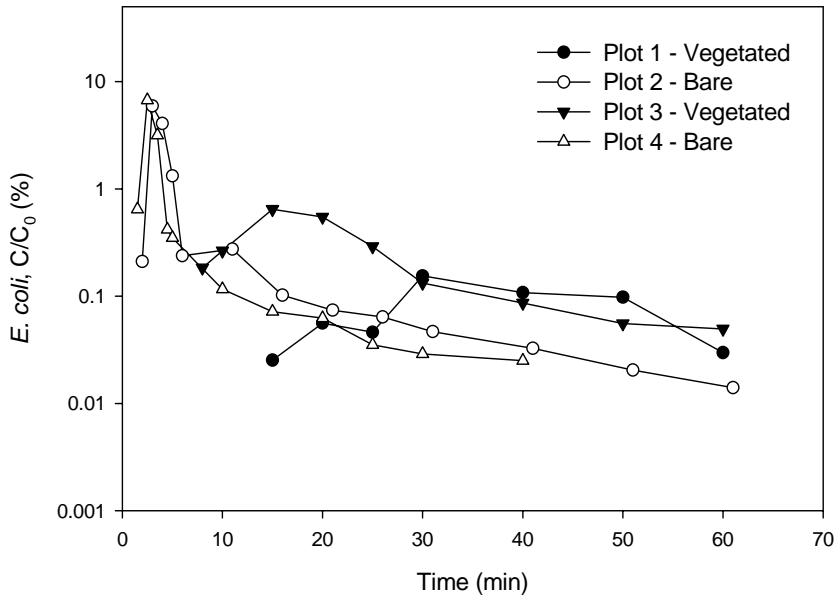


Figure 4.26. Average relative concentrations of *E. coli* measured with time in runoff collected at 620 cm downslope from the source of slurry application in all plots under wet initial soil moisture conditions. *E. coli* was not detected in the first three runoff samples ( $t = 6$ ,  $t = 8$ , and  $t = 10$  min) from Plot 1, and in the first runoff sample ( $t = 6$  min) from Plot 3.

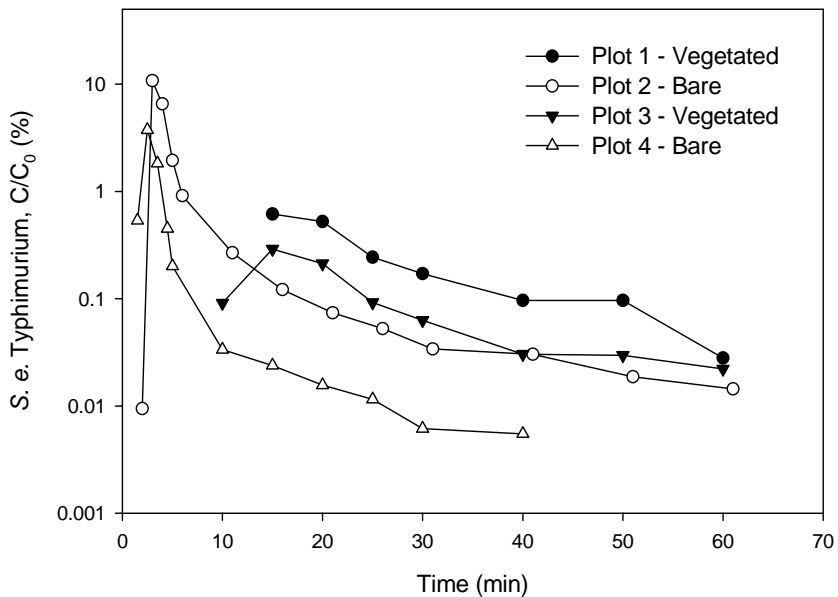


Figure 4.27. Average relative concentrations of *S. e. Typhimurium* measured with time in runoff collected at 620 cm downslope from the source of slurry application in all plots under wet initial soil moisture conditions. *S. e. Typhimurium* was not detected in the first three runoff samples ( $t = 6$ ,  $t = 8$ , and  $t = 10$  min) from Plot 1, and in the first runoff sample ( $t = 6$  min) from Plot 3.

In addition, as explained in Figures 4.24 through 4.27, no pathogens were detected in several early runoff samples collected from the vegetated plots (Plots 1 and 3) at both distances from the source of slurry, indicating that early runoff was only locally generated and did not yet contain the slurry. This trend was more pronounced in funnel 1 in the vegetated Plot 3, as it is indicated in Figure 3.3 (Section 3.1.5), where no pathogens were ever detected throughout the simulation, indicating that this funnel collected only locally generated runoff as opposed to runoff originating from the site of manure application.

As Figures 4.24 and 4.26 indicate, relative concentrations of *E. coli* in each treatment at both locations from the slurry were very similar, except at 620 cm (Figure 4.26) in which relative concentrations in Plot 3 were substantially higher than in Plot 1 during the first 30 min of simulations despite the fact that runoff rates in these plots were very similar during the simulations (Figure 4.2).

As Figures 4.25 and 4.27 indicate, relative concentrations of *S. e. Typhimurium* in bare plots at both locations from the slurry were higher in Plot 2 than in Plot 4 despite the fact that runoff rates in both of these plots were very similar during the simulations (Figure 4.2). This was also observed in the vegetated plots in which relative concentrations of *S. e. Typhimurium* at both locations from the slurry were higher in Plot 1 than in Plot 3 despite the fact that runoff rates in both of these plots were very similar during the simulations (Figure 4.2).

As Figures 4.24 to 4.27 indicate, relative concentrations of both pathogens in runoff decreased with respect to time at both distances from the source of slurry, especially in the bare plots. In the bare plots, there was a rapid decrease in relative

concentrations within the first 15 min of simulations, while in the vegetated plots relative concentrations typically increased to a peak several minutes after the initiation of runoff before decreasing. This trend was even more pronounced for *E. coli* measured at 620 cm in the vegetated Plot 1 in which relative concentrations were observed to increase for the first 30 min of simulation before reaching a peak and start to decrease.

Similar to what was observed in the simulations under dry conditions (Section 4.3.1), relative concentrations in runoff from bare plots were initially substantially higher than from vegetated plots; however, after approximately 15 min of simulations, relative concentrations observed in runoff from vegetated plots were comparable or higher than in bare plots. This may be attributed to the fact that most of the pathogens leave bare plots rapidly during initial portion of simulation, but movement of pathogens in vegetated filters is slow.

Figures 4.28 and 4.29 illustrate the differences in runoff rates between the bare and vegetated plots. Data points situated below the 1:1 line represent samples collected during the first 15 min of simulations. Both figures show that both pathogens reached both locations in the bare plots earlier than in the vegetated plots, and in higher relative concentrations.

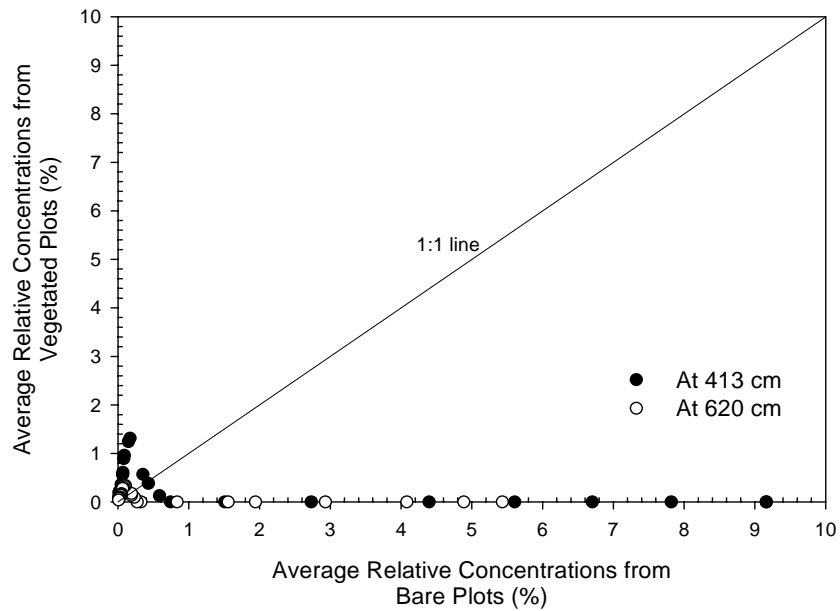


Figure 4.28. Average relative concentrations of *E. coli* measured in runoff at 413 and 620 cm from the source of slurry in vegetated vs. bare plots at identical time intervals during the experiments under wet initial soil moisture conditions.

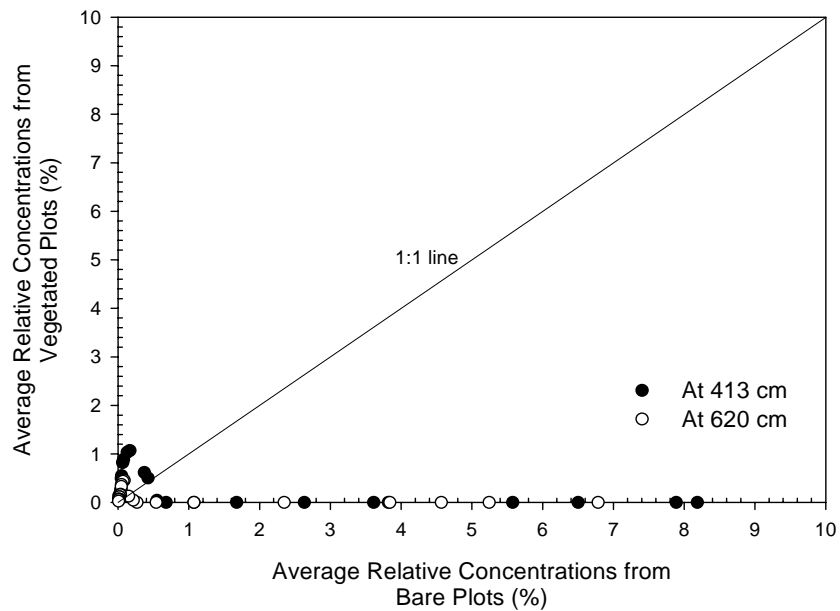


Figure 4.29. Average relative concentrations of *S. e. Typhimurium* measured in runoff at 413 and 620 cm from the source of slurry in vegetated vs. bare plots at identical time intervals during the experiments under wet initial soil moisture conditions.



Similar to what was observed in Section 4.3.1, after the first 15 min of simulations, an exponential decrease in relative pathogen concentrations was observed in both the vegetated and bare plots. This allowed for a comparison of pathogen reduction rates between bare and vegetated plots during this exponential phase. The changes in relative concentrations after 15 min of simulations were modeled as a single exponential with constant background according to Equation 3 (Section 4.3.1), which was as follow:

$$C/C_0 = [(C/C_{0(15)}) - b]e^{-k*(t-15)} + b \quad (3)$$

where,

$C/C_0$ : percent (%) relative concentration at time  $t$  (in minutes)

$C/C_{0(15)}$ : percent (%) relative concentration at  $t = 15$  min

$b$ : the background concentration (in percent) that does not change with time

$k$ : the time constant for the decrease or the “rate” of reduction with time

$t$ : point in time (in minutes) during the experiments

Table 4.8 shows the equations describing the exponential decrease in relative concentration after 15 min of simulation in each plot. High  $R^2$  values ( $> 0.98$ ) indicate a strong correlation between relative concentrations of both pathogens with time at different distances from the slurry application area, as represented by the exponential functions.

Table 4.8. Equations relating relative concentrations ( $C/C_0$ , %) of each pathogen in runoff to time  $t$  after 15 min of simulation at each distance in each plot under wet initial soil moisture conditions.

<i>E. coli</i>			
PLOT	Distance, cm	Equation, $C/C_0$ , %	$R^2$
Plot 1 (Vegetated)	413	$1.84e^{-0.11(t-15)} + 0.173$	0.998
	620 <sup>†</sup>	-	-
Plot 3 (Vegetated)	413	$0.66e^{-0.06(t-15)} + 0.017$	0.989
	620	$0.67e^{-0.09(t-15)} + 0.014$	0.981
Plot 2 (Bare)	413	$0.16e^{-0.18(t-15)} + 0.035$	0.996
	620	$0.10e^{-0.05(t-15)} + 0.007$	0.999
Plot 4 (Bare)	413	$0.09e^{-0.15(t-15)} + 0.042$	0.995
	620	$0.06e^{-0.09(t-15)} + 0.015$	0.999
<i>S. e. Typhimurium</i>			
PLOT	Distance, cm	Equation, $C/C_0$ , %	$R^2$
Plot 1 (Vegetated)	413	$1.73e^{-0.08(t-15)} + 0.052$	0.988
	620	$0.62e^{-0.08(t-15)} + 0.027$	0.982
Plot 3 (Vegetated)	413	$0.45e^{-0.08(t-15)} + 0.008$	0.981
	620	$0.28e^{-0.11(t-15)} + 0.016$	0.989
Plot 2 (Bare)	413	$0.20e^{-0.18(t-15)} + 0.038$	0.991
	620	$0.12e^{-0.11(t-15)} + 0.017$	0.997
Plot 4 (Bare)	413	$0.04e^{-0.09(t-15)} + 0.007$	0.998
	620	$0.02e^{-0.11(t-15)} + 0.004$	0.997

<sup>†</sup>An exponential decrease in relative concentrations of *E. coli* was not observed at 620 cm in Plot 1 after 15 min of simulation.

An ANOVA for fixed effects was performed to test whether or not distance (413 cm or 620 cm) from the source of slurry or cover condition (bare or vegetated) had any significant effect on the parameters  $k$ ,  $b$ , and  $C/C_{0(15)}$  of Equation 3. The statistical results, in the form of ANOVA tables, are presented in Tables 4.9 through 4.14.

The ANOVA tables showing the effect of distance and cover on the observed reduction rates ( $k$ ) of *E. coli* and *S. e. Typhimurium* are shown, respectively, in Tables 4.9 and 4.10. Data show that the rates ( $k$ ) governing the reduction in relative concentrations of both pathogens in runoff after 15 min of simulations were not affected by distance ( $p = 0.19$  for *E. coli* rates; and  $p = 0.86$  for *S. e. Typhimurium* rates) or by cover condition ( $p = 0.23$  for *E. coli* rates; and  $p = 0.17$  for *S. e. Typhimurium* rates).

Table 4.9. ANOVA table for the effect of distance and cover (at P value set at 0.05) on the observed *E. coli* rates of reduction ( $k$ ) obtained after 15 min of simulation under wet initial soil moisture conditions.

Source of Variation	Df <sup>†</sup>	Sum of Squares	Mean Square	F Value	Pr (F)
Distance	1	0.003676220	0.003676220	2.460191	0.1918415
Cover	1	0.002975104	0.002975104	1.990992	0.2310691
Residuals	4	0.005977130	0.001494282		

<sup>†</sup>Df = degrees of freedom

Table 4.10. ANOVA table for the effect of distance and cover (at P value set at 0.05) on the observed *S. e. Typhimurium* rates of reduction ( $k$ ) obtained after 15 min of simulations under wet initial soil moisture conditions.

Source of Variation	Df <sup>†</sup>	Sum of Squares	Mean Square	F Value	Pr (F)
Distance	1	0.000032401	0.000032401	0.033458	0.8620500
Cover	1	0.002481601	0.002481601	2.562544	0.1703208
Residuals	5	0.004842066	0.000968413		

<sup>†</sup>Df = degrees of freedom

Based on the study by Stout et al. (2005), it was not expected that  $k$  would be affected between the two locations from the slurry since a short distance separated them from each other. In the above study, although they did not investigate the effect of cover, they found that the rate of fecal coliform (FC) transport in runoff through vegetated filter strips was not affected by distance and the rates were similar at two locations (1 m apart from each other) from the source of dairy manure. However, if  $k$  observed after 15 min of simulations was to be affected by cover condition, this study could not verify such effect and further studies would have been necessary.

The ANOVA tables showing the effect of distance and cover on the observed background levels ( $b$ ) of *E. coli* and *S. e. Typhimurium* are shown, respectively, in Tables 4.11 and 4.12. Results indicate that background levels ( $b$ ) of both pathogens were not affected by distance ( $p = 0.28$  for *E. coli* background levels; and  $p = 0.46$  for *S. e. Typhimurium* background levels). Although Figures 4.23 to 4.26 suggest that  $b$  levels were higher in the vegetated plots than in the bare plots, particularly based on levels observed in Plot 1, Tables 4.11 and 4.12 indicate that  $b$  was not affected by cover condition ( $p = 0.47$  for *E. coli* levels; and  $p = 0.49$  for *S. e. Typhimurium* levels).

Table 4.11. ANOVA table for the effect of distance and cover (at P value set at 0.05) on the observed *E. coli* background levels (*b*) obtained after 15 min of simulations under wet initial soil moisture conditions.

Source of Variation	Df <sup>†</sup>	Sum of Squares	Mean Square	F Value	Pr (F)
Distance	1	0.00508096	0.00508096	1.522149	0.2848315
Cover	1	0.00211108	0.00211108	0.632435	0.4709862
Residuals	4	0.01335207	0.003338018		

<sup>†</sup>Df = degrees of freedom

Table 4.12. ANOVA table for the effect of distance and cover (at P value set at 0.05) on the observed *S. e. Typhimurium* background levels (*b*) obtained after 15 min of simulations under wet initial soil moisture conditions.

Source of Variation	Df <sup>†</sup>	Sum of Squares	Mean Square	F Value	Pr (F)
Distance	1	0.000205031	0.000205031	0.6278301	0.4640700
Cover	1	0.000181451	0.000181451	0.5556253	0.4895638
Residuals	5	0.001632856	0.0003265712		

<sup>†</sup>Df = degrees of freedom

The ANOVA tables showing the effect of distance and cover on observed relative concentrations of *E. coli* and *S. e. Typhimurium* at 15 min of simulations ( $C/C_{0(15)}$ ) are shown, respectively, in Tables 4.13 and 4.14. Results indicate that relative concentrations of both pathogens at 15 min of simulation ( $C/C_{0(15)}$ ) were not affected by distance ( $p = 0.29$  for *E. coli* relative concentrations; and  $p = 0.34$  for *S. e. Typhimurium* relative concentrations). Although Figures 4.23 to 4.26 suggest that  $C/C_{0(15)}$  were higher in the vegetated plots than in the bare plots, particular to what was observed at 413 cm, Tables 4.13 and 4.14 indicate that  $C/C_{0(15)}$  were not affected by cover condition ( $p = 0.07$  for *E. coli* relative concentrations; and  $p = 0.10$  for *S. e. Typhimurium* relative concentrations).

Table 4.13. ANOVA table for the effect of distance and cover (at P value set at 0.05) on relative concentrations of *E. coli* observed at 15 min of simulations ( $C/C_{0(15)}$ ) in the experiments under wet initial soil moisture conditions.

Source of Variation	Df <sup>†</sup>	Sum of Squares	Mean Square	F Value	Pr (F)
Distance	1	0.371595	0.371595	1.457397	0.2938486
Cover	1	1.497262	1.497262	5.872274	0.0725057
Residuals	4	1.019886	0.254971		

<sup>†</sup>Df = degrees of freedom

Table 4.14. ANOVA table for the effect of distance and cover (at P value set at 0.05) on relative concentrations of *S. e. Typhimurium* observed at 15 min of simulations ( $C/C_{0(15)}$ ) in the experiments under wet initial soil moisture conditions.

Source of Variation	Df <sup>†</sup>	Sum of Squares	Mean Square	F Value	Pr (F)
Distance	1	0.256099	0.256099	1.129364	0.3365205
Cover	1	0.940481	0.940481	4.147403	0.0972996
Residuals	5	1.133819	0.2267639		

<sup>†</sup>Df = degrees of freedom

In addition to statistical analyses using ANOVA for fixed effects, standard Two-Sample t-Tests were performed on the mean reduction rates ( $k$ ) of both pathogens to test whether or not the means were significantly different between the two treatments (bare and vegetated). The standard Two-Sample t-Test analysis indicated that the mean *E. coli* rate of reduction observed in the bare plots ( $0.12 \text{ min}^{-1}$ ) was not significantly different from the mean rate observed in the vegetated plots ( $0.08 \text{ min}^{-1}$ ) ( $p = 0.39$ ), regardless of distance away from the source of slurry. The same analysis indicated that the mean *S. e. Typhimurium* rate of reduction observed in the bare plots ( $0.12 \text{ min}^{-1}$ ) was not significantly different from the mean rate observed in the vegetated plots ( $0.09 \text{ min}^{-1}$ ) ( $p = 0.13$ ), regardless of distance away from the source of slurry.

This study could not verify that cover condition had a significant effect on the parameters  $k$ ,  $b$ , and  $C/C_{0(15)}$  describing the exponential decrease in relative concentrations of both pathogens in surface runoff observed under wet initial soil moisture conditions. The same above statistical results were also obtained under dry conditions (Section 4.3.1), except for relative concentrations of *E. coli* observed at 15 min of simulation ( $C/C_{0(15)}$ ), which were found to be affected by cover condition and were significantly higher in the vegetated plots than in the bare plots when subjected to dry conditions.

As previously discussed, the inability to detect significant effects and/or significant differences between treatments, particularly on parameters  $b$  and  $C/C_{0(15)}$ , however, could be due to the limitations of the experimental design. The lysimeter site where simulations were conducted could accommodate only four plots that were reasonable in size for inclusion of spatial heterogeneity; thereby severely limiting the number of replications per treatment (two vegetated and two bare plots). One should note, however, that decreasing the size of experimental plots in order to obtain statistical replications often hinders the area scale of experiments, thus limiting inclusion of spatial heterogeneity effect in the observations. In addition, as previously described, the two vegetated plots differed in three important characteristics: slope, soil texture at 20 cm depth, and topography. This contributed to large within-treatment variability, hence diminishing the likelihood of detecting statistically significant differences.

#### 4.3.2.1 Relationship between relative concentrations of *Escherichia coli* and *Salmonella enterica* Typhimurium in surface runoff

Linear regression analysis was performed between relative concentrations of *E. coli* vs. *S. e.* Typhimurium in surface runoff for each plot at each location.

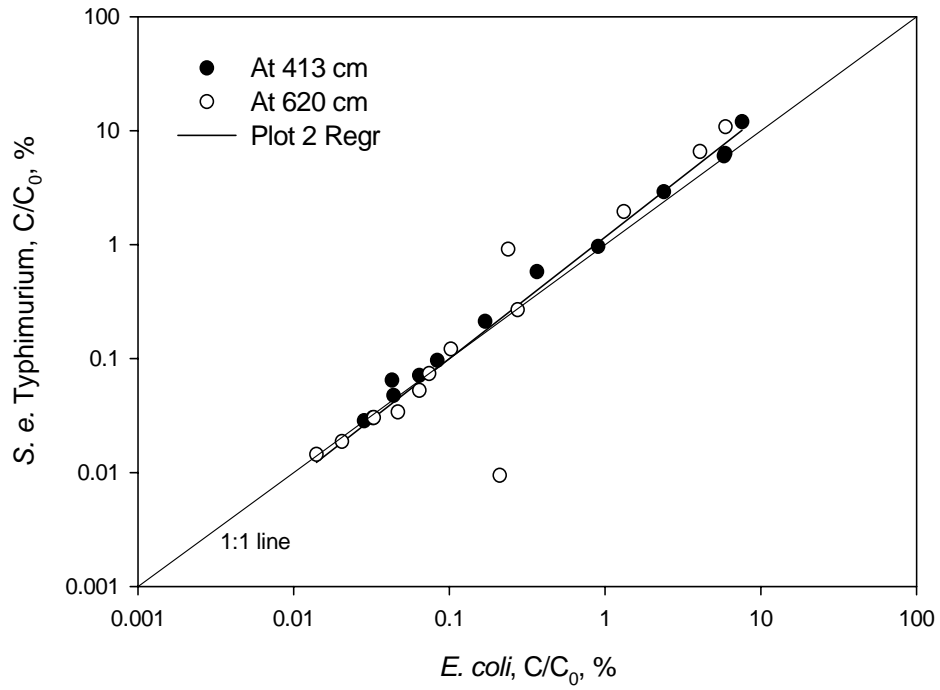
Correlation coefficient values (R) were determined based on Pearson correlation analysis since it is appropriate for variables that are continuous and can be analyzed statistically at various levels of significance (p). In this study, the R values were analyzed against critical values representing three levels of p: 0.10, 0.05, and 0.01.

Strong correlations were observed between relative concentrations of *E. coli* vs. *S. e.* Typhimurium in runoff from the bare plots (Figures 4.30 and 4.31) at both distances from the manure source (p = 0.01), indicating that both pathogens were transported similarly on a bare surface. These results are consistent with the ones obtained under dry initial soil moisture conditions (Section 4.3.1.1), indicating that initial groundwater table levels may not affect the transport pattern between these pathogens on bare surfaces.

A strong correlation was also observed between relative concentrations of *E. coli* vs. *S. e.* Typhimurium in runoff from both vegetated plots (Figures 4.32 and 4.33) at 413 cm (p = 0.01). Similar strong correlation was also observed between relative concentrations of *E. coli* vs. *S. e.* Typhimurium at 620 cm in Plot 3 (p = 0.01); however, such a strong correlation was not observed at 620 cm in Plot 1 (p > 0.10). These results are consistent with the ones obtained under dry initial soil moisture conditions (Section 4.3.1.1), indicating that initial groundwater table levels may not affect the transport pattern between these pathogens on vegetated surfaces.



As previously discussed, it is unclear why the transport of *E. coli* vs. *S. e.* Typhimurium in Plot 1 was not comparable at 620 cm. The pathogens are essentially identical with respect to size, shape, and density. Note, however, that there can be differences among these genera (and between strains within the genera), with respect to the presence of surface appendages (e.g., fimbria, pili) (Ofek et al., 2003).

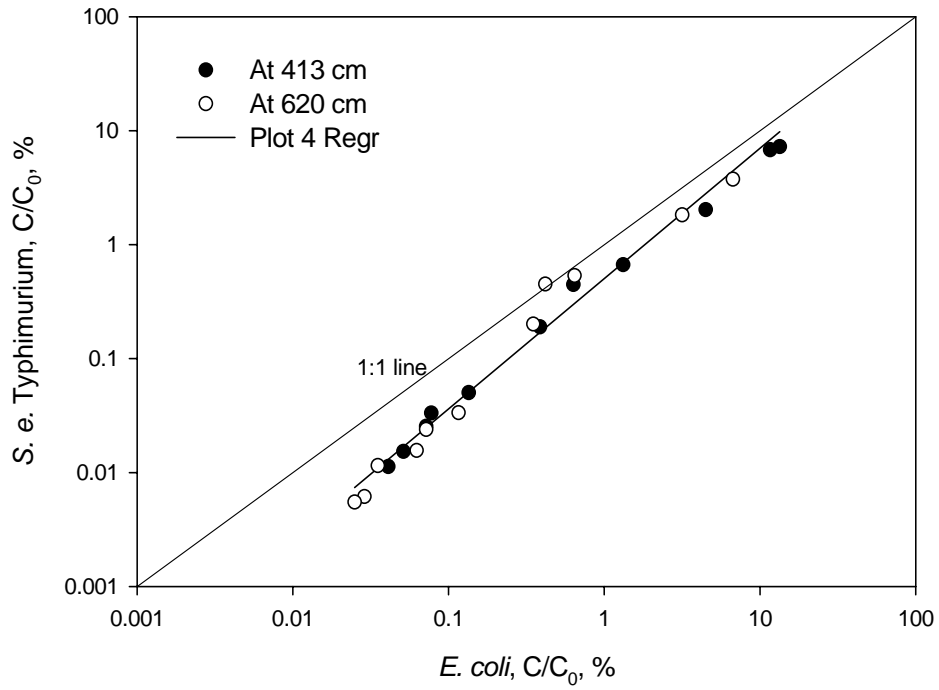


$$S_{413 \text{ cm}} = 0.08 + 1.02E_{413 \text{ cm}} \quad R = 0.973^{***}$$

$$S_{620 \text{ cm}} = 0.06 + 1.11E_{620 \text{ cm}} \quad R = 0.996^{***}$$

$$S_{\text{overall}} = 0.06 + 1.07E_{\text{overall}} \quad R = 0.970^{***}$$

Figure 4.30. Regressions of *S. e. Typhimurium* concentration ratios ( $C/C_0$ ) to *E. coli* concentration ratios ( $C/C_0$ ) in runoff from Plot 2 (bare) for experiment under wet initial soil moisture conditions. \*\*\* Statistically significant at  $p = 0.01$ .

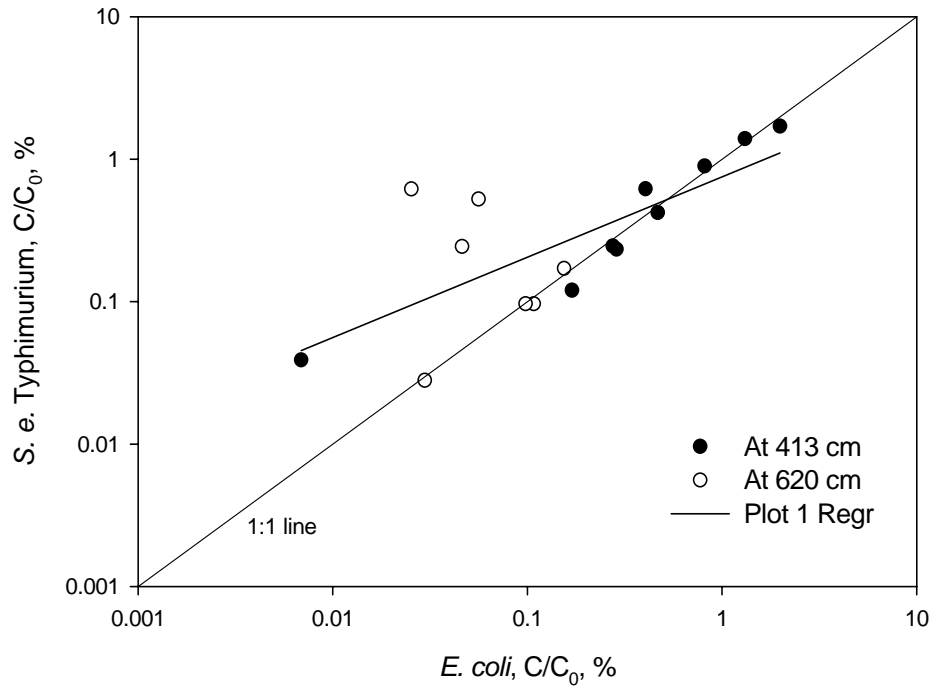


$$S_{413 \text{ cm}} = -0.33 + 1.10E_{413 \text{ cm}} \quad R = 0.998^{***}$$

$$S_{620 \text{ cm}} = -0.24 + 1.21E_{620 \text{ cm}} \quad R = 0.997^{***}$$

$$S_{\text{overall}} = -0.30 + 1.14E_{\text{overall}} \quad R = 0.997^{***}$$

Figure 4.31. Regressions of *S. e. Typhimurium* concentration ratios ( $C/C_0$ ) to *E. coli* concentration ratios ( $C/C_0$ ) in runoff from Plot 4 (bare) for experiment under wet initial soil moisture conditions. \*\*\* Statistically significant at  $p = 0.01$ .

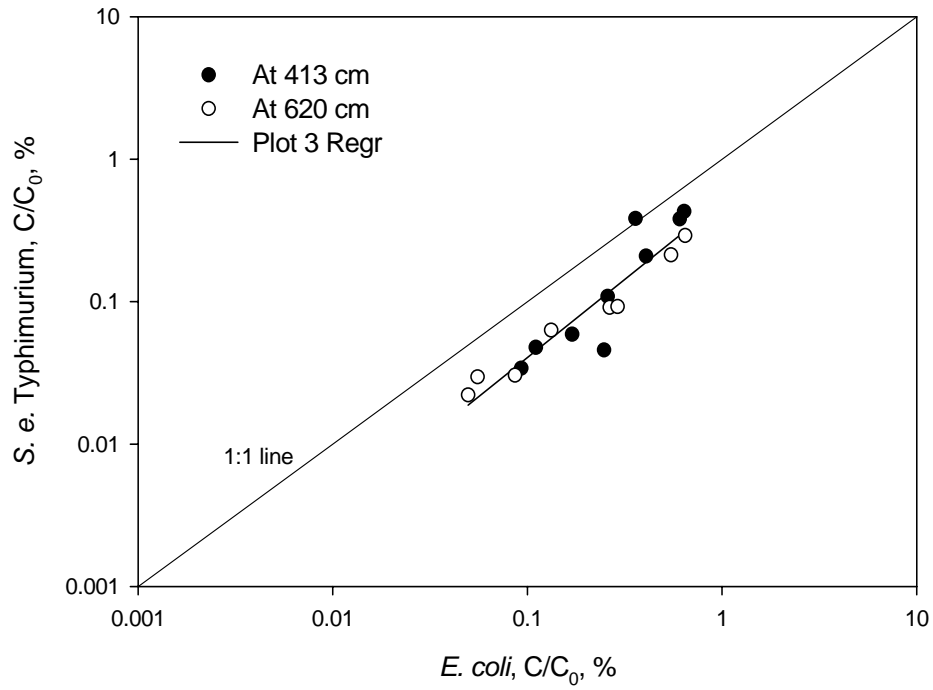


$$S_{413 \text{ cm}} = -0.08 + 0.70E_{413 \text{ cm}} \quad R = 0.981^{***}$$

$$S_{620 \text{ cm}} = -1.05 - 0.22E_{620 \text{ cm}} \quad R = 0.108$$

$$S_{\text{overall}} = -0.13 + 0.56E_{\text{overall}} \quad R = 0.929^{***}$$

Figure 4.32. Regressions of *S. e. Typhimurium* concentration ratios ( $C/C_0$ ) to *E. coli* concentration ratios ( $C/C_0$ ) in runoff from Plot 1 (vegetated) for experiment under wet initial soil moisture conditions. \*\*\* Statistically significant at  $p = 0.01$ .



$$S_{413 \text{ cm}} = -0.13 + 1.36E_{413 \text{ cm}} \quad R = 0.914^{***}$$

$$S_{620 \text{ cm}} = -0.44 + 0.93E_{620 \text{ cm}} \quad R = 0.988^{***}$$

$$S_{\text{overall}} = -0.30 + 1.10E_{\text{overall}} \quad R = 0.895^{***}$$

Figure 4.33. Regressions of *S. e. Typhimurium* concentration ratios ( $C/C_0$ ) to *E. coli* concentration ratios ( $C/C_0$ ) in runoff from Plot 3 (vegetated) for experiment under wet initial soil moisture conditions. \*\*\* Statistically significant at  $p = 0.01$ .

#### 4.3.2.2 Relationship between relative concentrations of each surrogate pathogen and Bromide in surface runoff

Linear regression analysis was performed between relative concentrations of each pathogen vs. Bromide (Br) in surface runoff for each plot at each location. Correlation coefficient values (R) were determined based on Pearson correlation analysis, since it is appropriate for variables that are continuous and can be analyzed statistically at various levels of significance (p). In this study, the R values were analyzed against critical values representing three levels of p: 0.10, 0.05, and 0.01.

Strong correlations were observed between the relative concentrations of each pathogen vs. Br in runoff from the bare plots (Figures 4.34 to 4.37) at both distances from the source of slurry ( $p = 0.01$ ). These results are consistent with the ones obtained under dry initial soil moisture conditions (Section 4.3.1.2), indicating that initial groundwater table levels may not affect the transport pattern between each of these pathogens and Br on bare surfaces.

As previously discussed (Section 4.3.1.2), since Br is inert and does not sorb to organic matter or soil particles (Walton et al., 2000), these results indicate that the bare plots offered little resistance to the transport of either pathogen. In addition, these results indicate that pathogens were either predominantly in the liquid phase of the swine slurry, or that the manure solids (including pathogens) consisted of suspended colloids, which were all transported at similar rates. This is consistent with previous studies indicating that bacterial cells attached to particles during runoff can be very low (Davies and Bavor, 2000; Borst and Selvakumar, 2003; Muirhead et al., 2005). These results, however, are very different from those reported for bovine manure (Roodsari, 2004). In contrast to swine slurry (4% solids), bovine manure

typically contains a higher solid content (10%), consisting of substantial amounts of residual dietary fiber. Consequently, a fraction of the bacteria must first “release” from the manure solids before they can be transported (Guber et al., 2006).

Figures 4.34 through 4.37 show that, in general, relative concentrations of Br in surface runoff from bare plots were higher than for pathogens (data points appear mostly below the 1:1 line). Note that only pathogens in the aqueous phase of runoff were measured; pathogens attached to soil particles in runoff were not measured. These results then suggest that some fraction of pathogens were attached to sediment in runoff, resulting in higher relative Br concentrations.

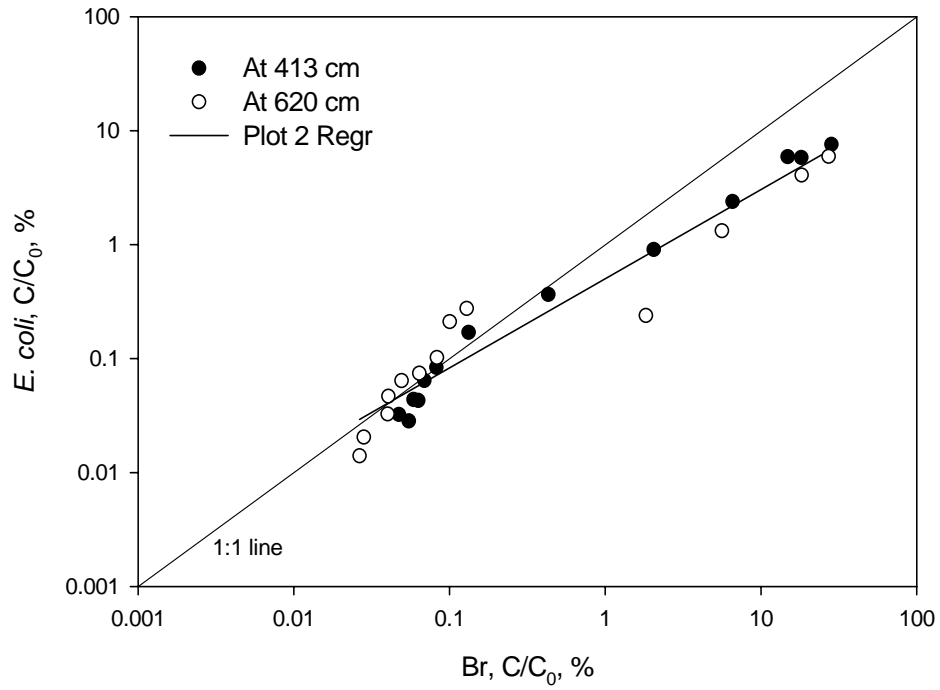
Strong correlations were observed between the relative concentrations of each pathogen and Br in runoff from the vegetated plots (Figures 4.38 to 4.41) at 413 cm ( $p = 0.01$ ). However, and similar to what was observed under dry conditions, correlations observed at 620 cm differed between the two pathogens and between the two vegetated plots. In Plot 1, a strong correlation between the relative concentrations of *S. e. Typhimurium* and Br was observed ( $p = 0.01$ ), but not between *E. coli* and Br ( $p > 0.10$ ). In Plot 3, the relative concentrations of each pathogen and Br were strongly correlated ( $p = 0.01$ ). Figures 4.38 through 4.41 also show that the relative concentrations of Br in surface runoff were consistently higher than for pathogens (data points appear mostly below the 1:1 line). Collectively, these results indicate that the mechanism by which vegetated surfaces affect pathogen transport are different than for Br.

Note that runoff did not initiate in the vegetated plots until 3-4 minutes after the start of rainfall simulations and that low infiltration was subsequently observed in

these plots (Figure 4.2). High cumulative recoveries of Br from the vegetated plots (see Table 4.17) are consistent with low infiltration. However, after the initiation of runoff, substantially higher relative Br concentrations were observed in runoff from vegetated plots than relative pathogen concentrations. Since it is unlikely that infiltration rates for pathogens were higher than for Br, these data suggest that pathogens were selectively retained in the vegetated plots, presumably as a result of sorption to plant litter/organic matter.

The primary difference between wet and dry initial soil moisture conditions was in vegetated plots where some correlations observed at 620 cm became stronger under wet conditions. This was particularly pronounced in Plot 3 where pathogen vs. Br correlations ( $p = 0.10$ ) observed under dry conditions (Figures 4.22 and 4.23) became stronger ( $p = 0.01$ ) under wet conditions (Figures 4.40 and 4.41). In Plot 1, this was observed only between *S. e. Typhimurium* and Br where a weak correlation ( $p = 0.05$ ) observed under dry conditions (Figure 4.21) became stronger ( $p = 0.01$ ) under wet conditions (Figure 4.39); but not between *E. coli* and Br where a significant correlation was never observed at 620 cm ( $p > 0.10$ ). These results suggest that the increase in runoff volumes under wet conditions (Figure 4.2) may have offset some retention mechanisms in the vegetated plots, but such process had less of an effect for *E. coli* in Plot 1. Therefore, the primary retention mechanism in Plot 1 may have been adsorption by litter and/or vegetation, whereas in Plot 3 it may have been infiltration. Another possibility is that external sources of *E. coli*, such as from wildlife fecal material (birds, foxes), were more present on Plot 1 and that caused relative concentrations of *E. coli* to oscillate, and, thus, correlations with Br to be lost.



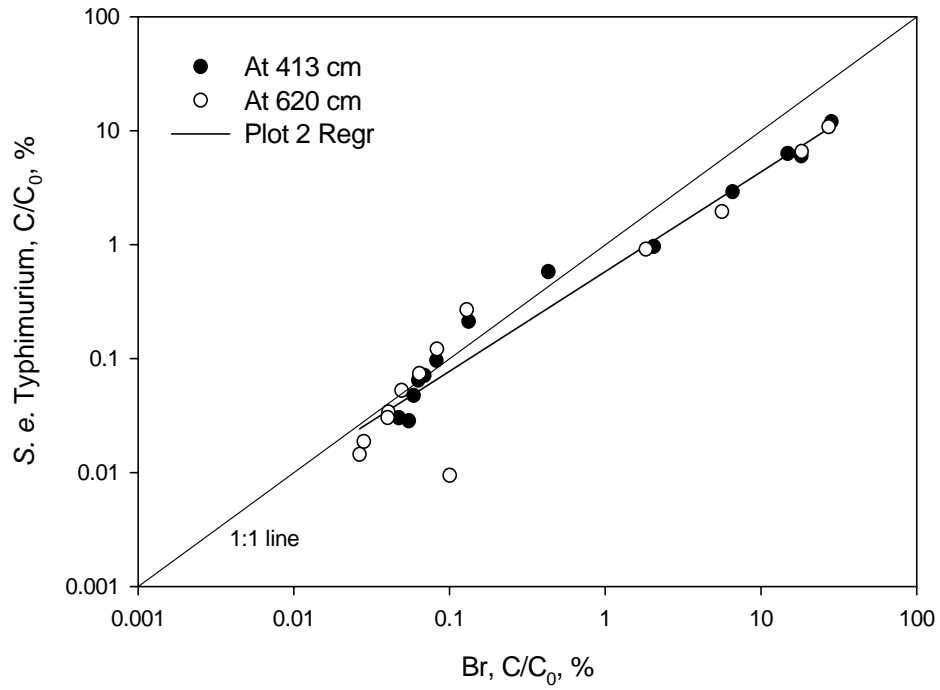


$$E_{413 \text{ cm}} = -0.27 + 0.84Br_{413 \text{ cm}} \quad R = 0.983^{***}$$

$$E_{620 \text{ cm}} = -0.35 + 0.72Br_{620 \text{ cm}} \quad R = 0.999^{***}$$

$$E_{\text{overall}} = -0.30 + 0.78Br_{\text{overall}} \quad R = 0.972^{***}$$

Figure 4.34. Regressions of *E. coli* concentration ratios ( $C/C_0$ ) to Bromide concentration ratios ( $C/C_0$ ) in runoff from Plot 2 (bare) for experiment under wet initial soil moisture conditions. \*\*\* Statistically significant at  $p = 0.01$ .

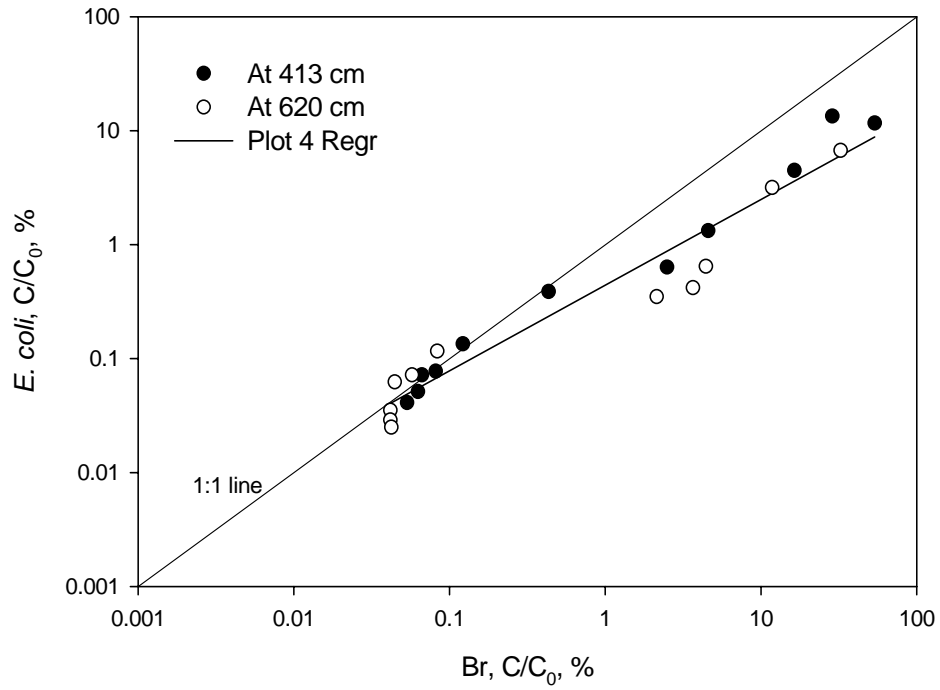


$$S_{413 \text{ cm}} = -0.20 + 0.85Br_{413 \text{ cm}} \quad R = 0.993^{***}$$

$$S_{620 \text{ cm}} = -0.28 + 0.88Br_{620 \text{ cm}} \quad R = 0.998^{***}$$

$$S_{\text{overall}} = -0.24 + 0.87Br_{\text{overall}} \quad R = 0.995^{***}$$

Figure 4.35. Regressions of *S. e. Typhimurium* concentration ratios ( $C/C_0$ ) to Bromide concentration ratios ( $C/C_0$ ) in runoff from Plot 2 (bare) for experiment under wet initial soil moisture conditions. \*\*\*Statistically significant at  $p = 0.01$ .

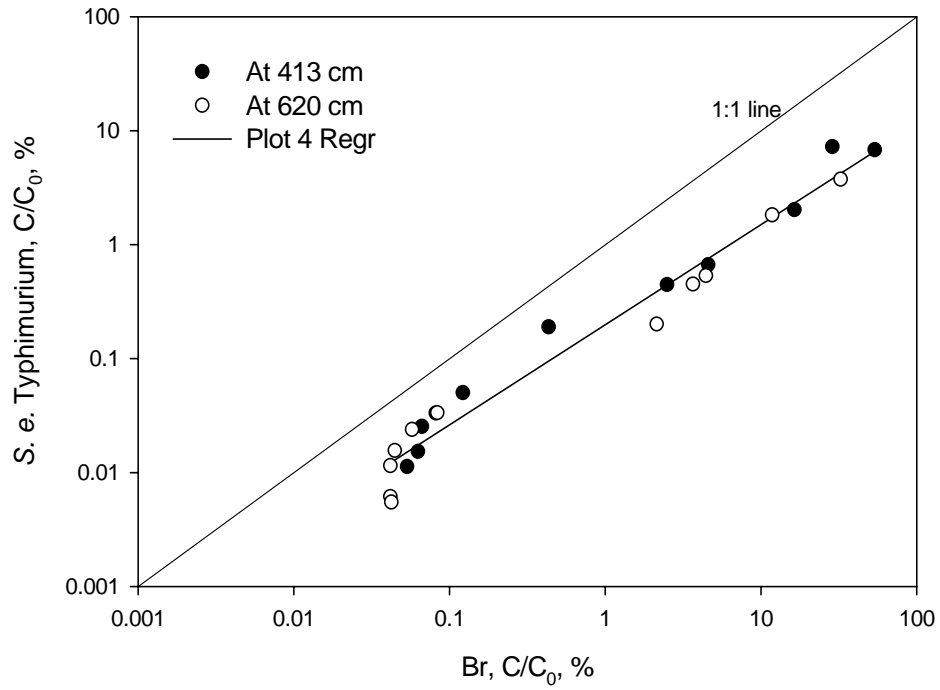


$$E_{413 \text{ cm}} = -0.27 + 0.79Br_{413 \text{ cm}} \quad R = 0.916^{***}$$

$$E_{620 \text{ cm}} = -0.46 + 0.69Br_{620 \text{ cm}} \quad R = 0.992^{***}$$

$$E_{\text{overall}} = -0.36 + 0.75Br_{\text{overall}} \quad R = 0.925^{***}$$

Figure 4.36. Regressions of *E. coli* concentration ratios ( $C/C_0$ ) to Bromide concentration ratios ( $C/C_0$ ) in runoff from Plot 4 (bare) for experiment under wet initial soil moisture conditions. \*\*\* Statistically significant at  $p = 0.01$ .

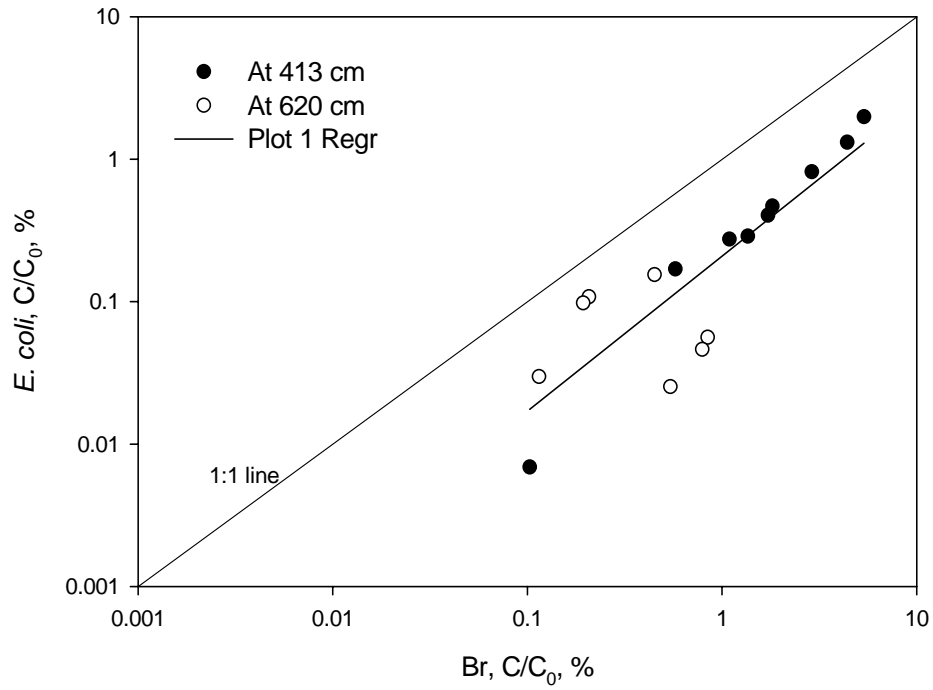


$$S_{413 \text{ cm}} = -0.63 + 0.87Br_{413 \text{ cm}} \quad R = 0.930^{***}$$

$$S_{620 \text{ cm}} = -0.78 + 0.86Br_{620 \text{ cm}} \quad R = 0.993^{***}$$

$$S_{\text{overall}} = -0.70 + 0.88Br_{\text{overall}} \quad R = 0.937^{***}$$

Figure 4.37. Regressions of *S. e. Typhimurium* concentration ratios ( $C/C_0$ ) to Bromide concentration ratios ( $C/C_0$ ) in runoff from Plot 4 (bare) for experiment under wet initial soil moisture conditions. \*\*\*Statistically significant at  $p = 0.01$ .

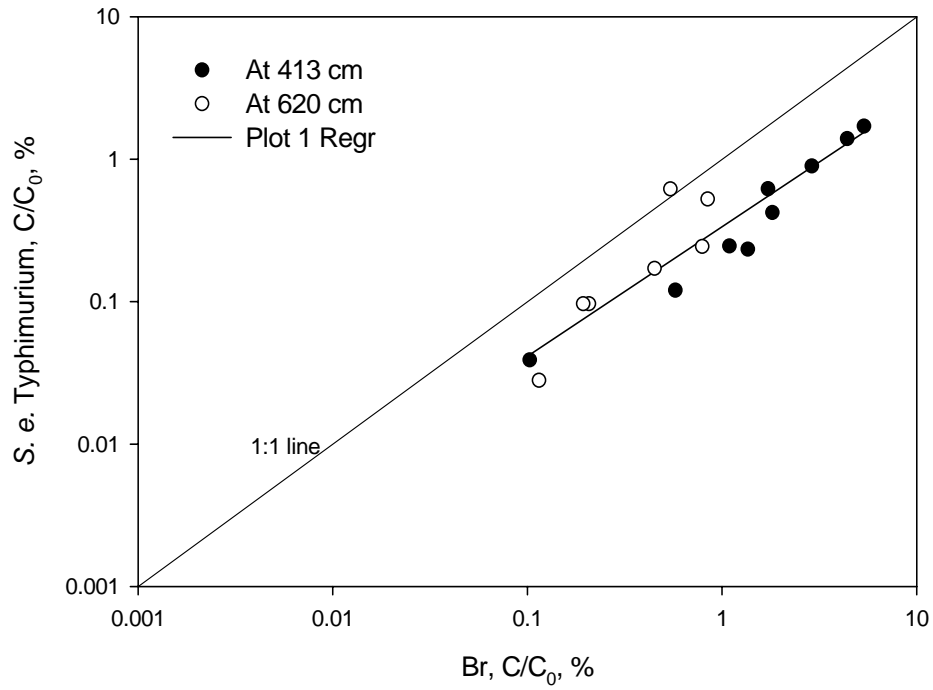


$$E_{413 \text{ cm}} = -0.68 + 1.36Br_{413 \text{ cm}} \quad R = 0.985^{***}$$

$$E_{620 \text{ cm}} = -1.24 - 0.07Br_{620 \text{ cm}} \quad R = 0.306$$

$$E_{\text{overall}} = -0.68 + 1.09Br_{\text{overall}} \quad R = 0.978^{***}$$

Figure 4.38. Regressions of *E. coli* concentration ratios ( $C/C_0$ ) to Bromide concentration ratios ( $C/C_0$ ) in runoff from Plot 1 (vegetated) for experiment under wet initial soil moisture conditions. \*\*\* Statistically significant at  $p = 0.01$ .

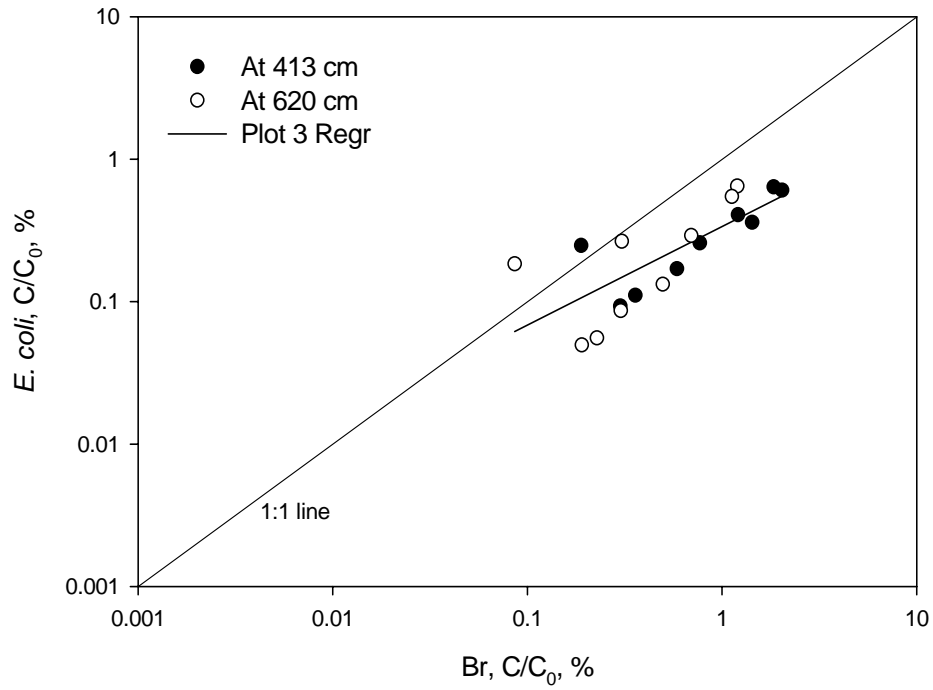


$$S_{413 \text{ cm}} = -0.57 + 0.99Br_{413 \text{ cm}} \quad R = 0.991^{***}$$

$$S_{620 \text{ cm}} = -0.22 + 1.26Br_{620 \text{ cm}} \quad R = 0.817^{***}$$

$$S_{\text{overall}} = -0.47 + 0.91Br_{\text{overall}} \quad R = 0.960^{***}$$

Figure 4.39. Regressions of *S. e.* Typhimurium concentration ratios ( $C/C_0$ ) to Bromide concentration ratios ( $C/C_0$ ) in runoff from Plot 1 (vegetated) for experiment under wet initial soil moisture conditions. \*\*\*Statistically significant at  $p = 0.01$ .

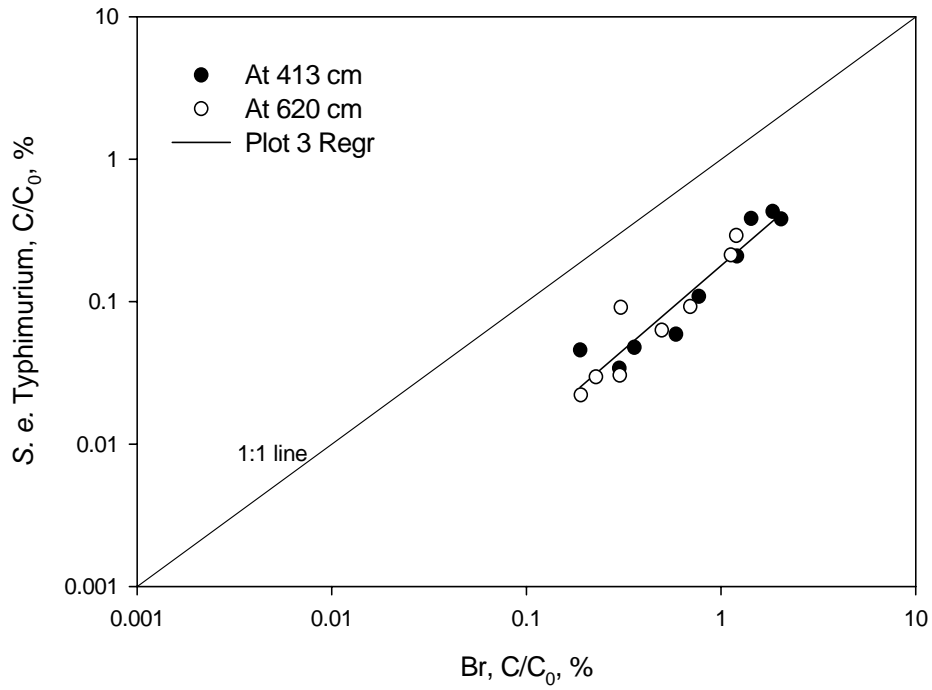


$$E_{413 \text{ cm}} = -0.49 + 0.66Br_{413 \text{ cm}} \quad R = 0.944^{***}$$

$$E_{620 \text{ cm}} = -0.44 + 0.74Br_{620 \text{ cm}} \quad R = 0.924^{***}$$

$$E_{\text{overall}} = -0.47 + 0.69Br_{\text{overall}} \quad R = 0.889^{***}$$

Figure 4.40. Regressions of *E. coli* concentration ratios ( $C/C_0$ ) to Bromide concentration ratios ( $C/C_0$ ) in runoff from Plot 3 (vegetated) for experiment under wet initial soil moisture conditions. \*\*\* Statistically significant at  $p = 0.01$ .



$$S_{413 \text{ cm}} = -0.76 + 1.15Br_{413 \text{ cm}} \quad R = 0.963^{***}$$

$$S_{620 \text{ cm}} = -0.73 + 1.23Br_{620 \text{ cm}} \quad R = 0.954^{***}$$

$$S_{\text{overall}} = -0.75 + 1.18Br_{\text{overall}} \quad R = 0.964^{***}$$

Figure 4.41. Regressions of *S. e. Typhimurium* concentration ratios ( $C/C_0$ ) to Bromide concentration ratios ( $C/C_0$ ) in runoff from Plot 3 (vegetated) for experiment under wet initial soil moisture conditions. \*\*\*Statistically significant at  $p = 0.01$ .



#### 4.4 Cumulative recoveries

##### 4.4.1 Dry initial soil moisture conditions

Cumulative recoveries obtained from the experiments under dry initial soil moisture conditions are shown in Tables 4.15 and 4.16, respectively, for the vegetated and bare plots. Data show that cumulative recoveries of applied rainfall as runoff were lower in the vegetated plots than those in the bare plots. Cumulative recoveries were 51.8% and 75.3%, respectively, for vegetated Plots 1 and 3 (Table 4.15), and 96.5% and 98.8%, respectively, for bare Plots 2 and 4 (Table 4.16). On average, surface runoff from vegetated plots was approximately 34% less than from bare plots. As shown in the hydrographs (Figure 4.1), the difference in recovery was primarily due to the much lower rates of infiltration in the bare plots during the first 30 minutes of simulations.

Cumulative recoveries of applied Br in surface runoff from the vegetated plots were lower than from the bare plots. Cumulative recoveries were 13.8% and 35.9%, respectively, for vegetated Plots 1 and 3 (Table 4.15), and 62.6% and 96.2%, respectively, for bare Plots 2 and 4 (Table 4.16). Note that cumulative Br recoveries in runoff from Plot 2 (Table 4.16) were extrapolated based on concentrations measured at the gutter (at 620 cm from the source of slurry) in runoff from Plot 4 during the first 7 min of simulation. Runoff samples were collected immediately after the initiation of runoff in Plot 4 (2 min), whereas runoff samples in Plot 2 were not collected until 5 min after the initiation of runoff. Extrapolation notwithstanding, it is still likely that Br recoveries in Plot 2 were underestimated.

Table 4.15. Cumulative recoveries obtained from the vegetated plots for experiments under dry initial soil moisture conditions.

Component recovered	PLOT 1 (Vegetated)		PLOT 3 (Vegetated)	
Water, cm				
Rainfall	8.3		8.1	
Runoff, %	<b>51.8</b>		<b>75.3</b>	
Bromide, g				
Applied	26		26	
In Runoff, %	<b>13.8</b>		<b>35.9</b>	
Bacteria, CFU				
	Salmonella	E. coli	Salmonella	E. coli
Applied	1.17E+11	8.02E+10	8.47E+10	5.81E+10
In Runoff	1.06E+09	2.45E+09	9.09E+09	6.40E+09
In Runoff, %	<b>0.9</b>	<b>3.1</b>	<b>10.7</b>	<b>11</b>

Table 4.16. Cumulative recoveries obtained from the bare plots for experiments under dry initial soil moisture conditions.

Component recovered	PLOT 2 (Bare)		PLOT 4 (Bare)	
Water, cm				
Rainfall	8.5 <sup>†</sup>		8.2	
Runoff, %	<b>96.5</b>		<b>98.8</b>	
Bromide, g				
Applied	26		26	
In Runoff, %	<b>62.6<sup>‡</sup></b>		<b>96.2</b>	
Bacteria, CFU				
	Salmonella	E. coli	Salmonella	E. coli
Applied	9.74E+10	5.61E+10	5.05E+10	7.10E+10
In Runoff	1.11E+10	1.55E+10	2.06E+10	1.95E+10
In Runoff, %	<b>11.4<sup>‡</sup></b>	<b>27.6<sup>‡</sup></b>	<b>40.8</b>	<b>27.5</b>

<sup>†</sup>Simulation in this plot lasted 62 min with a rainfall rate of 8.2 cm h<sup>-1</sup>

<sup>‡</sup>These values for Plot 2 were extrapolated based on concentrations measured at the gutter (620 cm) in Plot 4 during the first 7 min of simulation in this plot. See text for discussion.

Cumulative Br recovery in surface runoff from Plot 1 (13.8%) was lower than from Plot 3 (35.9%). These results are consistent with water recoveries and reflect the

fact that the two plots differed with respect to slope, soil texture at 20 cm depth, and topography (Section 4.1.1).

Cumulative recoveries of applied *E. coli* and *S. e. Typhimurium* in surface runoff were also lower from vegetated plots than from bare plots. Cumulative recoveries of *E. coli* were 3.1% and 11%, respectively, from vegetated Plots 1 and 3 (Table 4.15), and 27.6% and 27.5%, respectively, from bare Plots 2 and 4 (Table 4.16); while cumulative recoveries of *S. e. Typhimurium* were 0.9% and 10.7%, respectively, from vegetated Plots 1 and 3 (Table 4.15), and 11.4% and 40.8%, respectively, from bare Plots 2 and 4 (Table 4.16). Based on the averages within each treatment, total *E. coli* and total *S. e. Typhimurium* recovered in surface runoff from vegetated plots were, respectively, approximately 21% and 20% lower than that recovered from bare plots. Cumulative recoveries of *E. coli* and *S. e. Typhimurium* with time in vegetated vs. bare plots are shown in Figure 4.42. Data clearly show that vegetated filter strips were very effective in reducing pathogen runoff.

Note that the total pathogen load in runoff from bare plots is undoubtedly an underestimate, since only pathogens in the aqueous phase were measured, meaning that percent recoveries could have been higher than those depicted in Figure 4.42 if solid phase transport was measured. Previous studies indicate that bacteria typically partition between water and soil particulates (Tyrrel and Quinton, 2003; Roodsari, 2004; Muirhead et al., 2005). Consequently, considering the very low infiltration rates in the bare plots, most of the unaccounted for pathogens applied to bare plots were likely associated with the sediment. In addition, as previously noted for Br above, the pathogen recoveries from Plot 2 were undoubtedly underestimated.

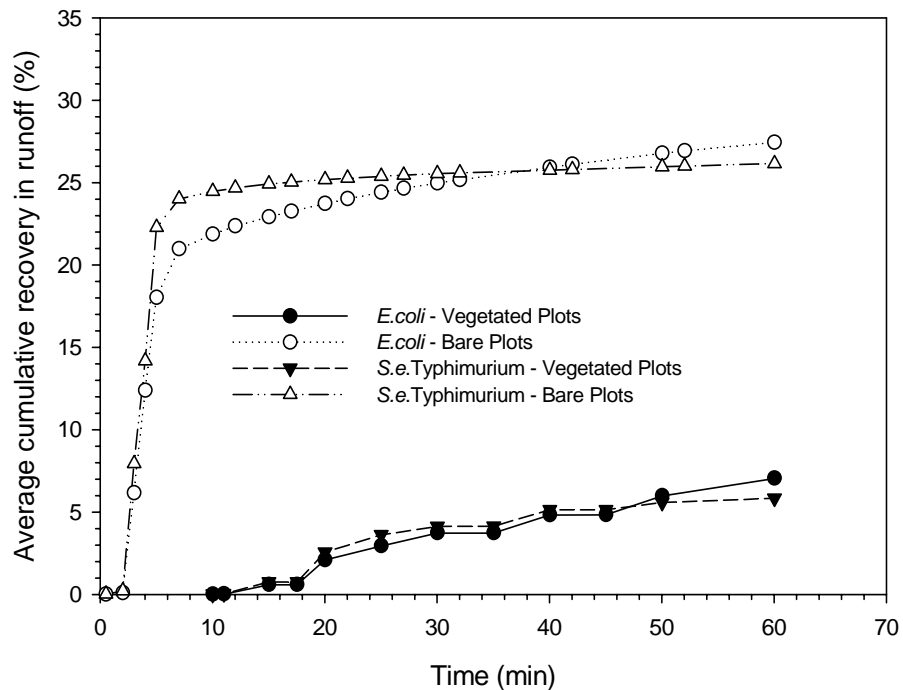


Figure 4.42. Average percent cumulative recovery in runoff of each pathogen for simulations under dry initial soil moisture conditions.

According to Crane et al. (1983) and Stout et al. (2005), several mechanisms are responsible for the ability of vegetation to minimize the transport of pathogens in surface runoff. These include: enhanced infiltration, sorption of pathogens to plant litter or organic matter, and deposition of pathogens attached to soil particles due to decreased surface water flow. In this study, deposition likely was of little importance since applied pathogens were only briefly exposed to soil. However, both infiltration and sorption played major roles in minimizing pathogen transport, although in varying degrees depending on the plot.

Table 4.15 shows that Plot 1 was more effective in reducing surface runoff of pathogens as compared to Plot 3. As previously discussed, both infiltration rates and

litter densities/organic matter were higher in Plot 1, allowing for greater infiltration of pathogens into the soil profile as well as greater retention onto plant litter. However, since neither soil samples nor litter samples were analyzed, no quantitative data on the adsorption of pathogens to soil particles or plant litter was available.

#### 4.4.2 Wet initial soil moisture conditions

Cumulative recoveries obtained from the experiments under wet initial soil moisture conditions are shown in Tables 4.17 and 4.18, respectively, for the vegetated and bare plots. Data show that cumulative recoveries of applied rainfall as runoff were similar between the vegetated and bare plots. Cumulative recoveries were 95.1% and 98.8%, respectively, for vegetated Plots 1 and 3 (Table 4.17), and 95.2% and 100%, respectively, for bare Plots 2 and 4 (Table 4.18). On average, surface runoff from vegetated plots was only 0.6% lower than from bare plots. As shown in the hydrographs (Figure 4.2), the similarity in recovery was primarily due to similar infiltration during the simulations under wet initial soil moisture conditions.

Cumulative recoveries of applied Br in surface runoff were lower in the vegetated plots than in the bare plots. Cumulative recoveries were 104.1% and 87.3%, respectively, for vegetated Plots 1 and 3 (Table 4.17), with an average of 95.7%; and 100.9% and 128.2%, respectively, for bare Plots 2 and 4 (Table 4.18), with an average of 114.6%. These results show that Br recovery in vegetated plots was lower than in the bare plots, indicating positive effect of vegetation in reducing this chemical loss in surface runoff.

Table 4.17. Cumulative recoveries obtained from the vegetated plots for experiments under wet initial soil moisture conditions.

Component recovered	PLOT 1 (Vegetated)		PLOT 3 (Vegetated)	
Water, cm				
Rainfall	8.2		8.2	
Runoff, %	<b>95.1</b>		<b>98.8</b>	
Bromide, g				
Applied	26		26	
In Runoff, %	<b>104.1</b>		<b>87.3</b>	
Bacteria, CFU				
	Salmonella	E. coli	Salmonella	E. coli
Applied	5.76E+10	6.16E+10	1.07E+11	6.08E+10
In Runoff	2.32E+10	1.61E+10	1.61E+10	2.12E+10
In Runoff, %	<b>40.3</b>	<b>26.1</b>	<b>15</b>	<b>34.9</b>

Table 4.18. Cumulative recoveries obtained from the bare plots for experiments under wet initial soil moisture conditions.

Component recovered	PLOT 2 (Bare)		PLOT 4 (Bare)	
Water, cm				
Rainfall	8.3 <sup>†</sup>		8.2 <sup>‡</sup>	
Runoff, %	<b>95.2</b>		<b>100<sup>‡</sup></b>	
Bromide, g				
Applied	26		26	
In Runoff, %	<b>100.9</b>		<b>128.2<sup>‡</sup></b>	
Bacteria, CFU				
	Salmonella	E. coli	Salmonella	E. coli
Applied	4.63E+10	6.49E+10	1.14E+11	7.02E+10
In Runoff	2.08E+10	2.03E+10	2.10E+10 <sup>‡</sup>	2.46E+10 <sup>‡</sup>
In Runoff, %	<b>44.9</b>	<b>31.3</b>	<b>18.4</b>	<b>35.0</b>

<sup>†</sup>Simulation in this plot lasted 61 min with a rainfall rate of 8.2 cm h<sup>-1</sup>

<sup>‡</sup>These values have been extrapolated for a 60 min simulation. Simulation in Plot 4 was terminated after 40 min because the soil was completely saturated and subsurface flow was observed to be emerging into the gutter.

Cumulative recoveries of applied pathogens in surface runoff were similar between vegetated and bare plots, particularly for *E. coli*. Cumulative recoveries of *E. coli* were 26.1% and 34.9%, respectively, from vegetated Plots 1 and 3 (Tables 4.17),

and 31.3% and 35.0%, respectively, from bare Plots 2 and 4 (Table 4.18); while cumulative recoveries of *S. e. Typhimurium* were 40.3% and 15%, respectively, from vegetated Plots 1 and 3 (Table 4.17), and 44.9% and 18.4%, respectively, from bare Plots 2 and 4 (Table 4.18). On average, 30.5% and 27.7%, respectively, of the applied *E. coli* and *S. e. Typhimurium* were recovered in surface runoff from the vegetated plots, while 33.2% and 31.7%, respectively, of the applied *E. coli* and *S. e. Typhimurium* were recovered in surface runoff from the bare plots. These recovery data also indicate the positive effect of vegetation in reducing pathogen loss in surface runoff even under wet initial soil moisture conditions.

Based on the averages within treatments, total *E. coli* and *S. e. Typhimurium* recovered in surface runoff from vegetated plots were, respectively, approximately 3% and 4% lower than that recovered from bare plots. These results are substantially different from the ones obtained under dry initial soil moisture conditions (Section 4.4.1) in which total *E. coli* and total *S. e. Typhimurium* recovered in surface runoff from vegetated plots were, respectively, approximately 21% and 20% lower than that recovered from bare plots.

The higher pathogen cumulative recoveries under wet conditions than dry conditions are consistent with the hydrographs, which show that there was less infiltration in vegetated plots under wet conditions. Less infiltration resulted in reduced amounts of pathogens transported into the soil profile and increased their loss in surface runoff. Cumulative recoveries of *E. coli* and *S. e. Typhimurium* with time in vegetated vs. bare plots are shown in Figure 4.43. Results show that initial loss of

pathogens in vegetated plots was much lower than in the bare plots, but, with time, vegetated plots tended to behave almost like bare plots.

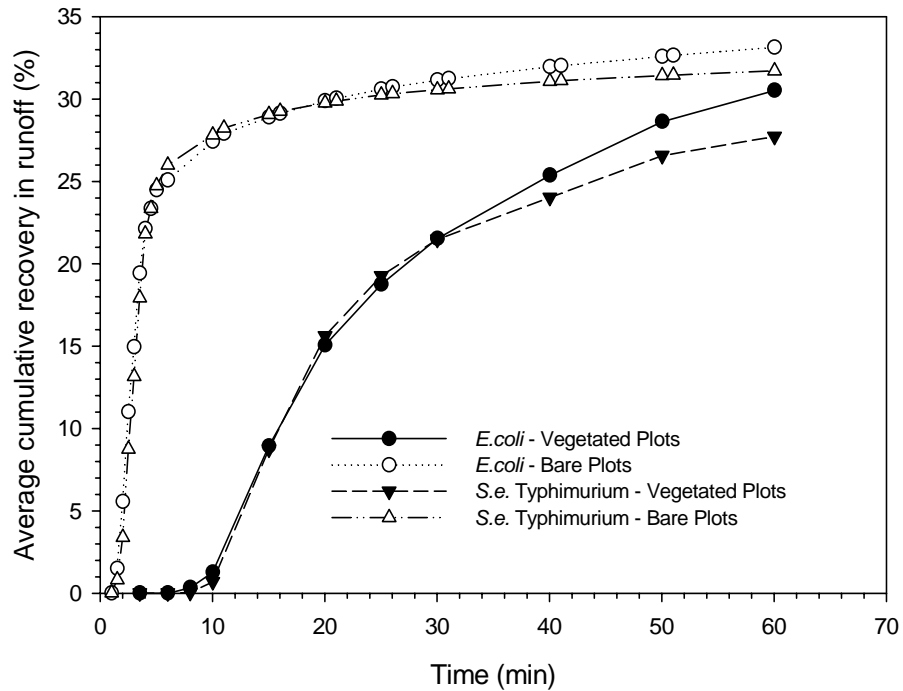


Figure 4.43. Average percent cumulative recovery in runoff of each pathogen for simulations under wet initial soil moisture conditions.

The primary difference between wet and dry initial soil moisture conditions was observed in vegetated plots where cumulative recoveries of rainfall, Br, and pathogens were substantially higher under wet conditions. On average, cumulative recoveries of rainfall and Br were, respectively, approximately 33% and 71% higher under wet than dry conditions. On average, cumulative recoveries of *E. coli* and *S. e. Typhimurium* were, respectively, approximately 23% and 22% higher under wet than dry conditions. These results are consistent with hydrographs, which show that there



was less infiltration in vegetated plots under wet conditions. Less infiltration resulted in reduced amounts of Br and pathogens transported into the soil profile and increased amounts lost in surface runoff.

## Conclusions and Recommendations

This study showed that initial soil moisture conditions – induced by the depth to the groundwater table – affected the efficiency of vegetated plots at attenuating surface runoff. Under dry conditions, in which the groundwater table was at least 50 cm below the surfaces, average cumulative recoveries of applied rainfall as runoff were 98% and 64%, respectively, for bare and vegetated plots, a decrease of 34%. Under wet conditions, in which the groundwater table was approximately 20 cm below the surfaces, average cumulative recoveries of applied rainfall were 97.6% and 97%, respectively, for bare and vegetated plots, a decrease of only 0.6%. Surface runoff in the vegetated plots increased 33% from dry to wet conditions, indicating that less infiltration was allowed in these plots and more of the applied rain was lost as surface runoff when the groundwater table in these plots was closer to the surface.

Initial conditions of groundwater table in the vegetated plots also affected their performance at attenuating surface runoff transport of pathogens from land-applied swine slurry. Under dry conditions, average cumulative recoveries of *Escherichia coli* in the liquid phase of runoff were 27.6% and 7.05%, respectively, for bare and vegetated plots, while average cumulative recoveries of *Salmonella enterica* Typhimurium in the liquid phase of runoff were 26.1% and 5.8%, respectively, for bare and vegetated plots. Under wet conditions, average cumulative recoveries of *E. coli* in the liquid phase of runoff were 33.2% and 30.5%, respectively, for bare and vegetated plots, while average cumulative recoveries of *S. e.* Typhimurium in the liquid phase of runoff were 31.7% and 27.7%, respectively, for

bare and vegetated plots. On average, cumulative recoveries *E. coli* and *S. e.* Typhimurium in runoff from the vegetated plots had an increase, respectively, of 23% and 22% from dry to wet conditions. These results were consistent with infiltration rates, which were lower in the vegetated plots under wet conditions. Less infiltration resulted in high runoff and in reduced amounts of pathogens transported into the soil profile and increased amounts of them lost in surface runoff.

This study not only shows that initial water table depth affected infiltration rates in the vegetated plots but also that infiltration was the major mechanism controlling overland transport of pathogens from land-applied swine slurry. It shows that 5%-sloped vegetated filter strips (VFS) with initial groundwater table depth of at least 50 cm are effective at mitigating runoff transport of pathogens from swine slurry even under extreme rainfall events. However, the effectiveness of these VFS is reduced if initial groundwater table depth is 20 cm or less since it prevents infiltration processes to occur. Therefore, parameters for designing VFS in the State of Iowa to reduce microbial transport in overland flow should be based on those that optimize infiltration rates even under extreme rainfall events.

One parameter that can be taken into consideration in order to optimize infiltration rates within VFS is their surface conditions before seeding. Non uniform or rough surfaces, that have depressions or channels, should be graded to more uniform and smooth surfaces in order to allow the flow of water to be shallow and uniform, thus avoiding the establishment of areas of concentrated flow conditions. In areas of concentrated flow conditions, flow velocity increases, which decreases the available time for infiltration to occur, thus increasing runoff rates.

Another parameter that can be considered is the type of vegetation. Some studies suggest that thick, deep rooted plants, such as eastern gamagrass, are preferable over thin, shallow rooted plants since the biological properties of thick, deep rooted plants help in the processes of loosening up soils (Perrygo et al., 2002). These processes enhance the formation of macropores and, most importantly, of macropore flow conditions, which are viewed as an important means for the development of preferential flow conditions that can significantly increase infiltration rates within soil profiles.

In the study by Perrygo et al. (2002), not only they demonstrated that final infiltration rates in Matawan-Hammonton loam soils planted with eastern gamagrass were significantly higher than in those planted with thinner, shallower-rooting tall fescue, but also that eastern gamagrass greatly improved the physical and hydraulic characteristics of these soils. In addition, the authors concluded that VFS constructed with eastern gamagrass might be very effective for reducing surface runoff from agricultural fields by enhancing infiltration rates. One should note that the selection of plants should also be based on their compatibility to climate conditions, type of soils, and topography.

Last, implementation of water table management strategies (through drainage systems) may be another parameter that can be considered in order to enhance infiltration rates within VFS. These strategies have been designed to lower possible high groundwater table levels for agricultural production purposes, and they can be separated, at least, into three systems: subsurface drainage, controlled drainage, and controlled drainage-subirrigation (Shirmohammadi et al., 1992).

According to Shirmohammadi et al. (1992), under subsurface and controlled drainage systems, there is the risk that groundwater table might reach levels that are too low to sustain vegetation, particularly during drought periods, and a supplemental water input (surface irrigation or natural rainfall events) would be necessary in order bring groundwater table to adequate levels. Under controlled drainage-subirrigation (CD-SI) system there is still the risk of groundwater table reaching levels that are too low, but the need for surface irrigation or natural rainfall events can be eliminated since under CD-SI water can be pumped/irrigated into the soil system.

According to Wright et al. (1990), another advantage with CD-SI system is that it has the potential to treat and reduce other nonpoint source pollutants, such as nutrients (net nitrogen), in areas with high groundwater table conditions. Nonetheless, in order to design feasible and efficient water table management systems, several elements have to be taken into consideration, such as the feasibility of the site; detailed field investigation; design computations; system layout and installation; and operation and management (Shirmohammadi et al., 1992).

This study was not conclusive regarding the correlations between each pathogen and Bromide (Br) in surface runoff collected at 620 cm from the source of slurry in the vegetated plots. At such distance, correlations differed between the two pathogens and between the two vegetated plots (Plots 1 and 3). This study showed significant correlations between relative concentrations of *S. e. Typhimurium* and Br in both vegetated plots and under both wet and dry conditions. However, the correlations between relative concentrations of *E. coli* and Br in runoff was less clear- for Plot 3 there was always a significant correlation while for Plot 1 there was none,

regardless of initial soil moisture conditions. This study shows that it is possible to have a significant correlation between relative concentrations of Br and pathogens in runoff from VFS - but not always. However, correlation studies of this nature are significant and it is important to understand what drives or disrupts the correlation between Br and pathogens in runoff from VFS. Based on well-understood correlations and on proper equations describing the relationships, relative concentrations of Br in runoff can approximate what will happen with pathogens in runoff. That would allow less costly and less labor-intensive studies.

For this study, there were only two replications per treatment: two vegetated and two bare plots. Statistically, it would have been better to have more plots to understand the variability in the results. For that matter, there are two ways to increase the statistical sample: 1) divide the existing plots into several smaller plots, or 2) increase the number of plots. Option 1 would not have been a realistic approach because the new plots would have been much too small and artificial to represent realistic natural conditions. The size of the plots is important in order to represent the spatial variability. For example, if the plot is too small it may not show the realistic distributions of flow paths. Therefore, if resources are available, a much better approach is option 2- more plots of reasonable size that can better represent natural conditions and have enough replications.

## **APPENDIX A**

**Time intervals at which runoff was collected, measured and sampled during all experiments**

Table A.1. Time intervals at which runoff was measured and sampled at the two distances from the applied swine slurry after the 60-min rainfall simulation initiated during the two sets of experiments.

Plots <sup>†</sup>	Distance (cm)	Time intervals (min)												
		Dry initial soil moisture conditions												
Plot 1	413	17.5	20	25	30	35	40	45	50	60				
	620	17.5	20	25	30	35	40	45	50	60				
Plot 2 <sup>‡</sup>	413	7	12	17	22	27	32	42	52	62				
	620	7	12	17	22	27	32	42	52	62				
Plot 3	413	11	15	20	25	30	40	50	60					
	620	11	15	20	25	30	40	50	60					
Plot 4	413				5	10	15	20	25	30	40	50	60	
	620	2	3	4	5	10	15	20	25	30	40	50	60	
		Wet initial soil moisture conditions												
Plot 1	413	6	8	10	15	20	25	30	40	50	60			
	620	6	8	10	15	20	25	30	40	50	60			
Plot 2 <sup>§</sup>	413	2	3	4	5	6	11	16	21	26	31	41	51	61
	620	2	3	4	5	6	11	16	21	26	31	41	51	61
Plot 3	413	6	8	10	15	20	25	30	40	50	60			
	620	6	8	10	15	20	25	30	40	50	60			
Plot 4 <sup>¶</sup>	413	1.5	2.5	3.5	4.5	5	10	15	20	25	30	40		
	620	1.5	2.5	3.5	4.5	5	10	15	20	25	30	40		

<sup>†</sup> Plots 1 and 3 were vegetated, while Plots 2 and 4 were bare.

<sup>‡</sup> This experiment lasted 62 min.

<sup>§</sup> This experiment lasted 61 min.

<sup>¶</sup> This experiment lasted 40 min.



## **APPENDIX B**

**Runoff and Bromide data collected at 413 cm from the source of slurry during experiments under dry initial soil moisture conditions**

Table B.1. Mean, standard deviation (STDEV), and coefficient of variance (C.V.) of runoff volumes and Bromide (Br) concentrations in runoff measured at 413 cm from the source of slurry in Plot 1 (vegetated) during the simulations under dry initial soil moisture conditions.

Time (min)	Mean at 413 cm		STDEV at 413 cm		C. V. (%)	
	Runoff Volume (mL)	Br Concentration (ppm)	Runoff Volume (mL)	Br Concentration (ppm)	Runoff Volume	Br Concentration
17.5	813.33	17.58	597.02	22.35	73.40	127.11
20	845.63	11.71	292.54	11.95	34.59	102.03
25	2093.33	11.94	684.79	13.49	32.71	113.01
30	2593.33	9.14	963.40	10.29	37.15	112.63
35	2396.67	6.48	805.01	6.92	33.59	106.75
40	2430.00	6.02	657.80	7.02	27.07	116.64
45	2456.67	5.25	627.40	5.87	25.54	111.78
50	2876.67	5.23	1307.15	5.83	45.44	111.65
60	5850.00	6.14	2667.86	6.94	45.60	113.03

Table B.2. Mean, standard deviation (STDEV), and coefficient of variance (C.V.) of runoff volumes and Bromide (Br) concentrations in runoff measured at 413 cm from the source of slurry in Plot 3 (vegetated) during the simulations under dry initial soil moisture conditions.

Time (min)	Mean at 413 cm		STDEV at 413 cm		C. V. (%)	
	Runoff Volume (mL)	Br Concentration (ppm)	Runoff Volume (mL)	Br Concentration (ppm)	Runoff Volume	Br Concentration
11	266.67	6.46	275.38	6.04	103.27	93.60
15	1276.67	22.48	1188.12	23.60	93.06	104.98
20	3311.67	24.35	3121.60	29.69	94.26	121.93
25	3683.33	11.44	3579.92	11.01	97.19	96.25
30	3900.00	7.29	3675.60	7.10	94.25	97.34
40	8216.67	4.20	8008.80	4.30	97.47	102.33
50	10166.67	3.64	9408.68	2.89	92.54	79.39
60	10645.00	3.17	9525.14	2.14	89.48	67.67

Table B.3. Mean, standard deviation (STDEV), and coefficient of variance (C.V.) of runoff volumes and Bromide (Br) concentrations in runoff measured at 413 cm from the source of slurry in Plot 2 (bare) during the simulations under dry initial soil moisture conditions.

Time (min)	Mean at 413 cm		STDEV at 413 cm		C. V. (%)	
	Runoff Volume (mL)	Br Concentration (ppm)	Runoff Volume (mL)	Br Concentration (ppm)	Runoff Volume	Br Concentration
7	1816.67	300.28	940.44	375.58	51.77	125.08
12	1990.00	5.54	808.02	4.59	40.60	82.84
17	2035.00	2.33	888.95	2.36	43.68	101.65
22	1880.00	1.44	877.10	1.41	46.65	97.63
27	1865.00	1.15	961.99	1.02	51.58	88.47
32	1826.67	1.00	962.57	0.79	52.70	79.83
42	3533.33	0.93	1569.50	0.66	44.42	71.05
52	3783.33	0.90	1692.52	0.56	44.74	62.52
62	3550.00	0.96	1796.52	0.47	50.61	49.41

Table B.4. Mean, standard deviation (STDEV), and coefficient of variance (C.V.) of runoff volumes and Bromide (Br) concentrations in runoff measured at 413 cm from the source of slurry in Plot 4 (bare) during the simulations under dry initial soil moisture conditions.

Time (min)	Mean at 413 cm		STDEV at 413 cm		C. V. (%)	
	Runoff Volume (mL)	Br Concentration (ppm)	Runoff Volume (mL)	Br Concentration (ppm)	Runoff Volume	Br Concentration
5	3730.00	417.96	1770.00	282.34	47.45	67.55
10	4330.00	6.17	1870.00	3.33	43.19	53.97
15	4325.00	1.93	625.00	1.16	14.45	60.07
20	7875.00	1.34	1325.00	0.68	16.83	50.75
25	7250.00	1.02	150.00	0.45	2.07	44.59
30	6800.00	0.94	0	0.42	0	45.15
40	13175.00	0.87	1175.00	0.38	8.92	43.98
50	12915.00	0.87	815.00	0.38	6.31	43.27
60	12225.00	0.99	575.00	0.45	4.70	45.28

## **APPENDIX C**

**Runoff and Bromide data collected at 413 cm from the source of slurry during experiments under wet initial soil moisture conditions**

Table C.1. Mean, standard deviation (STDEV), and coefficient of variance (C.V.) of runoff volumes and Bromide (Br) concentrations in runoff measured at 413 cm from the source of slurry in Plot 1 (vegetated) during the simulations under wet initial soil moisture conditions.

Time (min)	Mean at 413 cm		STDEV at 413 cm		C. V. (%)	
	Runoff Volume (mL)	Br Concentration (ppm)	Runoff Volume (mL)	Br Concentration (ppm)	Runoff Volume	Br Concentration
6	523.33	0.80	594.67	0.90	113.63	112.52
8	866.67	2.06	859.32	1.49	99.15	72.41
10	2098.67	34.44	1649.17	58.03	78.58	168.49
15	6266.67	107.21	4619.88	177.46	73.72	165.53
20	6583.33	87.90	5037.44	114.16	76.52	129.87
25	6966.67	57.87	5404.01	70.77	77.57	122.30
30	6363.33	36.28	4743.00	48.88	74.54	134.72
40	13550.00	21.82	11134.74	30.03	82.18	137.61
50	14500.00	27.12	12574.18	42.26	86.72	155.81
60	15173.33	11.52	13290.60	16.55	87.59	143.64

Table C.2. Mean, standard deviation (STDEV), and coefficient of variance (C.V.) of runoff volumes and Bromide (Br) concentrations in runoff measured at 413 cm from the source of slurry in Plot 3 (vegetated) during the simulations under wet initial soil moisture conditions.

Time (min)	Mean at 413 cm		STDEV at 413 cm		C. V. (%)	
	Runoff Volume (mL)	Br Concentration (ppm)	Runoff Volume (mL)	Br Concentration (ppm)	Runoff Volume	Br Concentration
6	163.33	1.52	125.03	0.11	76.55	7.24
8	1323.33	3.78	1096.10	3.75	82.83	99.14
10	1863.33	28.57	1596.32	36.88	85.67	129.12
15	4916.67	36.86	4153.41	53.60	84.48	145.41
20	4696.67	40.72	4010.71	50.58	85.39	124.21
25	4780.00	24.15	4256.76	27.70	89.05	114.69
30	6083.33	15.37	6342.02	15.10	104.25	98.19
40	13733.33	11.73	14424.40	10.93	105.03	93.14
50	12933.33	7.18	13110.91	5.60	101.37	78.05
60	12940.00	6.00	13475.71	4.76	104.14	79.43

Table C.3. Mean, standard deviation (STDEV), and coefficient of variance (C.V.) of runoff volumes and Bromide (Br) concentrations in runoff measured at 413 cm from the source of slurry in Plot 2 (bare) during the simulations under wet initial soil moisture conditions.

Time (min)	Mean at 413 cm		STDEV at 413 cm		C. V. (%)	
	Runoff Volume (mL)	Br Concentration (ppm)	Runoff Volume (mL)	Br Concentration (ppm)	Runoff Volume	Br Concentration
2	483.33	362.39	161.97	519.60	33.51	143.38
3	770.00	567.32	315.12	433.20	40.92	76.36
4	785.00	297.03	247.54	225.98	31.53	76.08
5	786.67	131.45	200.33	119.83	25.47	91.16
6	853.33	40.99	217.79	32.01	25.52	78.10
11	4500.00	8.63	1100.00	8.43	24.44	97.79
16	4180.00	2.65	1576.45	1.47	37.71	55.37
21	4883.33	1.65	1615.81	1.00	33.09	60.64
26	4900.00	1.38	2042.06	0.78	41.67	56.64
31	4500.00	1.26	1905.26	0.71	42.34	56.14
41	8400.00	1.18	4297.66	0.61	51.16	51.54
51	7683.33	0.95	4204.86	0.35	54.73	36.70
61	6516.67	1.10	3875.67	0.48	59.47	43.72

Table C.4. Mean, standard deviation (STDEV), and coefficient of variance (C.V.) of runoff volumes and Bromide (Br) concentrations in runoff measured at 413 cm from the source of slurry in Plot 4 (bare) during the simulations under wet initial soil moisture conditions.

Time (min)	Mean at 413 cm		STDEV at 413 cm		C. V. (%)	
	Runoff Volume (mL)	Br Concentration (ppm)	Runoff Volume (mL)	Br Concentration (ppm)	Runoff Volume	Br Concentration
1.5	766.67	574.19	650.41	162.47	84.84	28.30
2.5	2206.67	1074.60	2127.54	582.93	96.41	54.25
3.5	1400.00	327.77	1188.23	187.34	84.87	57.15
4.5	1776.67	91.80	1640.56	57.16	92.34	62.27
5	953.33	49.88	932.76	32.31	97.84	64.77
10	9026.67	8.66	8458.82	5.28	93.71	60.97
15	8333.33	2.44	7684.62	0.81	92.22	33.35
20	8700.00	1.63	6878.95	0.46	79.07	28.45
25	8400.00	1.33	6315.06	0.36	75.18	26.89
30	8116.67	1.25	6117.67	0.50	75.37	39.94
40	14826.67	1.07	12747.40	0.45	85.98	42.38

## **APPENDIX D**

**Runoff and pathogen data collected at 413 cm from the source of slurry during experiments under dry initial soil moisture conditions**

Table D.1. Mean, standard deviation (STDEV), and coefficient of variation (C.V.) of runoff volumes and pathogen concentrations in runoff measured at 413 cm from the source of slurry in Plot 1 (vegetated) during simulations under dry initial soil moisture conditions.

<b>PLOT 1 - Vegetated</b>						
<i>S. e. Typhimurium</i>						
	<b>Mean at 413 cm</b>		<b>STDEV at 413 cm</b>		<b>C. V. (%)</b>	
<b>Time (min)</b>	<b>Runoff Volume (mL)</b>	<b>Concentration (CFU/mL)</b>	<b>Runoff Volume (mL)</b>	<b>Concentration (CFU/mL)</b>	<b>Runoff Volume</b>	<b>Concentration</b>
17.5	813.33	3.95E+03	597.02	2.64E+03	73.40	66.79
20	845.63	3.29E+03	292.54	1.24E+03	34.59	37.67
25	2093.33	2.69E+03	684.79	7.38E+02	32.71	27.42
30	2593.33	1.46E+03	963.40	5.87E+02	37.15	40.34
35	2396.67	1.18E+03	805.01	5.04E+02	33.59	42.79
40	2430.00	9.94E+02	657.80	6.53E+02	27.07	65.76
45	2456.67	8.25E+02	627.40	7.60E+02	25.54	92.15
50	2876.67	7.37E+02	1307.15	5.92E+02	45.44	80.31
60	5850.00	7.11E+02	2667.86	4.35E+02	45.60	61.18
<i>E. coli</i>						
	<b>Mean at 413 cm</b>		<b>STDEV at 413 cm</b>		<b>C. V. (%)</b>	
<b>Time (min)</b>	<b>Runoff Volume (mL)</b>	<b>Concentration (CFU/mL)</b>	<b>Runoff Volume (mL)</b>	<b>Concentration (CFU/mL)</b>	<b>Runoff Volume</b>	<b>Concentration</b>
17.5	813.33	1.05E+04	597.02	1.46E+04	73.40	139.43
20	845.63	7.06E+03	292.54	4.94E+03	34.59	69.90
25	2093.33	5.15E+03	684.79	2.68E+03	32.71	52.11
30	2593.33	4.80E+03	963.40	3.43E+03	37.15	71.43
35	2396.67	3.95E+03	805.01	1.85E+03	33.59	46.81
40	2430.00	3.90E+03	657.80	2.93E+03	27.07	75.08
45	2456.67	3.84E+03	627.40	2.89E+03	25.54	75.08
50	2876.67	3.19E+03	1307.15	2.80E+03	45.44	87.97
60	5850.00	3.03E+03	2667.86	2.53E+03	45.60	83.46



Table D.2. Mean, standard deviation (STDEV), and coefficient of variation (C.V.) of runoff volumes and pathogen concentrations in runoff measured at 413 cm from the source of slurry in Plot 3 (vegetated) during simulations under dry initial soil moisture conditions.

<b>PLOT 3 - Vegetated</b>						
<i>S. e. Typhimurium</i>						
	<b>Mean at 413 cm</b>		<b>STDEV at 413 cm</b>		<b>C. V. (%)</b>	
<b>Time (min)</b>	<b>Runoff Volume (mL)</b>	<b>Concentration (CFU/mL)</b>	<b>Runoff Volume (mL)</b>	<b>Concentration (CFU/mL)</b>	<b>Runoff Volume</b>	<b>Concentration</b>
11	266.67	1.59E+04	275.38	1.40E+04	103.27	88.22
15	1276.67	2.95E+04	1188.12	2.55E+04	93.06	86.63
20	3311.67	2.68E+04	3121.60	2.32E+04	94.26	86.62
25	3683.33	9.91E+03	3579.92	9.03E+03	97.19	91.09
30	3900.00	3.98E+03	3675.60	4.17E+03	94.25	104.91
40	8216.67	2.60E+03	8008.80	2.81E+03	97.47	108.22
50	10166.67	1.31E+03	9408.68	1.17E+03	92.54	89.46
60	10645.00	1.23E+03	9525.14	1.11E+03	89.48	90.14
<i>E. coli</i>						
	<b>Mean at 413 cm</b>		<b>STDEV at 413 cm</b>		<b>C. V. (%)</b>	
<b>Time (min)</b>	<b>Runoff Volume (mL)</b>	<b>Concentration (CFU/mL)</b>	<b>Runoff Volume (mL)</b>	<b>Concentration (CFU/mL)</b>	<b>Runoff Volume</b>	<b>Concentration</b>
11	266.67	8.43E+03	275.38	1.33E+04	103.27	158.05
15	1276.67	2.76E+04	1188.12	2.67E+04	93.06	96.58
20	3311.67	2.84E+04	3121.60	3.23E+04	94.26	113.88
25	3683.33	1.21E+04	3579.92	1.61E+04	97.19	132.70
30	3900.00	5.51E+03	3675.60	6.78E+03	94.25	123.13
40	8216.67	3.25E+03	8008.80	3.57E+03	97.47	109.88
50	10166.67	2.88E+03	9408.68	2.56E+03	92.54	88.76
60	10645.00	2.37E+03	9525.14	2.06E+03	89.48	86.60

Table D.3. Mean, standard deviation (STDEV), and coefficient of variation (C.V.) of runoff volumes and pathogen concentrations in runoff measured at 413 cm from the source of slurry in Plot 2 (bare) during simulations under dry initial soil moisture conditions.

<b>PLOT 2 - Bare</b>						
<i>S. e. Typhimurium</i>						
	<b>Mean at 413 cm</b>		<b>STDEV at 413 cm</b>		<b>C. V. (%)</b>	
<b>Time (min)</b>	<b>Runoff Volume (mL)</b>	<b>Concentration (CFU/mL)</b>	<b>Runoff Volume (mL)</b>	<b>Concentration (CFU/mL)</b>	<b>Runoff Volume</b>	<b>Concentration</b>
7	1816.67	2.83E+05	940.44	3.26E+05	51.77	115.22
12	1990.00	7.52E+03	808.02	5.30E+03	40.60	70.43
17	2035.00	1.95E+03	888.95	1.93E+03	43.68	98.78
22	1880.00	1.60E+03	877.10	1.83E+03	46.65	114.80
27	1865.00	1.14E+03	961.99	1.31E+03	51.58	114.71
32	1826.67	8.57E+02	962.57	9.21E+02	52.70	107.50
42	3533.33	6.23E+02	1569.50	6.81E+02	44.42	109.31
52	3783.33	5.53E+02	1692.52	4.65E+02	44.74	84.04
62	3550.00	3.53E+02	1796.52	4.04E+02	50.61	114.38
<i>E. coli</i>						
	<b>Mean at 413 cm</b>		<b>STDEV at 413 cm</b>		<b>C. V. (%)</b>	
<b>Time (min)</b>	<b>Runoff Volume (mL)</b>	<b>Concentration (CFU/mL)</b>	<b>Runoff Volume (mL)</b>	<b>Concentration (CFU/mL)</b>	<b>Runoff Volume</b>	<b>Concentration</b>
7	1816.67	1.67E+05	940.44	2.05E+05	51.77	122.77
12	1990.00	8.04E+03	808.02	7.33E+03	40.60	91.09
17	2035.00	3.36E+03	888.95	3.62E+03	43.68	107.62
22	1880.00	1.43E+03	877.10	1.47E+03	46.65	102.82
27	1865.00	1.23E+03	961.99	1.53E+03	51.58	123.83
32	1826.67	1.03E+03	962.57	1.23E+03	52.70	118.90
42	3533.33	5.87E+02	1569.50	7.09E+02	44.42	120.83
52	3783.33	5.80E+02	1692.52	7.72E+02	44.74	133.12
62	3550.00	4.60E+02	1796.52	6.08E+02	50.61	132.07

Table D.4. Mean, standard deviation (STDEV), and coefficient of variation (C.V.) of runoff volumes and pathogen concentrations in runoff measured at 413 cm from the source of slurry in Plot 4 (bare) during simulations under dry initial soil moisture conditions.

<b>PLOT 4 - Bare</b>						
<i>S. e. Typhimurium</i>						
	<b>Mean at 413 cm</b>		<b>STDEV at 413 cm</b>		<b>C. V. (%)</b>	
<b>Time (min)</b>	<b>Runoff Volume (mL)</b>	<b>Concentration (CFU/mL)</b>	<b>Runoff Volume (mL)</b>	<b>Concentration (CFU/mL)</b>	<b>Runoff Volume</b>	<b>Concentration</b>
5	3730.00	3.67E+05	1770.00	1.41E+05	47.45	38.46
10	4330.00	6.30E+03	1870.00	1.22E+03	43.19	19.40
15	4325.00	2.02E+03	625.00	7.47E+02	14.45	37.02
20	7875.00	1.20E+03	1325.00	4.31E+02	16.83	35.98
25	7250.00	7.59E+02	150.00	2.42E+02	2.07	31.86
30	6800.00	6.10E+02	0	2.91E+02	0	47.69
40	13175.00	4.73E+02	1175.00	2.57E+02	8.92	54.33
50	12915.00	3.40E+02	815.00	1.80E+02	6.31	53.02
60	12225.00	2.90E+02	575.00	1.11E+02	4.70	38.40
<i>E. coli</i>						
	<b>Mean at 413 cm</b>		<b>STDEV at 413 cm</b>		<b>C. V. (%)</b>	
<b>Time (min)</b>	<b>Runoff Volume (mL)</b>	<b>Concentration (CFU/mL)</b>	<b>Runoff Volume (mL)</b>	<b>Concentration (CFU/mL)</b>	<b>Runoff Volume</b>	<b>Concentration</b>
5	3730.00	2.42E+05	1770.00	1.06E+05	47.45	43.86
10	4330.00	9.95E+03	1870.00	7.16E+03	43.19	71.93
15	4325.00	6.86E+03	625.00	6.29E+03	14.45	91.69
20	7875.00	5.39E+03	1325.00	3.99E+03	16.83	73.98
25	7250.00	4.03E+03	150.00	3.37E+03	2.07	83.58
30	6800.00	3.58E+03	0	2.67E+03	0	74.58
40	13175.00	2.86E+03	1175.00	2.06E+03	8.92	71.81
50	12915.00	2.00E+03	815.00	1.06E+03	6.31	52.79
60	12225.00	1.77E+03	575.00	9.71E+02	4.70	54.74

## **APPENDIX E**

**Runoff and pathogen data collected at 413 cm from the source of slurry during experiments under wet initial soil moisture conditions**

Table E.1. Mean, standard deviation (STDEV), and coefficient of variation (C.V.) of runoff volumes and pathogen concentrations in runoff measured at 413 cm from the source of slurry in Plot 1 (vegetated) during simulations under wet initial soil moisture conditions.

<b>PLOT 1 - Vegetated</b>						
<i>S. e. Typhimurium</i>						
<b>Time (min)</b>	<b>Mean at 413 cm</b>		<b>STDEV at 413 cm</b>		<b>C. V. (%)</b>	
	<b>Runoff Volume (mL)</b>	<b>Concentration (CFU/mL)</b>	<b>Runoff Volume (mL)</b>	<b>Concentration (CFU/mL)</b>	<b>Runoff Volume</b>	<b>Concentration</b>
6	523.33	0	594.67	0	113.63	0
8	866.67	1.72E+03	859.32	2.98E+03	99.15	173.21
10	2098.67	2.74E+04	1649.17	4.75E+04	78.58	173.21
15	6266.67	7.56E+04	4619.88	1.15E+05	73.72	151.98
20	6583.33	6.16E+04	5037.44	6.86E+04	76.52	111.32
25	6966.67	3.96E+04	5404.01	5.09E+04	77.57	128.56
30	6363.33	1.87E+04	4743.00	2.68E+04	74.54	143.92
40	13550.00	1.09E+04	11134.74	1.60E+04	82.18	146.71
50	14500.00	1.03E+04	12574.18	1.59E+04	86.72	154.16
60	15173.33	5.31E+03	13290.60	7.96E+03	87.59	149.86
<i>E. coli</i>						
<b>Time (min)</b>	<b>Mean at 413 cm</b>		<b>STDEV at 413 cm</b>		<b>C. V. (%)</b>	
	<b>Runoff Volume (mL)</b>	<b>Concentration (CFU/mL)</b>	<b>Runoff Volume (mL)</b>	<b>Concentration (CFU/mL)</b>	<b>Runoff Volume</b>	<b>Concentration</b>
6	523.33	0	594.67	0	113.63	0
8	866.67	3.27E+02	859.32	5.66E+02	99.15	173.21
10	2098.67	1.92E+04	1649.17	3.32E+04	78.58	173.21
15	6266.67	9.41E+04	4619.88	1.49E+05	73.72	158.20
20	6583.33	6.22E+04	5037.44	6.54E+04	76.52	105.15
25	6966.67	3.86E+04	5404.01	4.43E+04	77.57	114.63
30	6363.33	2.22E+04	4743.00	3.04E+04	74.54	137.03
40	13550.00	1.30E+04	11134.74	1.84E+04	82.18	141.15
50	14500.00	1.36E+04	12574.18	2.16E+04	86.72	158.33
60	15173.33	8.04E+03	13290.60	1.30E+04	87.59	161.31

Table E.2. Mean, standard deviation (STDEV), and coefficient of variation (C.V.) of runoff volumes and pathogen concentrations in runoff measured at 413 cm from the source of slurry in Plot 3 (vegetated) during simulations under wet initial soil moisture conditions.

<b>PLOT 3 - Vegetated</b>						
<i>S. e. Typhimurium</i>						
Time (min)	Mean at 413 cm		STDEV at 413 cm		C. V. (%)	
	Runoff Volume (mL)	Concentration (CFU/mL)	Runoff Volume (mL)	Concentration (CFU/mL)	Runoff Volume	Concentration
6	163.33	0	125.03	0	76.55	0
8	1323.33	3.77E+03	1096.10	6.52E+03	82.83	173.21
10	1863.33	3.15E+04	1596.32	5.46E+04	85.67	173.21
15	4916.67	3.53E+04	4153.41	5.23E+04	84.48	147.99
20	4696.67	3.13E+04	4010.71	4.60E+04	85.39	147.23
25	4780.00	1.72E+04	4256.76	2.47E+04	89.05	143.96
30	6083.33	8.93E+03	6342.02	1.17E+04	104.25	131.23
40	13733.33	4.86E+03	14424.40	5.54E+03	105.03	114.09
50	12933.33	3.92E+03	13110.91	4.89E+03	101.37	124.61
60	12940.00	2.80E+03	13475.71	3.47E+03	104.14	123.67
<i>E. coli</i>						
Time (min)	Mean at 413 cm		STDEV at 413 cm		C. V. (%)	
	Runoff Volume (mL)	Concentration (CFU/mL)	Runoff Volume (mL)	Concentration (CFU/mL)	Runoff Volume	Concentration
6	163.33	0	125.03	0	76.55	0
8	1323.33	1.16E+04	1096.10	2.01E+04	82.83	173.21
10	1863.33	1.68E+04	1596.32	2.92E+04	85.67	173.21
15	4916.67	2.99E+04	4153.41	2.86E+04	84.48	95.80
20	4696.67	2.84E+04	4010.71	2.83E+04	85.39	99.77
25	4780.00	1.91E+04	4256.76	1.98E+04	89.05	104.06
30	6083.33	1.21E+04	6342.02	1.14E+04	104.25	94.41
40	13733.33	7.95E+03	14424.40	8.99E+03	105.03	112.97
50	12933.33	5.17E+03	13110.91	6.17E+03	101.37	119.24
60	12940.00	4.35E+03	13475.71	4.92E+03	104.14	113.11

Table E.3. Mean, standard deviation (STDEV), and coefficient of variation (C.V.) of runoff volumes and pathogen concentrations in runoff measured at 413 cm from the source of slurry in Plot 2 (bare) during simulations under wet initial soil moisture conditions.

PLOT 2 - Bare						
<i>S. e. Typhimurium</i>						
Time (min)	Mean at 413 cm		STDEV at 413 cm		C. V. (%)	
	Runoff Volume (mL)	Concentration (CFU/mL)	Runoff Volume (mL)	Concentration (CFU/mL)	Runoff Volume	Concentration
2	483.33	2.13E+05	161.97	3.16E+05	33.51	148.13
3	770.00	4.26E+05	315.12	2.90E+05	40.92	68.11
4	785.00	2.24E+05	247.54	1.50E+05	31.53	67.13
5	786.67	1.04E+05	200.33	1.00E+05	25.47	97.03
6	853.33	3.42E+04	217.79	2.54E+04	25.52	74.21
11	4500.00	2.06E+04	1100.00	1.70E+04	24.44	82.84
16	4180.00	7.54E+03	1576.45	5.22E+03	37.71	69.24
21	4883.33	3.43E+03	1615.81	3.17E+03	33.09	92.40
26	4900.00	2.53E+03	2042.06	2.26E+03	41.67	89.30
31	4500.00	2.31E+03	1905.26	2.08E+03	42.34	90.34
41	8400.00	1.69E+03	4297.66	1.42E+03	51.16	84.14
51	7683.33	1.08E+03	4204.86	1.02E+03	54.73	94.62
61	6516.67	1.01E+03	3875.67	7.69E+02	59.47	76.16

PLOT 2 - Bare						
<i>E. coli</i>						
Time (min)	Mean at 413 cm		STDEV at 413 cm		C. V. (%)	
	Runoff Volume (mL)	Concentration (CFU/mL)	Runoff Volume (mL)	Concentration (CFU/mL)	Runoff Volume	Concentration
2	483.33	2.90E+05	161.97	4.17E+05	33.51	144.06
3	770.00	3.78E+05	315.12	2.58E+05	40.92	68.06
4	785.00	2.94E+05	247.54	2.58E+05	31.53	87.67
5	786.67	1.19E+05	200.33	1.08E+05	25.47	90.41
6	853.33	4.51E+04	217.79	3.78E+04	25.52	83.95
11	4500.00	1.82E+04	1100.00	1.85E+04	24.44	101.41
16	4180.00	8.46E+03	1576.45	7.89E+03	37.71	93.24
21	4883.33	4.18E+03	1615.81	4.33E+03	33.09	103.57
26	4900.00	3.21E+03	2042.06	3.09E+03	41.67	96.33
31	4500.00	2.14E+03	1905.26	1.62E+03	42.34	75.88
41	8400.00	2.18E+03	4297.66	1.87E+03	51.16	85.55
51	7683.33	1.61E+03	4204.86	1.58E+03	54.73	97.76
61	6516.67	1.41E+03	3875.67	1.27E+03	59.47	89.59

Table E.4. Mean, standard deviation (STDEV), and coefficient of variation (C.V.) of runoff volumes and pathogen concentrations in runoff measured at 413 cm from the source of slurry in Plot 4 (bare) during simulations under wet initial soil moisture conditions.

<b>PLOT 4 - Bare</b>						
<i>S. e. Typhimurium</i>						
<b>Time (min)</b>	<b>Mean at 413 cm</b>		<b>STDEV at 413 cm</b>		<b>C. V. (%)</b>	
	<b>Runoff Volume (mL)</b>	<b>Concentration (CFU/mL)</b>	<b>Runoff Volume (mL)</b>	<b>Concentration (CFU/mL)</b>	<b>Runoff Volume</b>	<b>Concentration</b>
1.5	766.67	6.34E+05	650.41	4.11E+05	84.84	64.93
2.5	2206.67	5.96E+05	2127.54	3.26E+05	96.41	54.67
3.5	1400.00	1.77E+05	1188.23	1.09E+05	84.87	61.71
4.5	1776.67	5.83E+04	1640.56	5.27E+04	92.34	90.34
5	953.33	3.91E+04	932.76	3.90E+04	97.84	99.83
10	9026.67	1.67E+04	8458.82	1.69E+04	93.71	101.59
15	8333.33	4.40E+03	7684.62	3.73E+03	92.22	84.60
20	8700.00	2.92E+03	6878.95	2.37E+03	79.07	81.41
25	8400.00	2.23E+03	6315.06	2.03E+03	75.18	91.06
30	8116.67	1.34E+03	6117.67	1.23E+03	75.37	91.51
40	14826.67	9.88E+02	12747.40	9.13E+02	85.98	92.38

<b>PLOT 4 - Bare</b>						
<i>E. coli</i>						
<b>Time (min)</b>	<b>Mean at 413 cm</b>		<b>STDEV at 413 cm</b>		<b>C. V. (%)</b>	
	<b>Runoff Volume (mL)</b>	<b>Concentration (CFU/mL)</b>	<b>Runoff Volume (mL)</b>	<b>Concentration (CFU/mL)</b>	<b>Runoff Volume</b>	<b>Concentration</b>
1.5	766.67	7.24E+05	650.41	4.88E+05	84.84	67.37
2.5	2206.67	6.28E+05	2127.54	3.08E+05	96.41	48.98
3.5	1400.00	2.42E+05	1188.23	1.80E+05	84.87	74.59
4.5	1776.67	7.15E+04	1640.56	4.95E+04	92.34	69.30
5	953.33	3.42E+04	932.76	2.33E+04	97.84	68.21
10	9026.67	2.09E+04	8458.82	1.50E+04	93.71	72.02
15	8333.33	7.28E+03	7684.62	5.93E+03	92.22	81.50
20	8700.00	4.18E+03	6878.95	2.61E+03	79.07	62.48
25	8400.00	3.88E+03	6315.06	3.29E+03	75.18	84.92
30	8116.67	2.77E+03	6117.67	2.12E+03	75.37	76.70
40	14826.67	2.22E+03	12747.40	1.92E+03	85.98	86.55



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