RELEASE AND TRANSPORT OF BACTERIA AND NUTRIENTS FROM LIVESTOCK MANURE APPLIED TO PASTURELAND

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ABSTRACT

Transport of fecal bacteria and nutrients from point and nonpoint sources to surface water bodies is of significant concern in Virginia and the United States. In Virginia, 4,320 river miles are impaired for one or more beneficial use and 72% of the streams are impaired due to pathogen indicators (VDEQ, 2002). Land applications of manure from confined animal systems and by direct deposit by grazing animals are both major sources of fecal bacteria and nutrients in runoff. Therefore, an understanding of the overland transport mechanisms for fecal bacteria and nutrients is very important for the development of best management practices to reduce loading of pathogens and nutrients to surface water bodies. The objectives of this study were to quantify the release and transport potential of three fecal bacterial indicators: *E. coli*, *Enterococcus*, and fecal coliforms; and nitrogen and phosphorus from land applied manure during runoff events. Another objective was to identify the *Enterococcus* species present in dairy manure and determine which species have the highest potential to be transported by runoff.

Release plots were established to study the in-field bacteria and nutrient release. The bacteria and nutrients released from the plots are available to be transported to the edge of the field in runoff. Four manure treatments (turkey litter, liquid dairy manure, cowpies, and none or control) and three land type treatments: pasture with a history of poultry litter application (Turkey Farm), pasture with a history of liquid dairy manure application (Dairy Farm), and pasture with no prior manure application (Tech Research Farm) were studied. During a short but intense rainfall event, the highest bacterial release was measured under the cowpie treatment (*E. coli* concentrations ranging from 37,000 to >300,000 and FC concentrations ranging from 65,000 to >300,000).

Pasturelands with a history of previous manure applications did not release higher bacteria concentrations compared with pasturelands which had never received manure applications. Pasturelands with a history of land application of liquid dairy manure and turkey litter had 143% and 94% higher TSS concentrations available to be transported off the field during overland flow events because of the build up of organic material on the soil surface. TP concentrations released from the cowpie, liquid dairy, and turkey litter treatments were 3.12 mg/L, 3.00 mg/L, and 1.76 mg/L, respectively.

Transport plots were developed to measure the concentrations of fecal bacteria and nutrients present in overland flow at the edge of the field. The bacteria flow-weighted concentrations were highest in runoff samples from the plots treated with cowpies (200,000 CFU/100 mL of *E. coli* and 234,000 CFU/100 mL of FC). The turkey litter had the highest concentration of dissolved phosphorus in runoff from pasturelands (1.22 mg/L), but the cowpie treatment had the highest concentrations of sediment bound phosphorus in runoff (0.73 mg/L). All three treatments investigated in this study contributed to phosphorus loading in surface waters and could potentially increase the risk of eutrophication. Total nitrogen concentrations from the transport plots exceeded the threshold for likely eutrophication problems for all treatments and the total nitrogen concentrations from plots treated with cowpies exceeded the threshold for severe eutrophication problems.

The Biolog System, a method of bacterial source tracking, was used to identify the different species of *Enterococcus* present both in the cowpie source manure and in the runoff collected from the transport plots treated with cowpies. The source manure is dominated by the *Enterococcus mundtii* (55%), *Enterococcus gallinarum* (20%), *Enterococcus faecium* (10%), and *Enterococcus faecalis* (10%). *Enterococcus faecalis* had the highest percentage of isolates present in runoff with a total of 37%, followed by *Enterococcus mundtii* which was present in 21% of the runoff events and *Enterococcus gallinarum* and *Enterococcus faecium* (11%).

Improvements in understanding the bacterial release and overland processes will enhance modeling of bacteria and nutrient transport, and provide a basis for a more realistic evaluation of the impacts of management practices implementation. The data from this study will serve as a baseline to model the release and transport of fecal bacteria and nutrients from agricultural watersheds to surface waters.

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CHAPTER 1 INTRODUCTION

Transport of fecal bacteria and nutrients from point and nonpoint sources to surface water bodies is of significant concern in Virginia and the United States. Elevated concentrations of fecal bacteria in drinking water can be detrimental to human health; potential diseases include <u>Salmonellosis</u>, <u>Anthrax</u>, <u>Tuberculosis</u>, <u>Brucellosis</u>, and <u>Listeriosis</u> (Azevedo and Stout, 1974). Approximately eight percent of U.S. river miles are impaired by pathogens (USEPA, 1998). In Virginia, 4,320 river miles are impaired for one or more use, and 3,111 stream miles or 72% of the streams are impaired due to pathogen indicators (VDEQ, 2002). Among the possible source of impairments in Virginia, agriculture is ranked third behind unknown sources and natural sources, with over 1,000 river miles classified as being impaired by agriculture (VDEQ, 2002). Land application of manure from confined animal systems and by grazing animals is a major source of fecal bacteria and nutrients in runoff. Therefore, an understanding of the overland transport mechanisms for fecal bacteria and nutrients is very important for the development of best management practices to reduce loading of pathogens and nutrients to surface water bodies.

Computer simulation modeling is the primary approach used to develop Total Maximum Daily Loads (TMDL), even though insufficient data exist on several model input parameters related to the release and transport of fecal bacteria in runoff. Furthermore, previous bacteria and nutrient studies often focused on a single manure source and did not provide comparative results from different sources under similar climatic and landuse conditions. In addition, the detachment or release of fecal bacteria and nutrients from land applied sources is not well documented. Improvements in understanding the overland processes will enhance modeling of bacteria and nutrient transport, and provide a basis for a more realistic evaluation of the impacts of management practices implementation.

The overall goal of this study was to quantify the release and transport potential of three fecal bacteria indicators: *E. coli*, *Enterococcus*, and fecal coliform (FC); and nitrogen and phosphorus from land applied manure during runoff events. The specific objectives of this study were to identify differences in transport among various livestock manures by comparing edge-of-field bacterial levels in runoff from pasturelands treated with liquid dairy manure, poultry litter, and cowpies. In addition, this study evaluated bacteria and nutrients release rates for different types of manure applied to pasturelands with different history of previous manure applications. The data from this study will serve as a baseline to model the release and transport of fecal bacteria and nutrients from agricultural watersheds to surface waters.

1.1 Goals and Objectives

The goal of this study was to quantify in-field bacteria and nutrients release and transport from livestock manure during rainfall/runoff events.

The specific objectives of this study were to:

1. Determine bacteria and nutrient release rates for surface applied poultry litter, dairy manure, and cowpies. Determine if bacteria and nutrient release rates differ between pasturelands that have previously been receiving waste applications and those that have not.

2. Identify differences in bacteria and nutrient transport potential of various livestock manures by comparing edge-of-field bacteria and nutrient levels from plots treated with liquid dairy manure, dried poultry litter, and dairy cowpies.

3. Identify the enterococcal species present in dairy manure and determine which species have the highest potential to be transported by runoff.

1.2 Hypothesis

The following hypotheses were tested:

1 Bacteria and nutrient release rates were higher from pasturelands with a history of land application of waste.
2 Edge-of-field bacteria and nutrient concentrations were highest from plots treated with cowpies, followed by liquid dairy manure and turkey litter.

1.3 Thesis Organization

The objectives of this thesis were achieved through field and laboratory studies. Chapter 2 reviews the literature on bacteria, nutrients, and bacterial source tracking. Chapter 3 describes both the field and laboratory methods for completion of this research. Chapter 4 presents the results and discusses the findings. Chapter 5 includes an overall summary and conclusions of the research.

CHAPTER 2 LITERATURE REVIEW

This chapter provides background information on the behavior of bacteria and nutrients, in addition to a baseline for comparison of the results. The bacteria section covers the effect of bacteria on human health, background information on indicator species, and bacteria fate, transport and release. The bacterial source tracking section describes the most recent methods and provides background information on enterococci. The nutrient section also discusses the effects of nutrient loading on human health and the environment.

2.1 Bacteria

Land application of animal wastes is a useful method of both disposing of animal waste and supplying nutrients to crops and pastureland. When manure from confined animal facilities is applied to the land, bacterial survival is generally considered to be affected by the environment at the time of waste application and the method of waste application. Bacterial die-off can decrease transport potential and reduce bacterial loading into surface water bodies. Elevated bacterial concentrations in surface water bodies can be detrimental to the health of humans, animals, and the environmental ecosystem. Bacteria and viruses applied to the land along with organic wastes may die-off, regrow, become extracted by the water, associate with waste particles, or be retained or associated with soil particles (Reddy et al., 1981). Those that are extracted by water have to the potential to be leached into groundwater or transported into streams along with runoff. Bacteria and viruses associated with waste particles or be retained or associated with soil particles also have to the potential to be transported into streams along with runoff (Reddy et al., 1981).

2.1.1 Human Health

Animal manure contains many different types of organisms pathogenic to humans and animals. Pathogenic bacteria may be transferred to humans when over-applied to agricultural lands. Some potential diseases that can be transferred to humans from infected cattle are salmonellosis, leptospirosis, anthrax, tuberculosis, Johne's Disease, brucellosis, listeriosis, tetanus, tularemia, erysipelas, and colibacilosis (Azevedo and Stout, 1974). However, by utilizing best management practices and taking care to not overload the capacity of the land, land application of waste materials may be the best method of recycling organic wastes (Moore et al., 1982).

Morrison and Martin (1977) reviewed the health aspect of applying waste to the land. In their study, they suggested the following:

1. Crops that will be eaten raw or directly grazed should not receive manure, sludges, or slurries until adequate time is allowed for bacterial die-off;
2. The number of applications on a single site should be limited to reduce the chance of pathogenic bacteria build-up;
3. Areas with high-density population should not receive applications in order to prevent disease transmission by wind, insects, rodents, or flowing waters; and
4. Infected animals should be treated and isolated to prevent high levels of pathogenic bacteria in the animal waste.

2.1.2 Indicator Species

Pathogenic organisms present in animal waste can be transferred to humans via water. Total coliforms (TC), *Enterococcus*, *E. coli*, fecal coliform (FC), and fecal streptococci (FS) bacteria are common indicator species, which identify the potential presence of disease causing organisms. Tests for coliforms, the original indicator species, were originally developed to test drinking water that may have been contaminated by contagious organisms in wastes from human beings. Since specific pathogens are very difficult to trap and culture, the coliform groups were chosen because they are easy to detect, simple to culture, and indicative of fecal contamination from warm-blooded animals (Larsen et al., 1994). TC is not a reliable indicator since the category includes numerous nonfecal sources, making the indicator group too broad (USDA, 2000).

Fecal coliforms are a subgroup of TC and they originate specifically from intestinal tracts of warm-blooded animals. FC is currently the predominant indicator used to assess bacterial pollution in watersheds (USDA, 2000). *E. coli* is a member of the FC group. The presence of *E. coli* correlates with illness from swimming in both fresh and marine water. *E. coli* includes the O157:H7 strain which is toxin producing. FS differ from FC in that they are less dominant in feces, are not known to multiply in the environment, and die-off more rapidly. A subgroup of FS, *Enterococcus*, is commonly found in intestinal tracts of humans and other warm-blooded animals. The presence of *Enterococcus* correlates well with illness from both fresh and marine waters. The fecal streptococci include *Streptococcus faecalis, S. faecium,* and *S. avium* (USDA, 2000).

Indicator species are assumed to be affected by environmental conditions in the same manner as the pathogens in the soil (Crane et al., 1980). Dazzo et al. (1973) found that the die-off of *Salmonella enteritidis* is similar to fecal coliform die-off when exposed to comparable conditions. A study by Morrison and Martin (1977) concluded that indicator organisms are most likely more resistant to die-off than actual pathogens, so even if the data provided by indicator organisms is not comparable to the die-off of pathogens, the error is most likely on the side of public safety.

Indicator organisms are commonly used instead of the actual pathogens because of three reasons (Wang and Mankin, 2001):

1. Indicator bacteria are usually present in greater numbers than pathogens;
2. Indicator bacteria are easier to isolate; and
3. Indicator bacteria are much safer to work with than pathogens.

Ideal indicators have the following characteristics (Moore et al., 1982):

1. Exist in large numbers in the contributing source and at levels far greater than pathogens associated with the waste;

2. The die-off or re-growth of the indicator in the environment should parallel that of the fecal pathogen;
3. The indicator should only be found in association with the particular waste source and its presence, therefore, is a positive indication of contamination.

Table 1 presents the federal standards for primary contact with the most common indicator organisms. Primary contact water includes recreational uses such as fishing and swimming.

Table 1. Federal Criteria for primary contact with TC, FC, *E. coli*, FS, and *Enterococcus* (USDA, 2000)

Microbial Indicator	**Federal Criteria**
Total Coliforms	1,000 CFU/100 mL
Fecal Coliforms	200 CFU/100 mL
E. coli	126 CFU/100 mL
Enterococcus	33 CFU/100 mL

Epidemiological studies have revealed that *Enterococcus* levels are more closely associated with enteric disease than coliforms (USDA, 2000). The U.S. EPA has proposed a revision of the contact recreation bacterial water quality criteria from fecal coliforms to *E. coli* or *Enterococcus* (USEPA, 1986). Virginia (VDEQ, 2003) has revised its primary contact freshwater standard for *E. coli* to a geometric mean limit of 126 *E. coli*/100 mL or 235 *E. coli*/100 mL for a single sample maximum. Saltwater standards for *Enterococcus* have a geometric mean limit of 35 enterococci/100 mL or 104 enterococci/100 mL for a single sample maximum.

2.1.3 Source Bacteria Concentrations

Fecal bacteria concentrations vary widely among different sources. This variability may be due to animal age, ration, housing system, and manure management systems. The bacteria composition of the manure may be affected by the animal health, use of antibiotics or other inhibitory substances in the feed, environmental stresses on the animal, and the amount of cleaning and disinfection used in the livestock operations. Other differences may be due to the variations among testing methods and media preparations (Moore et al., 1982).

Map Tech, Inc. (2000) used the membrane filtration method to evaluate source manure for FC concentrations. Dairy cattle fecal deposits were found to have an average concentration of 4.3×10^5 CFU/g and a dairy storage pit was found to have an average concentration of 1.2×10^4 CFU/g. Geldreich et al. (1962) found the FC concentration in turkey manure to be 2.9×10^4 CFU/g.

Panhorst (2002) evaluated FC and *E. coli* concentrations in three dairy manure storage ponds immediately after agitation. Bacterial counts were highest immediately after agitation and gradually decreased over time. When the storage ponds were re-agitated, the counts once again increased. Panhorst (2002) also evaluated the *E. coli* and FC concentration in dairy milker fecal deposits beginning in May and repeated the procedure in July. The results are presented in Table 2. Differences among studies are most likely the result of the differences in site characteristics and/or environmental conditions.

Table 2. *E. coli* and Fecal coliform concentrations in dairy manure storage ponds and dairy milker fecal deposits (Panhorst, 2002)

	Month	*E. coli*	Fecal coliform
Dairy manure storage ponds	April to May	4.9×10^6 to 5.8×10^6 CFU/100 mL	5.3×10^6 to 6.5×10^6 CFU/100 mL
Dairy milker fecal deposits	May	1.9×10^5 to 2.95×10^5 CFU/g	2.0×10^5 to 3.05×10^5 CFU/g
Dairy milker fecal deposits	July	8.5×10^5 to 2.5×10^7 CFU/g	1.2×10^7 to 3.5×10^7 CFU/g

2.1.4 Bacterial Survival

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

Van Donsel et al. (1967) studied the effects of seasonal variation on the survival of FC and FS in soil. The survival of FC and FS was studied for several years at shaded and exposed outdoor soil plots. The 90 percent reduction for FC occurred after 3.3 days in the summer and 13.4 days in the autumn, while the FS 90 percent reduction times ranged from 2.7 days in summer to 20.1 days in the winter. Howell et al. (1995) found that fecal coliform mortality rates decreased as the sediment particle size became finer and as temperature decreased. The study did not find evidence of interaction between temperature and particle size in determining fecal bacteria persistence. Taylor and Burrows (1971) found that *E. coli* survived 7 to 8 days and *Salmonella dublin* persisted up to 18 days on growing pastures. Cutting the pastures reduced the bacterial survival time on the grasses, most likely through its effect on drying rates and increased exposure to solar radiation.

Several studies have also detected after-growth following land application of waste. This occurrence may be explained by the predominating factor approach, which suggests that when a single factor is the limiting or excessive variable in the bacterial environments, it causes a reduction in enteric indicator populations. Van Donsel et al. (1967) noticed after-growth of both tracer fecal coliforms and nonfecal coliforms. The after-growth was thought to be stimulated by nutrients remaining from the broth inoculum used to apply a cultured fecal coliform to the field plots. During nonfreezing conditions, an increase in the nonfecal coliforms appeared most often after a rainfall. The increase seemed to be related to the temperature conditions following the rain rather than the amount of rain. Very warm weather following a rain could cause up to 100-fold increase in the soil coliforms.

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

Several studies have found that many of the organisms applied with manure are filtered out in the soil. A study by Gerba et al. (1975) found that 92 to 97 percent of the bacteria filter out in the first centimeter of soil. Those remaining filtered out in the next 4 cm of soil. Gerba et al. (1975) also found that as bacteria and organic substances accumulate on the soil surface, the trapped bacteria become part of the filtration system, and increase the filtration properties of the soil.

Faust (1982) studied the relationship between bacterial concentration and landuse. Samples were taken on pastureland, cornfield, and forest sites. On the pastureland, total coliform (TC), fecal coliform (FC), total streptococci (TS), and fecal streptococci (FS) consisted of an even portion of sediment populations in the 0- to 1-cm layer from the surface of the soil. Bacterial levels generally declined with depth, except for TS concentrations. In the surface 1 cm, bacterial numbers per gram of pasture soil were 252 to 1,603 TC, 10 to 396 FC, 317 to 2,270 TS, 163 to 1,330 FS, and 1.6 to 65.1×10^4 aerobic heterotrophic bacteria (TVC). Cattle grazed the pastureland during the spring and fall and were absent from the field during the winter and summer. The FC levels were 22 to 396 FC MPN/g in the fall and spring and 10 to 50 FC MPN/g in the summer and winter. Manure storage may reduce bacterial survival by allowing die-off to occur before it is applied to the soil. The amount of bacteria deposited on the land is a function of the livestock as well as whether or not the waste is stored prior to spreading. Storage also allows manure to be spread under optimum climatic conditions. Waste spread on frozen soils may not infiltrate and bacterial survival may be prolonged due to the low temperatures (Moore et al., 1988). A study by Crane et al. (1983) showed a trend toward minimal bacterial losses from liquid applied waste systems and greatest losses for solid spread methods, but the differences were not significant (Crane et al., 1983 (unpublished data) – as cited in Moore et al., 1982).

2.1.5 Bacterial Transport in Overland Flow

Indicator organisms, present in soils along with pathogens, may contaminate surface water and groundwater through downward leaching with infiltrating water, movement with surface runoff, and transport by attachment to sediment and waste particles (Reddy et al., 1981). Pathogenic organisms are largely retained at or near the soil surface (Faust, 1982), thus increasing the potential for pollution of surface runoff water. Although concentrations in runoff may be high, agricultural runoff is diluted as it enters streams and water bodies, reducing the impact of high concentrations. Bacterial transport is highly dependent upon the hydrologic characteristics of the watershed. If bacteria sources are in areas that do not contribute to overland flow, the contribution to fecal pollution in the stream from that area will be minor (Baxter-Potter and Gilliland, 1988).

The transport of bacteria in overland flow is affected by rainfall duration and intensity, method of manure application, fecal deposit age, and adsorption of cells to soil particles. Because manure is less dense than soil, incorporating manure into soil increases the soil's interrill erodibility and thus the amount of bacteria detached by overland flow (Khaleel et al., 1979a). Low amounts of manure can enhance the condition of the soil by improving the soil aggregate size and water holding capacity of the soil. When manure is applied at higher application rates, the large contribution of monovalent ions from incorporated manure was found to be responsible for increasing soil erodibility in a study by Mazurak et al. (1975). Plots were disked to a depth of 10 cm. Soil detachment from manured plots (application rate of 415 t ha^{-1} yr^{-1}) was 89 mg/cm^3 while soil detachment from non-manured plots was 55 mg/cm^3.

The distribution of bacteria between that which is attached to sediment particles versus that which is dissolved and transported into water bodies via overland flow or leaching is not clearly understood. In addition, it is unclear if cells adsorbed to soil particles may offer resistance to transport by overland flow or if they are transported along with eroding soil particles. The outer surfaces of bacterial cells are normally negatively charged and are attracted to positively charged particles in the soil. However, electrical forces do not appear to be fully responsible for attachment. Bacterial attachment to soil particles may also occur because of secretion of adhesive substances, clay content, soil cation exchange capacity, organic matter content, pH, temperature, and soil moisture content (Moore et al., 1988). Mitchell and Chamberlin (1978) found that clays tend to adsorb coliforms more than silts or sands. Another study found that 90 to 95 percent of the coliforms entering a lake from upland watersheds were associated with 0.45 to 5 μm particles (Gannon et al. 1983).

Runoff from snowmelt or rainfall can carry viable bacteria from fresh manure into the stream. Doran and Linn (1979) found that runoff from a grazed pasture had fecal coliform concentrations 5-10 times higher than from an ungrazed pasture. Patni et al. (1985) found that bacterial contamination of surface runoff was greater during wetter periods of the season than during dryer periods.

Baxter-Potter and Gilliland (1988) summarized typical values from the literature for cornfields, pasture, and feedlots in a literature review of bacteria in runoff from agricultural lands (Table 3).

Crane et al. (1983) included a summary table of the results from previous investigations on bacterial runoff quality. Some of the investigations presented in the review from grazing systems and manure application runoff are presented in Table 4. In many investigations there was little difference in the bacterial concentration in runoff from areas where manure was applied and from control areas.

Table 3. Densities of indicator organisms present in runoff from three different landuses. Bacterial densities are presented in CFU/100 mL

Indicator organism	Literature Values (CFU/100 mL)
	Corn Field
F.C.	5,400-14,300
F.S.	16,200-39,000
	Pasture
F.C.	1,000-57,000
F.S.	1,750-172,000
	Feedlot
F.C.	1,350,000-79,000,000
F.S.	8,000,000-79,000,000

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

Table 4. Results from previous investigations on bacterial runoff quality (Crane et al., 1983)

Description of Study	Organism	Bacterial Numbers in Runoff		Reference
Surface applied manure on		Treated	Control	Kunkle, 1979
grass pasture (Vermont) with	F.C.	$0.1 - 1.0 \times 10^2$	$0.1 - 1.0 \times 10^4$	
control area runoff measured	F.S.	$1.0 - 5.0 \times 10^4$	$0.5 - 2.0 \times 10^5$	
	T.C.	$1.0 - 9.0 \times 10^4$	$1.0 - 9.0 \times 10^4$	
		Treated Area Runoff		
Manure soils application (SC)	F.C.	3.0×10^4		Janzen et al., 1974
Liquid manure application	F.C.	5.0×10^4		
Dairy waste application to		Treated Area Runoff	Control Area Runoff	
bermudagrass pasture plots				
applied as (Alabama)	F.C.	$1.1 - 4.0 \times 10^6$	9.9×10^5	McCaskey et al., 1971
a. Sprinkler irrigated	F.S.	$4.5 – 8.6 \times 10^6$	1.1×10^6	
effluent	T.C.	$6.5 - 40 \times 10^6$	2.0×10^6	
	F.C.	$1.4 – 21.7 \times 10^6$	9.9×10^5	
b. Slurry spread by	F.S.	$2.5 - 92 \times 10^6$	1.1×10^6	
tank wagon	T.C.	$1.1 – 2.2 \times 10^7$	2.0×10^6	
	F.C.	$1.1 – 38.5 \times 10^6$	9.9×10^5	
c. Solids application	F.S.	$1.4 – 16.3 \times 10^7$	1.1×10^6	
	T.C.	$1.8 - 24 \times 10^7$	2.0×10^6	
Runoff from land disposal		Treated Area Runoff		Robbins et al., 1971
areas from different animal				
types. (N. Carolina)				
a. poultry waste	F.C.	9.6×10^3		
application	F.C.	2.0×10^5		
b. beef waste application	F.C.	3.1×10^5		
c. control area runoff	F.C.	1.0×10^4		
Dairy resting pasture runoff		Resting Pasture		Barker and Sewell, 1973
(Tennessee)	F.C.	8.9×10^3		
	F.C.	2.0×10^4		
	T.C.	1.8×10^5		
	T.C.	7.6×10^4		

Grass filter strips are used as a management tool for soil erosion and reducing nutrient loading into water bodies. A study by Coyne et al. (1995) concluded that grass filter strips alone are unable to reduce FC concentrations in runoff to meet primary water contact standards of 200 FC/100mL. The grass filter strips were 9 m long and effective in removing 99% of the sediment in the surface runoff. Fecal coliform removal rates from the surface runoff were 74% and 43%.

2.1.6 Bacterial Release

Thelin and Gifford (1983) placed standard cowpies on a platform and rained on them to determine the release of fecal coliforms. Fecal deposits 5 days old or less released fecal coliform concentrations into the water on the order of millions per 100 mL. Fecal deposits that had not been rained on for up to 30 days released fecal coliform concentrations on the order of 40,000 per 100 mL. All counts were determined using the MPN method.

Kress and Gifford (1984) also evaluated fecal coliform release from cowpies. The study found that even cowpies that are 100 days old are still potential sources of FC contamination. The peak count on a 100-day-old deposit was 4,200 fecal coliforms per 100 mL of water using the MPN method. Approximately 1,000 one hundred-day old fecal deposits are equal to one 2-day old deposit. Rainfall intensity was determined to only be significant when the cowpies are completely dried (beginning at day 20). The peak coliform counts were found to be significantly lower when the fecal deposits had been previously wetted, indicating that the lower losses are due to the previous rainfall event.

Larsen et al. (1994) placed bovine feces at 0.0, 0.61, 1.37, and 2.13 m from a runoff collection point to evaluate the release of FC. At the 0.0-m distance from the fecal deposit, the runoff bacterial concentrations corresponded to a release of 17% of the total FC in the manure, or between 40×10^6 and 115×10^6 organisms/mL. These values were significantly higher than those measured at the 2.13-m distance from the fecal deposit, where less than 5% of the organisms applied to the plots were present in runoff.

2.1.7 Standard Cowpies

Hafez et al. (1969) found that fecal deposits from cattle were not uniformly distributed throughout a pasture. The non-uniform distribution can result in approximately 0.4-2.0% of the area covered by fecal deposits, annually. However, in certain areas, such as water troughs, gates, fence lines, and bedding areas, manure concentrations may be much higher. Cattle allowed to roam freely on pastureland will defecate an average of 12 times per day (Miner et al., 1992).

Thelin and Gifford (1983) developed standard cowpies to study fecal coliform release patterns. The fecal deposits were formed by taking freshly mixed fecal material and placing it in a mold with a diameter of 20.3 cm and a depth of 2.54 cm. Fecal deposits were placed in the mold until a weight of 0.9 kg was reached.

A study by Kress and Gifford (1984) also evaluated fecal coliform release from cowpies. This study used the same method for forming standard cowpies as the study by Thelin and Gifford. The average weight of fresh naturally occurring cowpies was determined by weighing 100 fresh deposits. The mean weight of the naturally occurring fecal deposits was 1.24 kg. To develop the standard cowpies, the fresh manure was collected and mixed in a cement mixer for 15 minutes. The mold procedure used by Thelin and Gifford (1983) was used as described above. The standard cowpies were tested against naturally occurring fecal deposits for peak release. The peak release regression from the naturally occurring fecal deposits was not significantly different than the regression for the standard cowpies. From this investigation, the authors were able to conclude that the standard cowpies did not change the release patterns.

2.1.8 Summary

When manure from confined animal facilities is applied to the land, bacterial survival is affected by the environmental conditions at the time of waste application and the method of waste application. Pathogenic bacteria may be transferred to humans when over-applied to agricultural lands. Some potential diseases that can be transferred to humans from infected cattle are salmonellosis, leptospirosis, anthrax, tuberculosis, Johne's Disease, brucellosis, listeriosis, tetanus, tularemia, erysipelas, and colibacilosis. Indicator organisms, present in soils along with pathogens, may contaminate surface water and groundwater through downward leaching with infiltrating water, movement with surface runoff, and transport by attachment to sediment and waste particles.

In Virginia, over 1,000 river miles are classified as being impaired by agriculture (VDEQ, 2002), and 72% of all impaired streams are impaired due to pathogen indicators. Land application of manure from confined animal production facilities and grazing animals are major sources of fecal bacteria in runoff. It is important to evaluate the transport and release of different source manures under similar field conditions so that an appropriate comparison may be made between the fecal contamination potential. In addition, the detachment or release of fecal bacteria and nutrients from land applied sources is not well-documented. Knowledge of the transport and release properties of different manure types will assist in providing sufficient data for model input parameters for the development of TMDLs using computer simulation models.

2.2 Bacterial Source Tracking

Bacterial Source Tracking (BST) procedures are used to determine the sources of fecal bacteria from environmental samples (such as from human, livestock, or wildlife origins). BST methods can be utilized in TMDL development for fecal bacteria. Once the source of fecal contamination has been determined, best management practices can be properly implemented within the watersheds to control fecal loading to streams and waterways.

Currently there is no BST method that has emerged as the best for all applications. The BST methods that have been developed can be divided into three basic groups: molecular, biochemical, and chemical. Molecular and biochemical methods require the development of a source library of samples collected from the watershed. The library is used to identify the bacterial sources found in contaminated waterways.

Molecular BST methods are often called DNA fingerprinting and are based on the different genetic makeup (genotype) of the existing strains or subspecies of fecal bacteria (Kern et al., 2000). The molecular method is based on the hypothesis that the distinctions between fecal bacteria from different animals and humans are due to selective pressures in the intestinal environments. Since intestinal environments are not the same, the fecal bacteria will develop with detectable differences that can then be related to the sources (Hagedorn, 2003a).

Biochemical BST methods (phenotype) are based on the effect of an organism's genes that actively produce a biochemical substance (Kern et al., 2000). There are several advantages to using biochemical methods rather than molecular methods (Hagedorn, 2003a). Biochemical methods require less training for laboratory personnel, and cost less for each isolate for both time and materials. A larger number of isolates can be performed in a much shorter time period using biochemical methods. While molecular methods are thought to be more precise, research results show that some biochemical methods may be just as precise. It is recommended that a non-molecular method be validated with a molecular method.

Chemical methods are designed to detect chemical compounds that are often associated with humans such as caffeine and optical brighteners (Hagedorn, 2003a). These tests are not used to detect any form of fecal bacteria, but may be used to confirm a suspicion that there is a human source in the area. Chemical methods do not provide any information on animal sources that may exist.

The nutritional pattern method is a biochemical method that uses the wide range of carbon and nitrogen sources used by bacteria for energy and growth. The Biolog System is a commercial system based on carbon source utilization profiles and can be used along with the Biolog library to identify over 2,000 species of microorganisms (Biolog, 2003). It is used to perform, score, and tabulate 96 carbon source utilization tests per isolate, which eliminates the judgment calls common in most other microbial source tracking methods (Hagedorn et al., 2003c). Biolog is widely used in the medical field for microbial identification. Figure 1 shows the Biolog System carbon source wells being inoculated for microbial identification. The method has been found to work well in a clinical laboratory setting, but may not be as accurate when evaluating environmental isolates. This is because there are many environmental factors in a watershed that can affect the bacterial nutrient requirements.

A study by Hagedorn et al. (2003c) used carbon source utilization as a form of phenotypic fingerprinting to classify enterococcal isolates from known fecal sources in four different geographical regions. The study concluded that the commercial Biolog System provided levels of correct classification from the enterococcal library that are in the upper range of those reported in the literature.

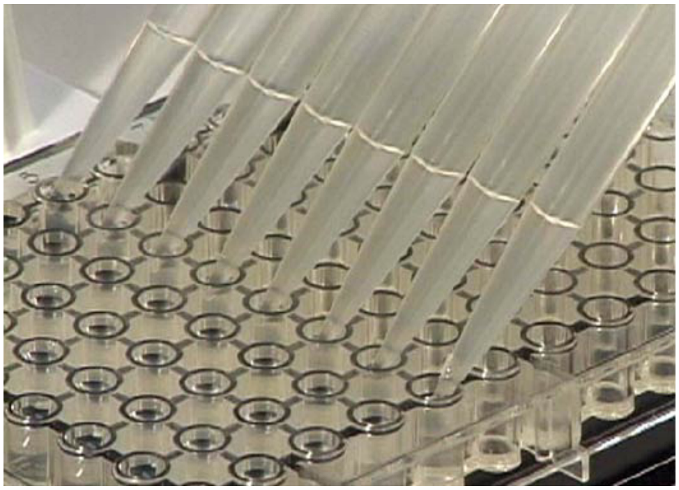

Figure 1. Carbon source wells being inoculated for microbial identification

2.2.1 Properties of Enterococci

More than 20 enterococcal species are now recognized (Aarestrup et al., 2002). The *Enterococcus* group is a subgroup of the fecal streptococci and are differentiated from other gram-positive cocci by their ability to grow at high pH (9.6 at 10), high temperature (45°C) and in high salt concentrations (6.5% sodium chloride) (Hagedorn, 2003b). *Enterococcus faecalis* and *Enterococcus faecium* are the most frequent species found in humans, and *Enterococcus faecalis* causes the majority of human enterococcal infections (McShan and Shankar, 2002). The identifying characteristics of enterococci are listed below (Hagedorn, 2003b):

1. Gram positive
2. Cocci shape
3. Nonmotile
4. Occur in pairs or short chains
5. Cells are one micrometer in diameter
6. Predominately inhabit human intestines
7. Faculative anaerobes (prefer anaerobic)
8. Complex and variable nutritional requirements
9. Resistant to many Gram positive antibiotics
10. Perform simple fermentation
11. Mechanism of pathogenicity unknown
12. Used as indicators of fecal pollution in the purification of water and dried and frozen foods
13. Members of genus streptoccous
14. Belong to Lancefield's serologic group D Streptococcus
15. Catalase negative
16. Can grow in 6.5% NaCl
17. Can grow at a pH range of 9.6 to 4.6
18. Can grow at temperatures ranging from 10 to 45°C
19. Optimum growth at 37°C
20. Sensitive to chlorination

2.2.2 Distribution of Enterococccal Species in Source Manure

Wheeler et al. (2002) evaluated human, Canada goose, cattle, deer, dog, chicken, and swine for the presence of *Enterococcus faecalis*. Of the 392 isolates that were considered to be *Enterococcus*, 5.6% were *Enterococcus Durans*, 15.6% were *Enterococcus Faecalis*, 25.0% were *Enterococcus faecium*, 21.9% were *Enterococcus Gallinarum*, and 31.9% were unidentifiable. The study determined that *Enterococcus faecalis* was limited to dogs, humans, and chickens and that other methods of BST could be used to differentiate between the three species.

Hagedorn et al. (2003c) used the commercial Biolog System to determine if carbon source utilization is an accurate method to identify sources of fecal pollution in water. Unknown source isolates were collected from three different streams in Virginia with human, livestock, and wildlife suspected contamination. Of the 64 isolates identified as *Enterococcus faecalis*, 54 were from human sources, 64 of 71 isolates identified as *Enterococcus gallinarum* were from non-human sources, and 51 of 72 isolates of *Enterococcus mundtii* were from non-human sources.

Bernstein et al. (2003) used the Biolog System to test 50 isolates from cow manure in California. Their study found *Enterococcus faecalis* to be 2% of the total isolates identified. Thirty eight percent of the isolates were identified as *Enterococcus casseliflavus,* 31% were identified as *Enterococcus flavescens*, and 12% were identified as *Enterococcus gallinarum*. An unpublished study by Hagedorn (C. Hagedorn, unpublished data, 2003. Blacksburg, Va.: Virginia Tech) used the Biolog System to test 53 isolates from cow manure in Delaware. This study found that 53% of the isolates were identified as *Enterococcus mundtii*, 21% were identified as *Enterococcus gallinarum*, 9% were identified as *Enterococcus casseliflavus* and *Enterococcus faecium*, 4% were identified as *Enterococcus flavescens*, and 2% were identified as *Enterococcus durans* and *Enterococcus faecalis*.

A study performed on the River Riato in Spain (Fernandex-Alovarex et. al, 1991) identified species of enterobacteria present in the water samples. The lowest enterobacterial count (MPN=10^3/100 mL) was obtained in April, but the count increased by three orders of magnitude by the month of May (10^6/100 mL). In September, the count decreased. The counts increased with the beginning and end of the sesteo, which is the season during which cattle are allowed to roam freely in the river. Species of enterobacteria isolated included: *Enterobacter cloacae, Ent. Intermedium, Citrobacter diversus,* and *Edwarsiella spp*. *Streptococcus bovis* was also isolated, but *Strep. faecalis* was not found in the samples.

Pourcher et al. (1991) used microfilter plates containing selective media to evaluate counts of *Enterococcus* from human and animal origin. From the human source, 44% of the isolates were identified as *Enterococcus faecium* and 37% were identified as *Enterococcus faecalis*. From cows, 25% were identified as *Enterococcus faecium* and 0% were identified as *Enterococcus faecalis*; from poultry 69% were identified as *Enterococcus. faecium* and 19% were identified as *Enterococcus faecalis.* The study concluded that *Enterococcus faecalis* was predominant in human and poultry feces.

Most regulatory agencies are interested in *E. coli* and *Enterococcus faecalis* as indicator bacteria (USEPA, 2001). If *Enterococcus faecalis* has a limited host range which includes humans, waters contaminated with human feces should be given first priority, while saving time and cost in identifying these waters. Wheeler et al. (2002) found that *Enterococcus faecalis* had a host range that was limited to humans, dogs, and chickens. *Enterococcus faecalis* was not present in Canada goose, cattle, deer, or swine. The discrepancy between this study and previous studies is most likely caused by different isolation and identification media. Wheeler et al. (2002) identified isolates based on selected defined biochemical characteristics including arginine hydrolysis, and carbon utilization of pyruvate, arabinose, L-sorbose, and raffinose. They found that false positives were caused by the excessive amounts of yeast extract, so new basal media for carbon source utilization and arginine hydrolysis was developed. When the yeast extract was lowered to 0.2 g/L, the false positives were eliminated.

2.2.3 Summary

Many different BST methods are currently being evaluated to determine the most accurate, cost effective method. The commercial Biolog System uses carbon source utilization and can be used along with the Biolog library to identify species of microorganisms. While many studies have evaluated the different species of enterococcus available in source manure, it is unknown which species are most transportable during a runoff event.

2.3 Nutrients

Land application of waste from confined animal production facilities is an effective method of disposing of animal waste while supplying nutrients to crops and pastureland. For many years, land application of manure from animal production facilities has been based on the nitrogen demand of the crops. When the nitrogen demands of the crop are met, excess phosphorus available in the source manure is not utilized by the crops and as a result, phosphorus has accumulated in the soils. When land for waste application is limited, excess waste is applied to the available area and agronomic application rates are not considered. The over-application of both phosphorus and nitrogen to the soils has resulted in the transport of nutrients, in the dissolved form and attached to eroding soil particles, in runoff to downstream water bodies.

Increased nutrient concentrations in surface waters can lead to eutrophication. "Eutrophication refers to the natural and artificial addition of nutrients to bodies of water and to the effect of these added nutrients on water quality" (Novotny and Olem, 1994). Properties of eutrophic waters include low oxygen levels, reduced aquatic species diversity, turbidity, and poor taste and odor in public water supplies (Hansen et al., 2002). The oxidation of excessive organic matter can depress dissolved O_2 levels below the respiration requirements of fish (Edwards and Daniel, 1994), resulting in fish kills. The amount of phosphorus lost from a field that can contribute to increased eutrophication of downstream water bodies can be less than 2 kg ha^{-1} yr^{-1} (1.8 lb ac^{-1} yr^{-1}), while the recommended application rates to corn in the Midwest can vary from 25 to 45 kg ha^{-1} yr^{-1} (Rehm et al., 1996). Novotny and Olem (1994) state that total phosphorus concentrations in lakes as low as 20 µg/L can lead to eutrophic properties. The eutrophication problem threshold for nitrogen concentrations in water bodies begins as low as 0.092 mg/L. Problems are likely to exist when nitrogen concentrations reach 0.92 mg/L, and severe problems are possible when the nitrogen concentrations reach 9.2 mg/L (USEPA, 1982).

Accumulation of nutrients in the soils can result in leaching of nitrate and dissolved phosphorus into groundwater. Nitrate easily moves with water through the soil and into groundwater systems that may be used for drinking purposes, during heavy rainfalls or in over-irrigated areas. The accumulation of phosphorus in the soils has also led to the infiltration of phosphorus into the groundwater.

Virginia DEQ (2003) water quality standards limit nitrate concentrations to 10 mg/L in public water supplies. The presence of nitrates in drinking water is known to cause blue baby syndrome, where the blood lacks the ability to carry enough oxygen to the cells in the body. This can occurs when mothers use tap water to mix formula for infants (ENN, 2001). A study by the University of Iowa (ENN, 2001) assessed nitrate exposure from drinking water for nearly 22,000 women between the age of 55 and 69 who lived in 400 Iowa communities and used the same drinking water supply for more than 10 years. The researchers found an increased risk for bladder cancer as the nitrate concentrations in the drinking water supplies increased. Women with an average drinking water nitrate level greater than 2.46 mg/L were 2.83 times more likely to develop bladder cancer than the women with lower nitrogen exposure levels. The Virginia (VDEQ, 2003) water quality standards for nitrogen in groundwater vary by physiographic region. Table 5 presents the nitrogen standards for groundwater in Virginia.

Table 5. Nitrogen groundwater quality standards for the different physiographic regions in Virginia (VDEQ, 2003)

	Coastal Plain	**Piedmont & Blue Ridge**	**Valley & Ridge**	**Cumberland Plateau**
pH	6.5 – 9	5.5 – 8.5	6 – 9	5 – 8.5
Ammonia Nitrogen	0.025 mg/L	0.025 mg/L	0.025 mg/L	0.025 mg/L
Nitrite Nitrogen	0.025 mg/L	0.025 mg/L	0.025 mg/L	0.025 mg/L
Nitrate Nitrogen	5 mg/L	5 mg/L	5 mg/L	0.5 mg/L

Allowable ammonia concentrations in surface waters vary based on the pH and temperature of the water body. In freshwater, the chronic ammonia criteria require that the thirty day average not be exceeded more than once every three years. The criteria are presented in Table 6 (VDEQ, 2003).

Table 6. Chronic ammonia criteria for freshwater based on pH and temperature

	Total Ammonia (mg/L)						
pH	**0°C**	**5°C**	**10°C**	**15°C**	**20°C**	**25°C**	**30°C**
6.5	3.02	2.82	2.66	2.59	2.53	2.5	2.5
6.75	3.02	2.82	2.66	2.59	2.53	2.5	2.5
7.0	3.02	2.82	2.66	2.59	2.53	2.5	2.5
7.25	3.02	2.82	2.66	2.59	2.53	2.5	2.5
7.5	3.02	2.82	2.66	2.59	2.53	2.5	2.5
7.75	2.80	2.60	2.47	2.38	2.35	2.3	2.4
8.0	1.82	1.71	1.62	1.57	1.55	1.56	1.59
8.25	1.03	0.97	0.93	0.91	0.90	0.91	0.95
8.5	0.58	0.55	0.53	0.53	0.53	0.55	0.58
8.75	0.34	0.32	0.31	0.31	0.32	0.35	0.38
9.0	0.20	0.19	0.19	0.20	0.21	0.23	0.27

2.3.1 Phosphorus

The transport of phosphorus from soils into surface water bodies depends on the climate, soil type, hydrology, soil phosphorus content, agronomic practices, landscape position (Whalen and Chang, 2001), the type of phosphorus applied, the rate and timing of the application, the frequency or timing of rainfall events after the application, the method of application, and the presence of a buffer zone (Withers et al., 2001). Nutrient losses from pasturelands depend upon the stocking density, length of grazing period, average manure loading rate, manure spreading uniformity by grazing livestock, and the disappearance of manure with time (Sweeten and Reddell, 1978). Potential problems may occur in areas where animals tend to congregate around feeding, watering, or resting areas that are in close proximity to streams or waterways (Khaleel et al., 1979b). Problems are also likely when cattle are allowed direct access to streams.

Average total phosphorus concentrations in manure can be 9 g/kg for dairy manure, 25 g/kg for poultry manure, 20 g/kg for poultry litter, and 30 g/kg for swine slurry (Barnett, 1994). These values are very dependent on animal diet, manure collection method, treatment, and storage (Sharpley and Moyer, 2000). The total phosphorus content in manure is a combination of both the organic and inorganic forms. Between 60 and 90% of P in manure is in the inorganic form (Barnett, 1994)

2.3.2 Phosphorus in soils

Hansen et al. (2002) classified soil phosphorus into 3 categories: soluble phosphorus, reactive phosphorus, and stable phosphorus. The soluble form is the most plant available, but often makes up less than 1% of the total phosphorus in the soil (Brady and Weil, 1999). Reactive phosphorus is in a constant equilibrium with the soluble phosphorus. When there is a decrease in the soluble phosphorus pool, phosphorus from the reactive pool will replace it through desorption, dissolution, and mineralization. The stable phosphorus is the largest portion of phosphorus in the soil and it is not biologically available. The reactive phosphorus and stable phosphorus forms both contain organic and inorganic phosphorus. In the short term, much of the phosphorus added to the soils remains plant available (Hansen et al., 2002). As time passes, the phosphorus will be used by crops, react with other soil constituents to form insoluble minerals, or be sorbed onto mineral or organic surfaces (Hansen et al., 2002). Most of the phosphorus removed from soils is removed either by crop uptake or by soil erosion (Novotny and Olem, 1994 pp335).

Phosphorus in the soils can be transported along with runoff either in the dissolved phase or along with eroded soil particles or the particulate phase (Hansen et al., 2002). Both dissolved phosphorus and a portion of the particulate phosphorus are available for biological uptake. The percent of the particulate phosphorus that is available for plant uptake ranges from 10 to 90%, with typical values around 20% (Hansen et al., 2002). The direct loss of applied phosphorus may be more significant than the transfer of soil in certain situations, such as when a runoff event occurs shortly after the application of fertilizer or manure. Up to 10% of the amount applied may be present in runoff (Hansen et al., 2002). The risk of direct loss decreases with subsequent rainfall events (Edwards and Daniel, 1994).

2.3.3 Phosphorus in runoff

Previous measurements of P transfer rates in runoff from land receiving manure applications are often variable and the difference can be related to differences in site conditions and runoff volumes. The differences do not appear to be due to the relative phosphorus availabilities of the materials applied (Withers et al., 2001).

Withers et al., 2001 applied triplesuperphosphate (TSP), liquid cattle manure (LCS), liquid anaerobically digested sludge (LDS), and dewatered sludge cake (DSC) to field plots cropped to cereals and compared the phosphorus transfer in surface runoff over a two-year period. The study noted that the LCS had high amounts of phosphorus extracted from the organic amendments by both NaOH and HCl, suggesting a dominance of inorganic forms. Large amounts of phosphorus were also extracted by water indicating that the LDS may also have the potential for rapid phosphorus release in storm runoff. Runoff from plots treated with LDS that was surface applied in the spring had a Flow-Weighted Concentration (FWC) of 3.99 mg/L total P during the first monitoring period. The study concluded that the risk of P transfer to waterways from agriculture is greatest from land application of LCS and TSP due to the lower phosphorus solubility in water and sodium bicarbonate.

Burwell et al. (1977) applied fertilizer at both the recommended rate and higher rate to corn on a contoured watershed. The concentrations of water-soluble N in surface runoff was highest at the beginning of the growing season and declined during the remainder of the year. The study also found the excessive phosphorus was often adsorbed by soil particles and did not travel in runoff except with soil erosion.

Heathman et al. (1995) applied poultry litter to bermuda grass field plots in Oklahoma. The rate of application was 11 Mg/ha (5 ton/ac). In one plot the manure was incorporated into the soil, to the second plot only litter was applied to half of the plot but at twice the application rate, the third plot was surface broadcast, and the fourth plot was the control. The study found that litter application increased the total phosphorus concentration in runoff for both the no-till (5.8 mg/L) and tilled treatments (6.1 mg/L).

The plot with the half-area application had total phosphorus values similar to the control (2.0 mg/L and 1.3 mg/L, respectively). This study indicates that the insertion of a buffer strip between land application areas and streams may significantly reduce phosphorus loading into surface waters.

A study by Edwards et al. (1994) found that increasing poultry litter application rates to fescue pastureland also increased the erodibility of the soil. The total solids yield also increased along with the rainfall intensity. The poultry litter particles have a relatively high erodibility when compared to soil particles. Edwards and Daniel (1994) constructed plots on fescuegrass and treated them with poultry litter, inorganic fertilizer, and no fertilizer. The poultry litter and inorganic fertilizer applications had similar nutrient contents. The plots received rain on days 7, 13, 36, and 68 after application. During the first simulation conducted after fertilizer applications, the plots with poultry litter treatments had lower concentrations of NO_3-N, NH_3-N, total phosphorus, and PO_4-P than inorganic fertilizer applications. The study concluded that this result was due to the composition of the nutrient sources and the mobility of the parameters. The concentrations of nutrients in the runoff from the plots treated with poultry litter were 0.08 kg/ha NO_3-N, 0.83 kg/ha NH_3-N, 2.06 kg/ha TKN, 0.75 kg/ha PO_4-P, and 1.07 kg/ha Total P. The plots that were treated with poultry litter only lost 1.4 and 2.3% of the applied N and P during the four simulated rainfall events.

2.3.4 Nitrogen

In soils and sediments, nitrogen exists in four basic forms (Novotny and Olem, 1994): ammonium, nitrate, organic phytonitrogen (in plants and plant residues), and protein nitrogen (in living and dead bacteria and small soil inhabitants). Most of the nitrogen reactions in the soil are microbial; therefore, the form of nitrogen present in soils is related to temperature, moisture content, and aeration. Nitrogen is mobile in soils and may leach into groundwater or reappear with groundwater discharge in the base flow of streams (Novotny and Olem, 1994).

Nitrogen can be classified as either reduced or oxidized. The reduced forms of nitrogen include organic nitrogen and ammonia nitrogen. Organic nitrogen consists of proteins derived from animal or plant material. Ammonia nitrogen is readily bio-available and can be used as a nutrient by algae, aquatic plants, or other bacteria. When present in water bodies, deionized ammonium is toxic to fish while ionized ammonia is a nutrient to algae and aquatic plants, and also exerts dissolved oxygen demand (Novotny and Olem, 1994). Total Kjeldahl Nitrogen (TKN) is the sum of organic nitrogen and ammonia.

The oxidized forms of nitrogen include nitrite nitrogen (NO_2-N) and nitrate nitrogen (NO_3-N). Nitrite nitrogen is usually produced from ammonia when specific bacteria and dissolved oxygen are present in the waste. Nitrite can be further oxidized to nitrate. Nitrate is readily bio-available and is not toxic to aquatic life. Total combined nitrogen is the sum of all forms of nitrogen in the water (organic nitrogen + ammonia + nitrite + nitrate).

2.3.5 Nitrogen in runoff

A study by Ross et al. (1978) determined that waiting one day between the application of liquid manure and a simulated rainfall event reduced the yield of nitrogen in runoff by between 80 and 97%. The study determined that this was due to reduced solids loss. The additional time before rainfall occurred allowed the dairy manure to dry and reduced the total solids concentrations in runoff.

McLeod and Hegg (1984) applied dairy manure, poultry manure, municipal sludge, and commercial fertilizer to fescue pasture at a rate of 112 kg N/ha. The overall nutrient loss in the runoff was found to be $< 4\%$ of TKN and $< 2.5\%$ of total P applied to the plots. The dairy manure application lost 2.5% of the NH_4-N, 29.4% of the NO_3-N, and 1.3% of the TP. The poulty manure application lost 4.9% of the NH_4-N, 20.6% of the NO_3-N, and 2.4% of the TP. These values are the mean loss per application for four runoff events.

Heathman et al. (1995) applied poultry litter to bermuda grass field plots in Oklahoma. The rate of application was 11 Mg/ha (5 t/acre). In one plot the manure was incorporated into the soil, to the second plot only litter was applied to half of the plot but at twice the application rate, the third plot was surface broadcast, and the fourth plot was the control. The study found that the litter application increased the total nitrogen concentration for both the no-till (15.4 mg/L) and tilled treatments (16.7 mg/L). The plot with the half-area application had total nitrogen values similar to the control (5.6 mg/L and 5.7 mg/L respectively). This study indicates that the insertion of a buffer strip between land application areas and streams may significantly reduce nitrogen loading into surface waters

2.3.6 Summary

Nutrients present in surface waters can cause eutrophication and lead to deadly diseases if present in drinking water. Nutrients are most often associated with agriculture. The land application of manure from confined animal production facilities and grazing animals can both be major sources of nutrient contamination in runoff. A comparative study of the land application of different wastes under similar conditions will assist in understanding the nutrient transport capabilities of this method of waste disposal. The data from this study will serve as a baseline from which the release and transport of nutrients from agricultural watersheds to surface waters can be modeled.

CHAPTER 3 RESEARCH METHODS

Field plots were constructed on existing pastureland in and around Blacksburg, Virginia. Two sets of plots were established; one set for the study of in-field bacterial and nutrient release and one set for the study of bacterial and nutrient transport. Release plots were used to measure fecal bacterial and nutrient concentrations available to be transported to the edge of the field in runoff. Four manure treatments (turkey litter, liquid dairy manure, cowpies, and none) and three land type treatments: pasture with a history of poultry litter application (Turkey Farm), pasture with a history of liquid dairy manure application (Dairy Farm), and pasture with no prior manure application (Tech Research Farm) were studied. A total of 36 release plots were constructed for three replications of the four manure treatments and three land type treatments.

The transport plots were used to measure the concentrations of fecal bacteria and nutrients present in overland flow at the edge of the field. The transport plots were only constructed at the Virginia Tech research farm due to the labor intensiveness of this component of the research. The transport of bacteria from plots applied with liquid dairy, dried poultry litter, and standard cowpies were compared to control plots on which no animal waste was applied. Two replications of each treatment (turkey litter, liquid dairy manure, cowpies, and control) necessitated the construction of eight plots.

3.1 Site Descriptions

Three sites were selected based on their land-use histories. The Tech Research Farm was selected because it does not have a history of receiving any form of manure applications. The Turkey Farm was selected because turkey manure is frequently applied to the land surrounding the farm. The Dairy Farm was selected because it is one of the few locations near Blacksburg where the dairy manure is applied to pastureland.

3.1.1 Virginia Tech Research Farm

The Tech Research Farm is used for hay production and does not receive liquid manure applications. The property was last fertilized in the spring of 2002. The area selected for the transport plots was dominated by fescue and red clover. Also present was orchardgrass and some broadleaf weeds (John Fike, personal communication, Blacksburg, Virginia, 2 Oct 2002.). The smaller release plots were located just south of the transport plots, also on hay production land. The small plots are predominately tall fescue with a fair amount of orchardgrass and plantain. There is not as much red clover in the area where the small plots were placed (John Fike, personal communication, Blacksburg, Virginia, 2 Oct 2002.).

3.1.2 Turkey Farm

The pastureland surrounding the Turkey Farm is rotationally grazed throughout most of the year. The turkey litter is land-applied on an as-needed basis. The area selected for the release plots had last received poultry litter applications in the fall of 2001. The selected pastureland is described as predominately fescue with a small amount of bluegrass, orchardgrass, and some broadleaf weeds (John Fike, personal communication, Blacksburg, Virginia, 2 Oct 2002.).

3.1.3 Dairy Farm

The land surrounding the Dairy Farm is rotationally grazed throughout most of the year. The liquid dairy manure is land-applied twice a year to both corn and pastureland. The site selected for the die-off and release plots received the last land-applied dairy waste in the fall of 2000 and has not received any additional applications since then. The selected pastureland is described as predominately fescue and bluegrass with some crabgrass, white clover, and broadleaf weeds (John Fike, personal communication, Blacksburg, Virginia, 2 Oct 2002.).

3.2 Release Plot Construction

Twelve release plots were constructed at each of the three sites for measurement of fecal bacterial concentrations available to runoff. Each release plot had the dimensions of 1 m by 1 m (3.3 ft by 3.3 ft). Pre-fabricated steel borders were placed into the soil along the plot boundaries to prevent water movement into or out of the plots (Figure 2). Runoff water from the plots drained through a small flume and was collected down-slope in a bucket. The flume was covered during the rainfall simulations to prevent collection of rainfall. The volume of the collected water was determined by weighing the bucket.

Figure 2. Krissy Yanosek, a research assistant at Virginia Tech, installs the release plot borders and flumes.

A dumpy level was used to determine the slope at each of the locations before the borders were installed. The slopes of the release plots are listed in Table 7.

Table 7. The slopes of the release plots

Plot[*]	Slope	Plot[†]	Slope	Plot[‡]	Slope
TRF-1	5.8%	**TKY-1**	8.5%	**DF-1**	11.3%
TRF-2	8.2%	**TKY-2**	9.1%	**DF-2**	6.4%
TRF-3	7.3%	**TKY-3**	6.7%	**DF-3**	5.2%
TRF-4	4.6%	**TKY-4**	7.9%	**DF-4**	8.5%
TRF-5	8.5%	**TKY-5**	8.8%	**DF-5**	8.8%
TRF-6	9.8%	**TKY-6**	8.5%	**DF-6**	7.9%
TRF-7	10.1%	**TKY-7**	8.2%	**DF-7**	8.5%
TRF-8	10.1%	**TKY-8**	8.2%	**DF-8**	10.1%
		TKY-9	7.9%	**DF-9**	9.8%
		TKY-10	6.1%	**DF-10**	9.2%
		TKY-11	7.9%	**DF-11**	8.5%
		TKY-12	9.1%	**DF-12**	7.6%

[*] Tech Research Farm; [†] Turkey Farm; [‡] Dairy Farm

3.3 Transport Plot Construction

An area at the Tech Research Farm was surveyed in a grid pattern using the Topcon Total Station to determine the location of the transport plots. Square corners were ensured using the 3/4/5 triangle method. After the area was marked, the Topcon Total Station was used to verify the lengths of the plots and to check the angles on the corners for accuracy. The staked out area was surveyed to develop a contour map. From the survey data, a contour map was developed using ArcView 3.2 (Figure 3).

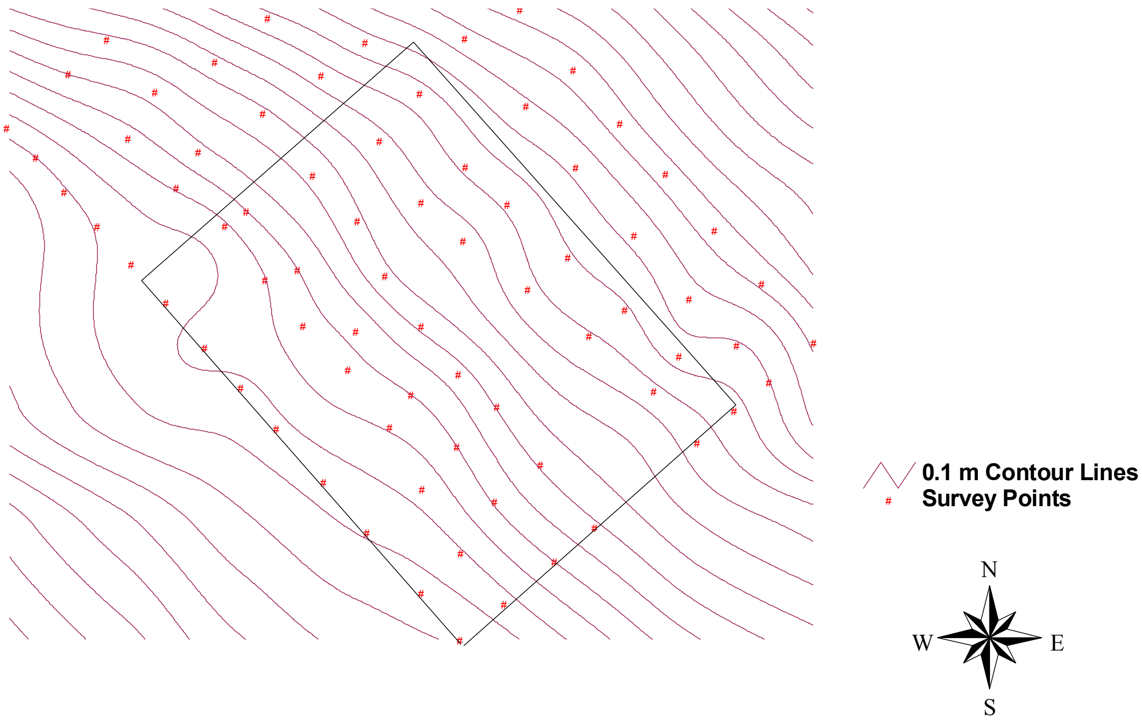

Figure 3. Contour map of Tech Research Farm, used to determine the location and slope of the transport plots

A total of eight transport plots were constructed at the Tech Research Farm. Each transport plot was 3-m (9.8-ft) wide by 18.3-m (60-ft) long on an approximate 5.5-percent slope. Plywood borders were placed to a depth of 15 cm along the plot boundaries to prevent water movement into or out of the plots. A "V" shaped outlet was placed at the down-slope end of each plot to direct runoff into a 0.15-m (6-inch) H-flume equipped with an FW-1 stage recorder for flow measurement. Flumes were stabilized with wood stakes in order to assure that they remained level throughout the experiment and allowed for precise flow measurements by the stage recorders (Figure 4). A stilling well was attached to the flumes. The FW-1 stage recorder recorded runoff depth continuously.

Figure 4. Installation of the FW-1 stage recorders, flumes, and stilling wells

3.4 Soil Properties

The soils at each of the sites were evaluated for particle size distribution and nutrient content. One hundred grams of soil were collected from each of the plots to define the soil horizon for the particle size analysis. Table 8 presents the results from the particle size analysis performed on the transport plot soil samples. Table 9 presents the results from the particle size analysis performed on the samples taken from the three sites where the release plots were located. Particle size analysis was performed by the Virginia Tech Soil Physics Laboratory.

Table 8. Particle size analysis on the transport plots located at Tech Research Farm

Soil Particle Diameter Category	T-1*	T-2	T-3	T-4	T-5	T-6	T-7	T-8
%Very Coarse Sand	2.2%	2.3%	1.5%	1.6%	1.5%	1.8%	2.1%	1.2%
%Coarse Sand	6.8%	6.8%	6.1%	6.8%	6%	5.4%	6%	5.3%
%Medium Sand	10.1%	9.8%	10.7%	10.5%	9.1%	8.7%	7.7%	6.8%
%Fine Sand	11.5%	11.2%	10.3%	11.1%	10.6%	9.1%	6.9%	7.2%
%Very Fine Sand	6.8%	6.5%	5.9%	7%	7.2%	6.4%	9%	5.2%
Total % Sand	**38%**	**37%**	**34%**	**37%**	**34%**	**31%**	**33%**	**26%**
%Coarse Silt	11.7%	11.6%	11%	7.5%	11%	15.6%	9.6%	14.7%
%Medium Silt	36.8%	37.2%	37.8%	38.3%	36.5%	36.1%	39.5%	39.8%
%Fine Silt	9.8%	9.9%	12.7%	11.9%	13%	11%	12.7%	13.6%
Total % Silt	**58%**	**58%**	**62%**	**58%**	**61%**	**63%**	**61%**	**68%**
Total % Clay	**4%**	**5%**	**4%**	**5%**	**5%**	**6%**	**6%**	**6%**
Textural Class	**Silt**	**Silt**	**Silt**	**Silt**	**Silt**	**Silt**	**Silt**	**Silt**

*Tech Research Farm, Plot 1

Table 9. Particle size analysis on samples taken from the release plots at the Tech Research Farm, the Turkey Farm, and the Dairy Farm

Soil Particle Diameter Category	Turkey Farm	Tech Research Farm	Dairy Farm
%Very Coarse Sand	2%	2.9%	7.2%
%Coarse Sand	11.4%	6.8%	4.6%
%Medium Sand	7.9%	10.8%	3.4%
%Fine Sand	6.3%	11%	2.7%
%Very Fine Sand	5.2%	6.7%	2.3%
Total % Sand	**33%**	**38%**	**20%**
%Coarse Silt	8.9%	9.2%	7.2%
%Medium Silt	40.8%	33.8%	43.7%
%Fine Silt	12.2%	10.6%	16.5%
Total % Silt	**62%**	**54%**	**68%**
Total % Clay	**5%**	**8%**	**12%**
Textural Class	**Silt**	**Silt**	**Silt**

Samples were also collected from each of the sites for nutrient analysis. This information was used to determine the manure application rates. Six samples were collected from each of the transport plots and mixed in a bucket to form a single composite sample. The sample were taken from the top 2 to 4 inches of soil in the pastureland. The Virginia Tech Soil Testing Laboratory analyzed the soil samples for a routine test and organic matter content. The organic matter was estimated by either the loss on ignition method or the Walkley-Black method. The results from the Soil Testing Laboratory are presented in Table 10.

Table 10. Nutrient levels in soil samples taken at the Tech Research Farm, the Turkey Farm, and the Dairy Farm. All values are in ppm.

Sample Location	pH	P[*]	K[†]	Ca[‡]	Mg[§]	OM[‖]	Zn[#]	Mn[**]	Cu[††]	Fe[‡‡]	B[§§]
T-1[‖‖]	5.7	10	72	564	106	3.3	1.7	21.7	1.4	15.5	0.5
T-2	5.8	9	63	598	114	3.8	1.6	22.8	0.4	13.7	0.4
T-3	5.6	11	80	584	116	3.7	1.9	26.5	0.3	13.1	0.4
T-4	5.6	15	94	540	113	3.6	1.7	34.5	0.3	15.1	0.4
T-5	5.4	8	95	434	95	3.2	1.2	25.9	0.3	14.7	0.3
T-6	5.5	6	71	453	105	3.2	1.2	21.9	0.3	16.4	0.3
T-7	5.6	5	86	465	98	3.2	0.9	17	0.2	14.2	0.3
T-8	5.7	6	101	534	120	3.1	0.8	14.1	0.2	9.8	0.3
Turkey Farm	6.7	114	158	1391	245	4.8	6.3	35.4	0.3	8.9	0.6
Dairy Farm	7	81	233	1199	233	4.8	4.1	16.5	0.5	7.2	0.8

[*]Phosphorus; [†]Potassium; [‡]Calcium; [§]Magnesium; [‖]Organic Matter; [#]Zinc; [**]Manganese; [††]Copper; [‡‡]Iron; [§§]Boron; [‖‖]Tech Research Farm, Plot 1

The state of Virginia requires phosphorus-based application of manure on crop and pasturelands. This method uses the residual phosphorus levels in the soil and the phosphorus levels in the manure to determine the manure application rate to the land. The P_2O_5 application rates recommended for Orchardgrass/Fescue-Clover Pastures on soil productivity groups I and II (VDCR, 1995) are presented in Table 11.

Table 11. **Recommended manure application rates based on the DCR, P based application rates**

Sample ID	Phosphorus in soil (kg/ha)	Phosphorus Level	Recommended P_2O_5 (kg/ha)
T-1[*]	11.2	M-	101
T-2	10.1	M-	101
T-3	12.3	M	90
T-4	16.8	M	90
T-5	9.0	M-	101
T-6	6.7	M-	101
T-7	5.6	L+	112
T-8	6.7	M-	101
Average Tech Research Farm	9.8	M-	99
Turkey Farm	127.8	VH	0
Dairy Farm	90.8	VH	0

[*] Tech Research Farm, Plot 1

3.5 Animal Waste Application

Because the turkey and dairy farms have a history of receiving land applications of manure, the phosphorus levels were much higher in these fields. The Department of Conservation and Recreation (DCR) Standards and Criteria (1995) recommendation is that no additional phosphorus be applied to the pasture. This situation is realistic and is faced by many farmers in southwest Virginia. For many years the manure application rates have been based on nitrogen content of the manure. This approach has overloaded the soils with phosphorus, creating very high levels in many crop and pasturelands. In the meantime, the confined animal operations continue to produce manure, so currently the best solution is to apply the manure at a rate slightly lower than the estimated crop uptake, or to restrict waste applications to every other or every third year. Pasture typically uptakes 28 kg/ha P_2O_5 (25 lbs/ac P_2O_5) or less per growing season. Based on this approach, the procedures were adjusted so that the manure would be applied to the plots at the rate of 56 kg/ha P_2O_5 (50 lbs/ac P_2O_5). This rate was selected based on the fact that farm equipment used to spread manure cannot spread evenly or accurately if the application rates are too low, so in many situations the farmers apply this minimum level. By applying phosphate at a rate of 56 kg/ha, manure can still be applied to the pasture every other year, remaining an option for disposal of animal waste. Over time, the lower application rate will reduce potential phosphorus loading in runoff and allow for the nutrients in the manure to be used by the grasses.

Previous animal waste analysis reports were obtained from the DCR and from the farm managers (Appendix A). The samples analyzed during previous waste application events were used to estimate the manure application rates for this study (Table 12). The application rate of manure is commonly estimated by using nutrient results from the previous season because the sample is collected for analysis during agitation or land application. Grab samples of the manure applied to the plots was sent to the Agricultural Service Laboratory at Clemson University for waste analysis. Table 12 compares the results from the previous manure tests to the manure samples collected prior to their application to the plots. The previously analyzed results accurately predicted the P_2O_5 concentrations in the manure.

Table 12. Concentrations of P_2O_5 in manure and the application rate and volume of the manure applied to the transport and release plots

Manure	P_2O_5 estimate based on samples from previous years	P_2O_5 estimate based on current waste samples	P_2O_5 applied to the plots	Application Rate	Transport Plots	Release Plots
Liquid Dairy	0.67 kg/1000 L	0.67 kg/1000 L	56 kg/ha	81,958.5 L/ha	450.1 L/plot	8.2 L/plot
Cowpie	2.0 kg/t	1.7 kg/t	50 kg/ha	29.4 t/ha	161.6 kg/plot (180 cowpies)	3.0 kg/plot (3 cowpies)
Turkey	20.4 kg/t	19.9 kg/t	54.7 kg/ha	2.8 t/ha	15.1 kg/plot	0.28 kg/plot

3.6 Animal Waste Collection and Application Methods

The dried turkey litter was collected from the Virginia Tech turkey barns. The litter, comprised of pine shavings and manure, was collected after a flock of turkeys were sent to market. The barns at Virginia Tech are cleaned between each set of birds for experimental purposes, so the litter was in the turkey barns for approximately 3 months. The litter was stacked under a covered shed (Figure 5) for a time period varying between 3 and 6 weeks before it was applied to the plots. Litter is typically stored before it is land-applied to the fields. Storage time depends on when the farmer has time to spread the litter or when the storage area is filled. The litter was uniformly broadcast onto the plots using small buckets

Figure 5. Turkey litter was stacked under a shed before it was applied to the plots

The liquid dairy manure applied to the plots was obtained from the Virginia Tech Dairy manure storage pond. The storage pond contents are agitated twice a year, in March and October, before land application. The agitation re-suspends the solids that accumulate on the bottom of the pond. The manure was pumped into an 1136 L (300 gal) tank and stored throughout the duration of the field experiment. The liquid manure was mixed in the tank before being drained into buckets and applied to the field plots.

“Standard” cowpies were constructed from fresh dairy cow deposits. Each cowpie was standardized by weight and shape, and randomly positioned by project personnel at various locations in the “cowpie” treatment plots. The size and shape of the “standard cowpies” was based on research by Thelin and Gifford (1983), who developed standard cowpies to study FC release patterns. The fresh deposits were formed by taking fresh manure and mixing it in a cement mixer for approximately 15 minutes. The manure was then placed in a mold with a diameter of 20.3 cm (8 in) and a depth of 2.54 cm (1 in). Fecal deposits were placed in the mold until a weight of 0.9 kg (2.0 lbs) was reached. The transport plots were divided into 1-m by 3-m (3.3-ft by 9.8-ft) sections. Approximately 9 cowpies were placed in each of the sections. A total of 360 cowpies were applied to the two transport plots. The three cowpies were randomly placed in each of the 1-m by 1-m (3.3-ft by 3.3-ft) release plots.

The cowpies applied to the transport plots were constructed from fresh solid dairy manure (Figure 6). The solid manure used to construct the cowpies was collected on the day before the rainfall simulation. Because of the large number of cowpies needed for the transport plots, the manure was collected after the dairy stalls were scraped early in the morning. This method allowed over 318 kg (700 lbs) of manure to be collected at once. The manure used to make the cowpies had some wood chip bedding from the dairy stalls mixed in with it. The scraped manure was representative of the wastes from many cows, and not based on a single animal. For the release plots, the manure used to construct the cowpies was also collected from the dairy barns on the day before the rainfall simulation. The manure for these plots was collected from individual, fresh cowpies found in the dairy stalls. Several cowpies were collected and mixed together. Thus, each cowpie was representative of several cows instead of a single animal.

Figure 6. A cowpie being applied to the transport plot

3.7 Rainfall simulation –Release Plots

A Tlaloc 3000 portable rainfall simulator, based on the design of Miller (1987), with a ½ 50WSQ Tee Jet nozzle was used to apply rain to the release plots. Figure 7 shows a diagram of the rainfall simulator's dimensions. The nozzle is placed in the center of the simulator at 305 cm (10 ft) above the surface of the plots. A pressure regulator is used to establish a water flow rate of 210 mL/s at the nozzle. Before each simulation, the nozzle is centered over the plot, and the simulator should be enclosed in tarps to minimize wind disturbance of rainfall intensity. This rainfall simulator has been developed as the standard simulator used to test the phosphorus index in various states. Rainfall simulations were conducted within 24 hours of the manure application to represent a worst case scenario. The plot was rained on until runoff water collected in the flume for 30 minutes as recommended by the National Phosphorus Research Project. After 30 minutes, the rainfall simulation ended and the runoff water sample was collected.

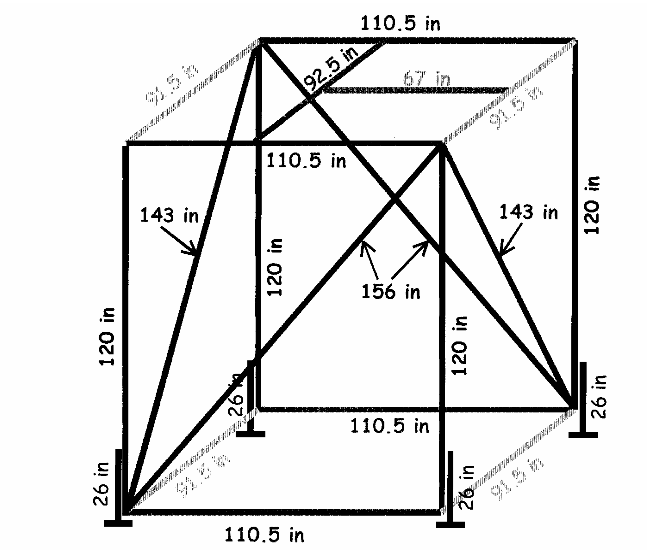

Figure 7. Portable rainfall simulator used to create rainfall over the release plots

The uniformity coefficient (UC) was determined, using Equation 1, for several different nozzles that were tested for both their rainfall intensity and distribution. A summary of the uniformity coefficients and the nozzles tested are presented in Table 13. The nozzle initially selected was the TeeJet ½ 30WSQ. Rainfall simulation began at Tech Research Farm using the selected nozzle. After 1,100 L (290 gal) of water were applied to the plots with no runoff it was concluded that the extremely dry soil conditions would require a nozzle with a higher rainfall intensity to produce the desired runoff. The TeeJet ½ 30WSQ nozzle was, therefore, replaced with the TeeJet ½ 50WSQ.

$$UC = 1 - \frac{x}{y} \tag{1}$$

Where:

$UC = Uniformity\ Coefficient$

$x = average\ absolute\ deviation\ from\ mean\ rainfall\ depth$

$y = average\ rainfall$

Table 13. The nozzles tested at different pressure settings to determine the suitable nozzle for the rainfall simulations

Test	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Run 7	Run 8
Date	9/19/2002	9/23/2002	9/23/2002	10/9/2002	10/10/2002	10/10/2002	10/21/2002	10/21/2002
Nozzle	1/2 50WSQ	1/2 50WSQ	1/2 50WSQ	1/2 20WSQ	1/2 20WSQ	1/2 20WSQ	1/2 30WSQ	1/2 30WSQ
Pressure	6 psi	5 psi	7 psi	7.5 psi	9 psi	14 psi	9 psi	13.5 psi
Flow	1.98 gpm	1.74 gpm	2.11 gpm	1.1 gpm	1.24 gpm	1.75 gpm	1.75 gpm	2.2 gpm
y[*]	2.0925	2.027	2.2075	0.611	0.94	1.2975	1.395	1.46
x[†]	0.639	0.8203	0.6545	0.079	0.122	0.1345	0.23	0.148
UC[‡]	69.5%	59.5%	70.4%	87.1%	87.0%	89.6%	83.5%	89.9%

[*] Average Rainfall; [†] Average absolute deviation from the mean rainfall depth; [‡] Uniformity coefficient

The uniformity coefficient for the inner rain gauges was determined for the ½ 50WSQ nozzle. This is representative of the plot area. Using this method, the UC for the ½ 50WSQ was determined to be 86.4%. Three rain gauges (RG-1, RG-2, and RG-3) were placed in the plots during each simulation. RG-1 was placed directly behind the plot border. RG-2 was placed in the center of the plots, and RG-3 was placed in front of the plot borders. The average uniformity coefficient for all simulations was 91.9% (Appendix B).

In addition to changing the nozzle, it was necessary to pre-wet the plots to enhance the runoff. Pre-wetting was accomplished by pouring approximately 5.1 cm (2 in) of water onto the surface of each of the plots before the manure treatments were applied. Ironically, an extremely dry summer and early fall at the beginning of the fieldwork period turned into a very wet fall. The plot pre-wetting was adjusted according to the weather conditions. Appendix B summarizes the small plot pre-wettings information.

3.8 Rainfall simulation – Transport Plots

Due to the unreliability of natural precipitation for short-term field research, the Department of Biological Systems Engineering's rainfall simulator (Dillaha et al., 1987) was used to generate storm events to produce runoff from the field plots. Figure 8 shows a picture of the rainfall simulation. Rainfall was applied at a uniform rate (approximately 4.45 cm/h) to all pasture plots. A series of rainfall simulations was conducted within 24 hours after manure application. The first simulation (S1) lasted approximately 3 hours. The rainfall continued until a steady state runoff resulted. The S1 simulation represented the bacterial transport during dry field conditions. Before the second simulation (S2) began (approximately 22 hours after the end of the first simulation, S1), soils were saturated. This was due to an overnight natural rainfall of approximately 2.9 cm (1.15 in) and the long simulated rainfall event during the first simulation. Therefore, the second rainfall simulation represented the transport characteristics of bacteria under very wet soil conditions.

Figure 8. The portable rainfall simulator rains on the transport plots

The uniformity of rainfall applications was measured using a network of volumetric rain gauges in and around each plot. The uniformity coefficient was determined for both rainfall simulations using Equation 1. The uniformity coefficients for the first and second rainfall events were 93% and 95.5%, respectively

3.9 Sampling Procedures

All runoff water was collected from the flume at the end of each of the release plots and weighed to determine the volume. The runoff water was collected in buckets and a single sample was taken from the total volume for bacterial and nutrient analyses. A total of 32 runoff samples were collected from the release plots.

Grab samples of runoff water were collected from the transport plots every 3 to 9 minutes during both simulated-storm events (Figure 9). The individual samples were collected and analyzed for bacterial and nutrient concentrations. The bacterial analysis was used to evaluate the temporal distribution of bacterial concentrations. A total of 68 samples were collected during S1 and 68 samples were collected during S2. The FW-1 stage recorders were used to create hydrographs of the runoff from each of the transport plots. A mark was made on the stage hydrograph when each sample was collected to accurately record the sampling time.

Figure 9. Becky Zeckoski, a research associate at Virginia Tech, collects a grab sample from the flume located at the bottom of a transport plot.

3.10 Additional Monitoring

Soil moisture content was measured before each rainfall simulation to determine the antecedent soil moisture conditions. Soil moisture content was measured by weighing moist soil samples, drying soil samples and obtaining the weight of the soil, and then using the following equations to obtain the moisture content (Lambe, 1969):

$$w\% = \frac{W_w}{W_s} \times 100$$

Where: (2)

$w\% = Water\ Content\ in\ Percent$
$W_w = Weight\ of\ Water\ in\ Soil\ Sample$
$W_s = Weight\ of\ Oven-dry\ Soil$

3.11 Laboratory Analysis

Runoff samples were collected from both the transport and release plots and analyzed for bacterial and nutrient content. Table 14 shows the samples collected and parameters analyzed.

Table 14. Samples collected and parameters analyzed

	E. coli	FC[*]	ENT[†]	DP[‡]	TP[§]	TSS[‖]	NO_3-N	NH_4-N	TKN[#]
Transport plot runoff	X	X	X	X	X	X	X	X	X
Release plot water samples	X	X	X	X	X	X	X	X	X

[*]Fecal coliform; [†]*Enterococcus*; [‡]Ortho-phosphate; [§]Total Suspended Solids; [‖]Total Kjeldahl Nitrogen

Samples were analyzed, immediately after collection, for FC, *E. coli*, and *Enterococcus* concentrations in runoff. The water samples were shaken 23 times before adding 1 mL of sample solution to 9 mL of the buffer solution. The buffer solution is comprised of magnesium chloride and potassium dihydrogen phosphate and is produced by the Hach Company (Hach, 2003). Soil and manure samples were gently beat with a mallet to break up the clumps before adding 10 grams of the soil or manure to 90 mL of the buffer solution. The samples were analyzed using the Spread Plate (Clesceri et al., 1998) and membrane filtration methods (Clesceri et al., 1998 and EPA, 2000).

The M-Enterococcus Agar was used to determine *Enterococcus* concentrations; the M-FC Agar was used to determine the FC; and the M-Tec agar was used to determine the *E. coli* concentrations. The plates were inverted and placed in a tub with a paper towel to increase the humidity. The enterococcal plates were incubated at 37°C for 48 hours. The FC plates were incubated at 44.5°C for 24 hours. The *E. coli* plates were incubated at 37°C for 2 hours and then incubated for 22 hours at 44.5°C.

Because of the large number of samples during the field experiment, only a single dilution was performed on the runoff samples. When the estimated dilution level was not correct, the sample was re-plated within 5 to 8 days. Samples were stored in a dark room at 4°C.

The nutrient analysis was performed using the Traacs 800 Continuous Flow Wet Chemistry Autoanalyzer. The nutrient analysis (Clesceri et al., 1998) included ammonia (EPA 350.1), nitrate (EPA 353.1), ortho-phosphate (EPA 365.1), total phosphorus (EPA365.4), Total Kjeldahl Nitrogen (EPA 351.2), and total suspended solids (EPA 160.2).

3.12 Biolog Analysis

The Biolog identification system was used to perform additional analysis on the *Enterococcus* plates that were obtained from the transport plots treated with cowpies. The Biolog System was used to identify the different species of enterococci present both in the cowpie source manure and in the runoff collected from the plots. The Biolog System identifies microorganisms based on carbon source utilization. The system contains 96 wells which each contain a single carbon compound and a metabolic dye. The isolates of enterococci were prepared to a standardized concentration and then were added to all 96 wells in a plate. After incubation, a purple color forms due to the dye in the wells where the isolate was able to use the carbon compound located in that well. The biolog database used cluster analysis to identify the isolate. The biolog library currently contains 300 known source isolates of enterococci.

The Biolog System was used to identify the species of enterococci that are most transportable. Twenty isolates were taken from plates that represent the beginning of runoff, the peak runoff point, and the end of runoff. Isolates were taken from each of these points for both rainfall simulations so that a comparison could be made between dry and wet soil conditions. In addition, twenty isolates were taken from the source plate to identify the enterococcal species present in the source manure.

The Biolog *Enterococcus* Protocol is a 4-day procedure as follows (Biolog Technical Manuel, 1999):

Day 1

- Pipette 200 μL of Enterococcosel Broth into each well of a 96 well microwell plate using an 8-channel pipetter and sterile tips.
- Touch the tip of a sterile toothpick to a single enterococci colony on the filter or spread plate and place the toothpick into a well of Entero Broth and gently agitate. Remove the toothpicks from the microwell plate and cover. Incubate at 37^{o} overnight.

Day 2

- Read the inoculated microwell plate for positive or negative reactions. Positive reactions turn the fluid dark brown to black and indicate a pure enterococci growth. Negative reactions leave the liquid a yellow color, but still may contain enterococcal growth.
- Place the tip of a sterile cotton tipped swab into each well then streak directly onto a Biolog Universal Growth media and 5% sheep's blood agar plate. Incubate overnight at 37^{o}.

Day 3

- Swab the surface of the blood agar plate with a sterile cotton tipped swab and then dunk the swab into a tube of Biolog GN/GP Inoculating fluid until the turbidity reaches 70% saturation.
- Place three drops of sodium thyglocylate into the inoculating fluid tube and invert to mix.

- Pour the contents of the tube into a plastic reservoir and, using an 8-channel pipetter, pipette 150 µL of inoculating fluid into each well of the GN-ENT microwell plate.
- Cover and incubate at 37^o overnight.

Day 4

- Place the Biolog microwell plate, with plate cover removed, into the plate reader. The Molecular Devices E Max precision Microplate reader with a 405 nm wavelength was used, along with the Biolog MicroLog System, Release 4.0 software.
- Print result and discard microwell plates.

3.13 Statistical Methods

Transport plots were analyzed using the Repeated Measures Design (Ott and Longnecker, 2001). The repeated measures design experiment is used to obtain different measurements corresponding to different time points following administration of a treatment. The response variable is the concentration of bacteria in the runoff leaving the plot. Tukey's pairwise comparison (Ott and Longnecker, 2001) was used to find significance among the treatments. Significance was determined at the $P<0.05$ significant level. The null hypothesis being tested is that there is no difference in the concentrations of the bacteria in surface runoff among the treatments.

$$\mu_{\text{turkey}} = \mu_{\text{cowpie}} = \mu_{\text{liquid dairy}} = \mu_{\text{control}}$$

The release plots were analyzed using a Generalized Randomized Block Design. The Tukey's pairwise comparison was used to find significance between the treatments. Significance was determined at the $P<0.05$ significant level. The null hypothesis being tested is that there is no difference in the concentrations of the bacteria in surface runoff among the treatments.

$$\mu_{\text{turkey}} = \mu_{\text{cowpie}} = \mu_{\text{liquid dairy}} = \mu_{\text{control}}$$

When the treatments were considered within each location, significance was determined when $P<0.1$.

CHAPTER 4 RESULTS AND DISCUSSION

The results and discussion section presents the results from the bacteria and nutrient field study and the bacterial source tracking laboratory experiment. The first section presents the sediment and runoff losses from the release and transport plots. The following section discusses the bacteria results from the release and transport plots and the bacterial source tracking study. The last section evaluates the nutrient results from the release and transport plots studies.

4.1 Runoff and Sediment Loss

The runoff and sediment losses from the release and transport plots are presented in the following section. Differences in the TSS concentrations at the three farms and among the different treatments are discussed.

4.1.1 Runoff Losses from the Release Plots

The release plots were designed to measure fecal bacterial and nutrient concentrations potentially available for transport to the edge of the field in runoff. Runoff water from the plots drained through a small flume and was collected down-slope in a bucket. The volume of the collected water was determined by weighing the bucket. Figure 10 presents the runoff results from the release plots.

Differences in the runoff among the release plots are due to varying antecedent soil moisture conditions prior to the start of the rainfall simulation at the different farms. As expected, higher runoff volumes occurred from wetter soils. A summary of rainfall conditions and runoff volumes collected from each of the plots is presented in Appendices B and C.

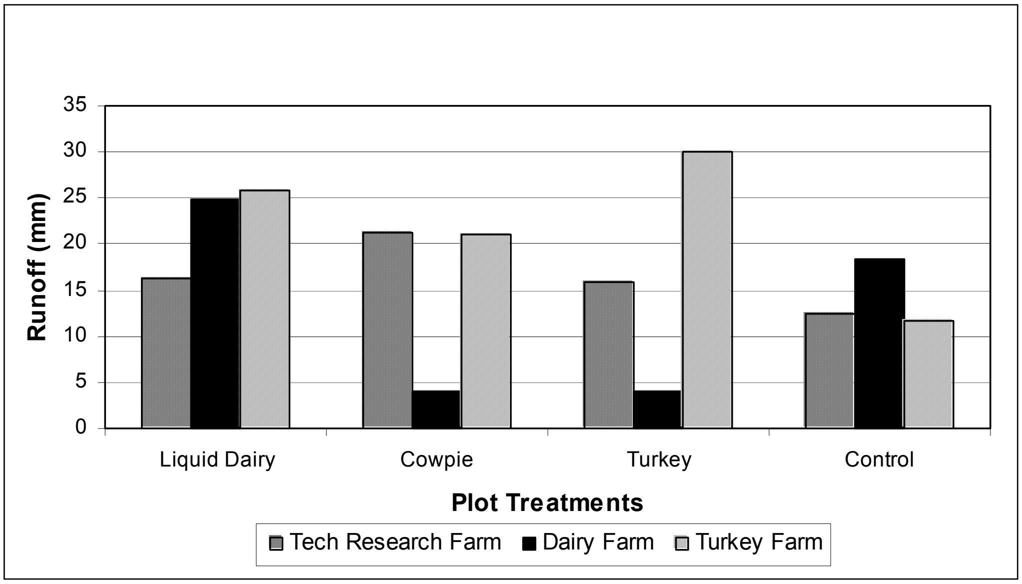

Figure 10. Runoff from the Release Plots

4.1.2 TSS Concentrations from the Release Plots

Table 15 presents the Total Suspended Solids (TSS) concentrations released from the plots. No statistical differences were found in the TSS concentrations released from the plots with different treatments. There were however, differences in the average TSS concentrations at the different farms. The Dairy Farm and the Turkey Farm had 143% and 94% higher respective TSS concentrations released than the Tech Research Farm. Higher TSS concentrations could result in a higher percentage of the TP is in the form of Sediment Bound Phosphorus (SBP) when background soil phosphorus concentrations are high. These findings show that farms with a history of land application of waste have higher TSS concentrations available to be transported off the field during overland flow events. Due to the very short overland flow length and the fact that the release plots were fully covered with grass, it is unlikely that the increased TSS concentrations were due to soil erosion. More likely, the build up of organic material on the soil surface contributed to increased TSS concentrations. This could results in higher erosion rates and increased loading of phosphorus to surface waters.

Table 15. Total Suspended Solids concentrations released from the plots

Treatment	Tech Research Farm (mg/L)	Dairy Farm (mg/L)	Turkey Farm (mg/L)	Average (mg/L)
Liquid Dairy	86	166	111	121
Cowpie	72	189	131	131
Turkey	32	135	147	104
Control	37	62	52	50
Average	**56.8**	**138.0**	**110.3**	**101.5**

4.1.3 Runoff Losses from the Transport Plots

The transport plot field experiments were designed to measure bacteria and nutrients concentrations and loadings transported to the edge of the field in runoff. Figure 11 shows the total runoff volume from each of the transport plots. Runoff volume increased during the second rainfall simulation (S2), due to the saturated ground conditions before the simulation began. The runoff from the plots varies among the plots due to differing soil conditions, manure treatments prior to the rainfall simulation, or different runoff start times. Statistical analysis was performed on the runoff volumes using the repeated measure method and Tukey's pairwise comparison (Ott and Longnecker, 2001). Although differences are noted, no statistical differences in the runoff volumes from the different treatments were found. There were also no significant differences between the runoff volumes during S1 and S2 simulations at the 0.05 error level. These results indicate that differences in the bacteria and nutrient concentrations are due to the different manure treatments, and not necessarily due to the differences in runoff volumes or other factors affecting runoff.

During S1, the plots treated with liquid dairy had the highest runoff volume, followed by the cowpie, turkey litter, and control treatments. The liquid dairy treatment increased the soil moisture content prior to the rainfall simulation, possibly accounting for the higher runoff volume during S1. During S2, the plots treated with cowpies had the highest runoff volume, followed by the control, liquid dairy, and turkey litter treatments. Appendix C presents the runoff that occurred from the plots at each sample point.

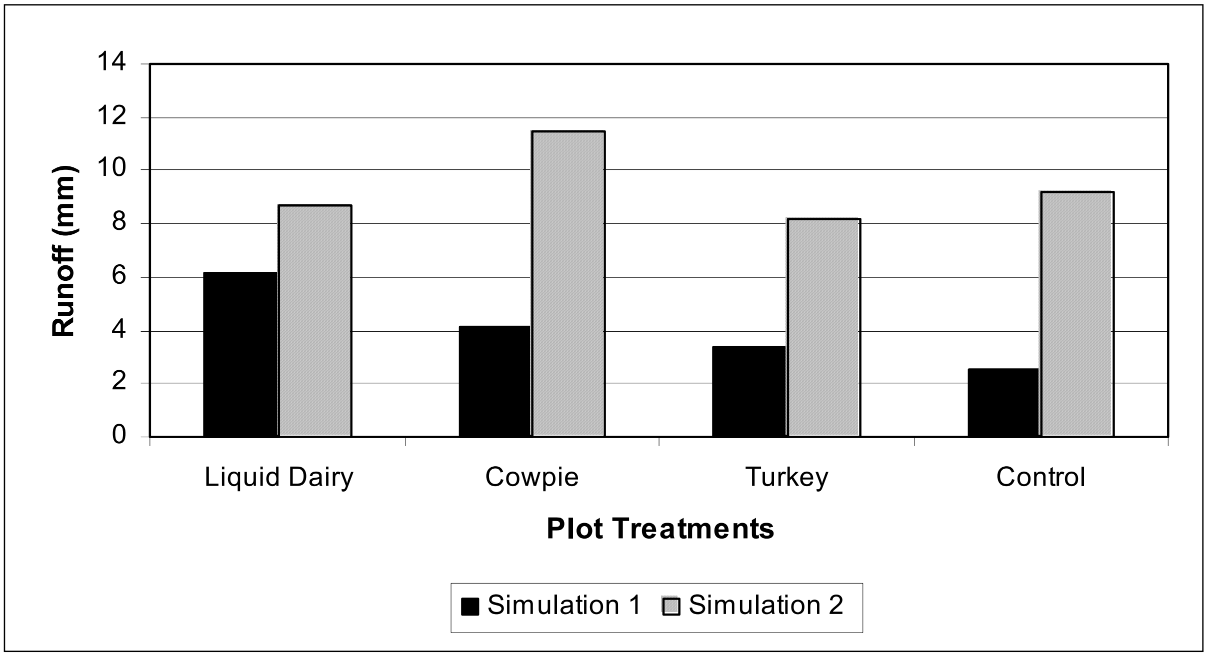

Figure 11. Runoff from the transport plots

4.1.4 TSS Concentrations from the Transport Plots

Flow-weighted concentrations (FWC) were calculated for the TSS in runoff from each of the transport plots (Table 16). The FWC were calculated by multiplying the sample concentration by the volume of runoff that occurred during that time period. These values were then summed and divided by the total volume of runoff from each plot. There were no statistical differences between the TSS concentrations between the two simulations or among any of the different treatments. Higher TSS concentrations in runoff do not necessarily correspond with higher bacterial or nutrient concentrations. Because the plots were rained on within 24 hours of the manure applications, the bacteria and nutrients did not have time to attach to eroding soil particles.

Table 16. Flow-weighted Total Suspended Solid concentrations present in runoff from the transport plots

	Total Suspended Solids – FWC*		
Treatment	**Simulation 1 (mg/L)**	**Simulation 2 (mg/L)**	**Average (mg/L)**
Liquid Dairy	59.9	83.4	71.7
Cowpie	176.7	54.6	115.7
Turkey	37.3	22.5	29.9
Control	85.2	29.1	57.1

*Flow-weighted Concentration

The addition of the manure to the plots decreased TSS concentrations from the liquid dairy and turkey plots when compared to the control. Gerba et al. (1975) reported that as bacteria and organic substances accumulate on the soil surface, they increase the filtration properties of the soil. This may explain the decrease in TSS concentrations from the liquid dairy and turkey litter plots during the first simulation. The cowpie treatment covered just the areas where the fecal deposits were located, but not the entire plot area. The cowpies had higher moisture content than the other waste types; therefore it is possible that after the raindrop impacts disintegrated the cowpies, they were more readily carried off the plots by runoff.

The FWC decreased during S2 for all treatments except the liquid dairy manure, compared with S1. This indicates that most of the TSS during S1 were in organic forms. During S2 much of the transportable organic materials had already been washed off of the plots, resulting in lower TSS concentrations.

4.2 Bacteria

This section discusses the results obtained from the bacterial analysis on the release and transport plots. Comparisons are made between the different manure treatments to determine which treatments have the greatest potential to release fecal bacteria for their potential transport with overland flow, and which source manure actually had the greatest concentrations of bacteria in overland flow. The percent of bacteria present in source manure that was released and was transported in runoff from the transport plots was also evaluated. Three indicator species, *E. coli*, fecal coliforms (FC), and *Enterococcus*, are evaluated so that results can be compared to previous studies, and the relationship between FC and the new indicators (*E. coli* and *Enterococcus*) can be evaluated.

Use of the commercial Biolog system as a method of Bacterial Source Tracking (BST) is also discussed. The Biolog system was used to identify the enterococcal isolates that were applied to the transport plots and then analyze which isolates have the highest potential to be transported by the runoff samples. This information could identify species of *Enterococcus* most commonly related to fecal pollution caused by cattle in a watershed.

The results discussed here represent a worst case scenario since the rainfall event occurred within 12 to 24 hours after land application of the waste and all cowpies were fresh. Bacteria concentrations are higher than they would be if the rainfall event had been delayed for days or weeks after the land application due to bacterial die-off. Usually this situation is avoided, but undoubtedly does occur at times.

4.2.1 Source Bacteria

The source manure bacteria concentrations for this study are presented in Table 1. The cowpie sample was not diluted to the correct level before die-off began to occur, so the concentrations in the cowpie source manure may have been much higher than the presented values.

Table 17. Source Manure concentrations

Manure Source	*E. coli* (CFU/gram)	Fecal Coliform (CFU/gram)	*Enterococcus* (CFU/gram)
Liquid Dairy	410,000	490,000	670,000
Cowpie	>300,000	>300,000	>300,000
Turkey Litter	3,000	3,000	3,600

4.2.2 Release Plots

The release plots were constructed to measure fecal bacterial concentrations potentially available for transport to the edge of the field in runoff. The release plots were constructed at three different farms to determine if bacterial release rates differ between pasturelands that have previously been receiving waste applications and those that have not.

Source Manure Applied to the Release Plots

The manure was applied to the release plots between 12 and 24 hours before the rainfall simulation. The number of colony forming units (CFU) applied to each plot is presented in Table 18. The CFU applied to each plot was determined by multiplying the bacterial concentration in the source manure (Table 17) by the manure application rate. Since the manure was applied to the plots based on the phosphorus application rate, the bacterial counts vary based on the concentration of the phosphorus in the manure.

Table 18. The number of CFU (colony forming units) applied to each of the release plots

Manure Source	*E. coli* (CFU/plot)	Fecal coliform (CFU/plot)	*Enterococcus* (CFU/plot)
Liquid Dairy	3.4×10^{10}	4.0×10^{10}	5.5×10^{10}
Cowpie	$>9.0\times10^{8}$	$>9.0\times10^{8}$	$>9.0\times10^{8}$
Turkey Litter	8.4×10^{5}	8.4×10^{5}	1.0×10^{6}

As stated before, the manure was applied to the land based on the phosphorus content. Thus, the manure sources which have lower phosphorus levels are applied to the land at higher application rates. Dietary supplements such as phytase and new diet management techniques are currently being used to reduce the phosphorus levels in animal waste, which allows farmers to spread manure at higher application rates without overloading the soils with phosphorus. This practice may result in higher application of bacteria to the soils as demonstrated in Table 18. The manure used in this study did not have phytase added to the diet, but dairy cattles' diets are carefully managed to reduce phosphorus concentration in manure.

Release Plot Fecal Bacteria and TSS Concentrations

The concentrations of bacteria and TSS in water collected from the release plots are presented in Table 19. The results presented are the average concentration for each treatment. The TSS concentrations do not appear to be directly related to either the waste treatment or the bacterial concentrations in the runoff. A large number of samples were processed at once, making it impossible to always estimate the correct dilution level within the allotted time. The highest dilution levels achieved were averaged and are presented in Table 19. The concentrations from each of the individual plots are presented in Appendix D.

The results from the Tech Research Farm were quite different than expected. The Tech Research Farm, which in the past had not received manure applications, had much higher concentrations of bacteria released from the control plots compared with the other farms. This anomaly could be due to a higher wildlife population in the area and the absence of cattle to discourage wildlife, or due to the build up of stable populations in the soil (Faust, 1982). The higher enterococcal concentrations are another indication of background concentrations since *Enterococcus* can survive much longer in the environment and are not necessarily indicative of recent fecal pollution.

Table 19. Concentrations of *Enterococcus*, fecal coliform, *E. coli*, and Total Suspended Solids released from the release plots

Tech Research Farm	*E.coli* (CFU/100 mL)	Fecal Coliform (CFU/100 mL)	*Enterococcus* (CFU/100 mL)	TSS[*] (mg/L)
Liquid Dairy	23,000 a**	35,000 a	17,000 b	86 a
Cowpie	152,500 a	159,500 a	285,000 a	72 a
Turkey	18,550 a	29,050 a	12,075 b	32 a
Control	21,300 a	29,350 a	23,000 b	38 a
Dairy Farm	***E.coli* (CFU/100 mL)**	**Fecal Coliform (CFU/100 mL)**	***Enterococcus* (CFU/100 mL)**	**TSS (mg/L)**
Liquid Dairy	55,000 b	>300,000 a	3,067 a	166 a
Cowpie	>300,000 a	>300,000 a	8,133 a	189 a
Turkey	28,000 bc	92,000 a	1,880 a	135 a
Control	1 bd	134 b	1 a	1 a
Turkey Farm	***E.coli* (CFU/100 mL)**	**Fecal Coliform (CFU/100 mL)**	***Enterococcus* (CFU/100 mL)**	**TSS (mg/L)**
Liquid Dairy	30,667 b	47,667 b	1,867 a	111 a
Cowpie	37,000 a	65,000 a	1,007 a	131 a
Turkey	4,733 b	9,000 b	507 a	1467 a
Control	1 b	167 b	23 a	52 a

[*] Total Suspended Solids

**Values followed by the same letter within each farm do not differ at the 10% level of significance according to Tukey's pairwise comparison.

At the Tech Research Farm, the plots that received the liquid dairy and turkey manure applications had lower bacteria concentrations than the control. The cowpie plots, consistent with the other sites, had the highest concentrations of bacteria in the runoff. The turkey plots resulted in less suspended solids in the runoff than the control, which may partially explain the reason for reduced bacterial loading from these plots.

E. coli Concentrations from the Release Plots

Figure 12 compares the average concentrations of *E. coli* in the water collected from the release plots at the three different farms using the data presented in Table 19. The plots treated with cowpies released the highest *E. coli* concentrations. In general, the plots treated with liquid dairy manure had higher *E. coli* concentrations than the plots treated with turkey litter. Statistical analyses performed on the treatments, which accounted for the different site locations, found statistical differences among all treatments except for the turkey treatment, which was not statistically different from the liquid dairy or control treatments at the 0.05 level. Statistical analyses were also performed to compare the treatments within each of the different sites at the 0.1 significance level. The 0.1 significance level is used when comparing the treatments within each site because of the selected method of statistical analysis. At the Dairy Farm, *E. coli* concentrations released from the cowpie treatment were significantly higher than those from all other treatments and the liquid dairy treatment was significantly higher than the control. At the Tech Research Farm, the concentrations released from the cowpie treatment were significantly higher than those from all other treatments.

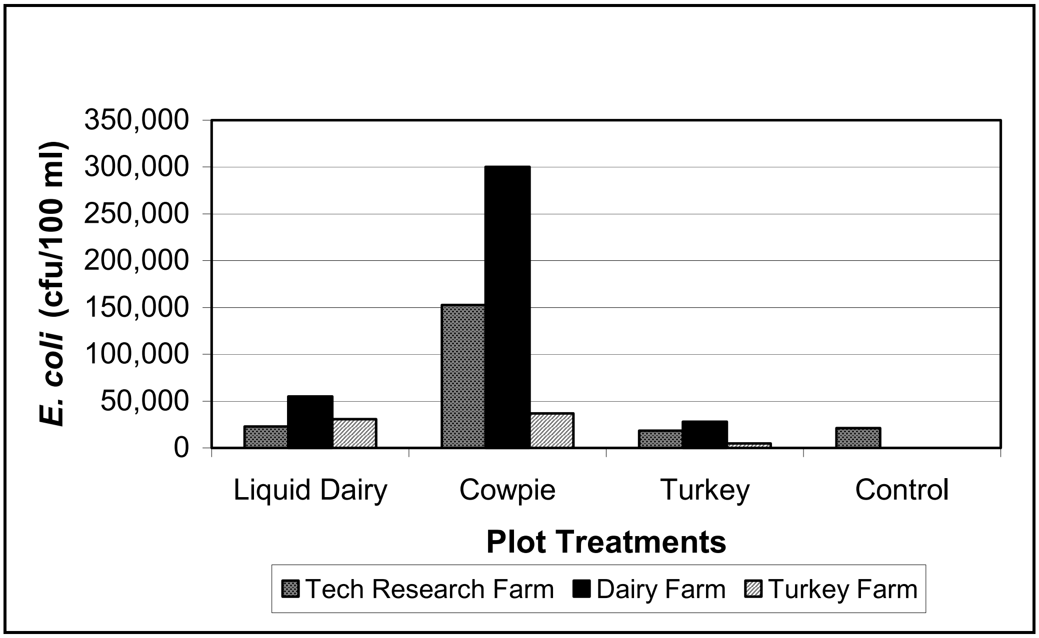

Figure 12. *E. coli* concentrations present in runoff water collected from release plots

FC Concentrations from the Release Plots

Figure 13 shows the concentration of FC released at the three different farms, previously presented in Table 19. The plots treated with cowpies, again, had higher FC concentrations in the runoff followed by the liquid dairy and turkey litter. Statistical analyses indicated significant differences among all treatments except for the turkey treatment, which was not statistically different from the control treatment at the 0.05 level. The FC release concentrations from the plots treated with cowpies ranged from 6.5×10^4 CFU/100 mL to 30×10^4 CFU/100 mL, which corresponds with the values reported by Larsen et al. (1994) who reported the FC release concentrations from bovine feces were between 40 × 10^4 and 115 × 10^4 organisms/100 mL.

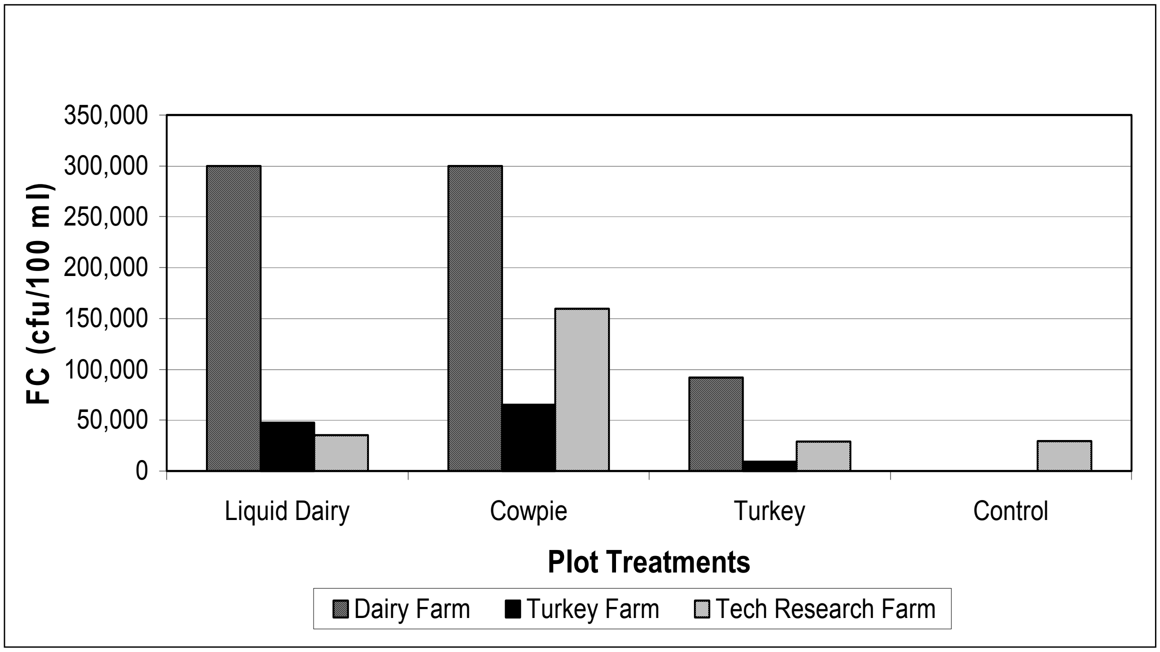

Figure 13. Fecal coliform concentrations present in runoff water collected from release plots

Statistical analyses were also performed to compare concentrations of FC in runoff among the treatments at each of the different sites at the 0.1 significance level. At the Dairy Farm, FC concentrations from all treatments were significantly higher than those from the control. The turkey treatment FC concentrations were also significantly lower than the liquid dairy and cowpie treatments. At the Tech Research Farm, the FC concentrations in cowpie treatment plots were significantly higher than those from all other treatments.

Enterococcus Concentrations from the Release Plots

The results from the enterococcal analysis from Table 19 are illustrated in Figure 14. A logarithmic scale is used to better show the differences among the different treatments. Statistical analyses performed on the *Enterococcus* concentrations in runoff collected from the release plots indicated significant differences in *Enterococcus* concentrations among all sites between the cowpie treatment and all other treatments at the 0.05 significance level. At the Tech Research Farm the *Enterococcus* concentrations from the cowpie treatment were significantly higher than those measured from all of the other treatments at the 0.1 level.

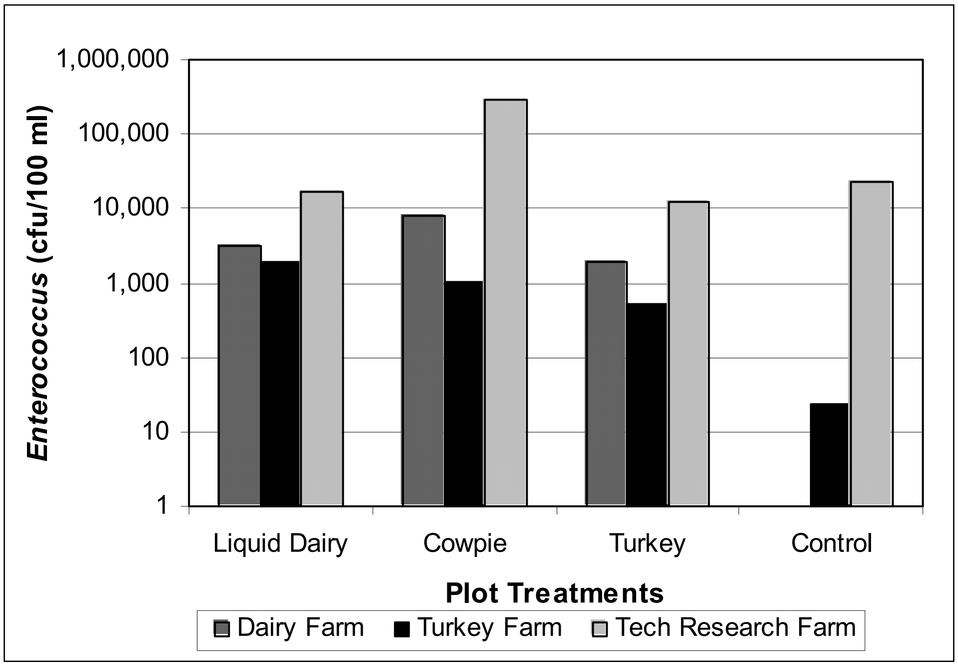

Figure 14. ***Enterococcus*** **concentrations present in runoff water collected from release plots**

4.2.3 Percent of Source Bacteria Released

By comparing the bacteria in the source manure that was applied to the release plots to the total bacteria collected in runoff from the release plots, the percent of the source bacteria that is initially released by rainfall was determined. The amount released would potentially be available to be transported to the edge of the field in overland flow. The total bacteria counts collected in runoff from the release plots were determined by multiplying the bacteria concentration in the runoff by the total volume of runoff. Table 20 shows the percent of bacteria released from the manure.

Table 20. Percent of bacteria that are released from the manure

Treatment	% *E. coli* released from waste	% Fecal Coliforms released from waste	% *Enterococcus* released from waste
Liquid Dairy	0.026%	~0.1%[*]	0.003%
Cowpie	~3.8%	~4.1%	~2.3%
Turkey	463.0%	1336.1%	87.3%

[*] Actual values may be less than or greater than the presented percentages samples were not diluted sufficiently.

A very low percentage of the source manure was present in the runoff from the liquid dairy treatments. Among the three bacteria species investigated, the percent of the FC released from the dairy waste was highest with 0.1% present in runoff. The percent released from the liquid dairy manure treatment is most likely low because the dairy manure was in a liquid form and infiltrated into the soil before the rainfall simulation was initiated. The percent released from the cowpie treatment ranged from 2.3% for *Enterococcus* and 4.1% for FC. These values were consistent among the three indicator species. As previously mentioned, the cowpie source manure and some of the water samples at the Dairy Farm were not diluted to the correct concentration level within the allotted time. Therefore, the percent released from the release plots may be more or less than the presented values. The turkey treatment had more bacteria released by rainfall than the amount applied to the plots in the source manure. There is a great degree of variability in bacterial content of turkey waste. The poultry litter that was applied to the plots may have been from a part of the pile that had more bacteria in it than the litter collected for the source bacterial analysis. Another possibility is background concentrations, although unlikely since the same was not observed on the plots treated with cowpies and liquid dairy manure.

4.2.4 Summary of Bacteria Release Study

In summary, the results from the release plots indicate that during a short but intense rainfall event, the cowpie treatment had the highest bacterial release rate, and had the greatest potential for bacteria to be transported with runoff into surface waters. The liquid dairy treatment had a slightly lower release rate, followed by the turkey litter treatment. Pasturelands with a history of previous manure applications did not result in higher bacterial release. The percent of source bacteria released was highest from the turkey litter treatment, but this was due to variability in the bacteria concentrations in the turkey litter.

In general, the FC concentrations in the effluent from the release plots were higher than the *E. coli* or *Enterococcus* concentrations. This result is understandable because FC can include many different fecal bacterial species while the *E. coli* agar is selective for a single species. The *Enterococcus* agar is selective for 20 species, which indicates that enterococcal bacteria are not as readily released as FC and *E. coli*. There are fewer enterococcal bacteria potentially available for transport in overland flow. Federal standards for primary contact also reflect these differences among the three indicator species. Standards for fecal coliforms are 200 CFU/100 mL while standards for *E. coli* are 126 CFU/100 mL, and standards for *Enterococcus* are 33 CFU/100 mL.

4.2.5 Transport Plots

The transport plot field experiments were designed to measure fecal bacteria concentrations transported to the edge of the field in runoff. The results were evaluated to determine if bacterial transport rates differ among different manure applications.

Source Manure Applied to Transport Plots

The number of CFU applied to each plot is presented in Table 21. The CFU applied to each plot was determined by multiplying the concentration of the source manure (Table 17) by the manure application rate. Since the manure was applied to the plots based on the phosphorus application rate, the bacterial counts varied based on the concentration of the phosphorus in the manure.

Table 21. The number of CFU (colony forming units) applied to each of the transport plots

Manure Source	*E. coli* (CFU/plot)	Fecal Coliform (CFU/plot)	*Enterococcus* (CFU/plot)
Liquid Dairy	1.8×10^{11}	$2.2\text{E}\times10^{11}$	3.0×10^{11}
Cowpie	$>4.8\times10^{10}$	$>4.8\times10^{10}$	$>4.8\times10^{10}$
Turkey	4.5×10^{7}	4.5×10^{7}	5.4×10^{7}

Fecal Bacterial Concentrations from the Transport Plots

The runoff samples from one of the plots treated with cowpies during S1 were not diluted sufficiently within the time allotted before die-off occurs and prior to sample analysis. A large number of samples were processed at once, making it impossible to always estimate the correct dilution level within the allotted time. The highest dilution levels achieved were averaged and are presented in Table 22. The concentrations from each of the individual samples are presented in Appendix D.

Table 22. Flow-weighted bacterial concentrations in runoff from the transport plots for rainfall simulations S1 and S2

Treatment	*E. coli* (CFU/100 mL)			FC* (CFU/100 mL)			*Enterococcus* (CFU/100 mL)		
	S1†	S2‡	Average	S1	S2	Average	S1	S2	Average
Liquid Dairy	31,294	5,526	18,410 b*	74,073	6,817	40,445 a	9,341	3,179	6,260 a
Cowpie	200,047	73,235	136,641 a	234,288	96,045	165,166 a	187,406	50,465	118,936 a
Turkey	9,275	16,450	12,863 b	16,719	18,953	17,836 a	6,757	6,521	6,639 a
Control	16	11	13 b	51	36	43 a	6	2	4 a

*Fecal coliforms; †Simulation 1; ‡Simulation 2

*Values followed by the same letter within each farm do not differ at the 5% level of significance according to Tukey's pairwise comparison.

Relationship between Fecal Bacteria and TSS Concentrations

The trends in the TSS concentrations (Table 16) were compared to the trends for the bacteria concentrations in the runoff from the transport plots (Table 22). In general, the plots treated with cowpies and liquid dairy manure had lower bacteria concentrations in the runoff during S2 than S1. The opposite occurred for the turkey litter, except for the

Enterococcus concentrations. The TSS concentrations, however, decreased during S2 simulations compared with S1 for the cowpies and turkey litter treatments (Table 16), but they increased for the plots treated with liquid dairy manure. These results indicate that higher TSS concentrations in runoff do not necessarily correspond with higher bacteria concentrations. Sufficient time may not have passed between the application of the manure and the runoff event for bacteria to become attached to sediment.

E. coli Concentrations from the Transport Plots

Figure 15 presents the *E. coli* results from Table 22 in a graphical form. The *E. coli* FWC decreased for the liquid dairy and cowpie treatments during S2, compared with the S1 values. For the turkey treatment, however, the *E. coli* concentrations increased during S2. This increase can be partly due to the nature of the poultry waste. The liquid dairy and cowpie wastes are more easily transported, while the turkey litter is dry and may require a more significant runoff event to release the bacteria for transport off of the plots. The runoff from the cowpie plots clearly had the highest *E. coli* FWC for both simulations. Statistical analysis was performed using the repeated measure method and Tukey's pairwise comparison (Ott and Longnecker, 2001). Statistical differences were only found between the *E. coli* concentrations in the runoff from the cowpie and the control plots. There were no statistical differences in *E. coli* concentrations between the two rainfall events.

Virginia DEQ standard for primary contact *E. coli* is 126 CFU/100 mL. The concentrations reported in this study are much greater since they represent the edge of the field levels as opposed to in-stream concentrations. In-stream concentrations are expected to be lower due to dilution effects and die-off.

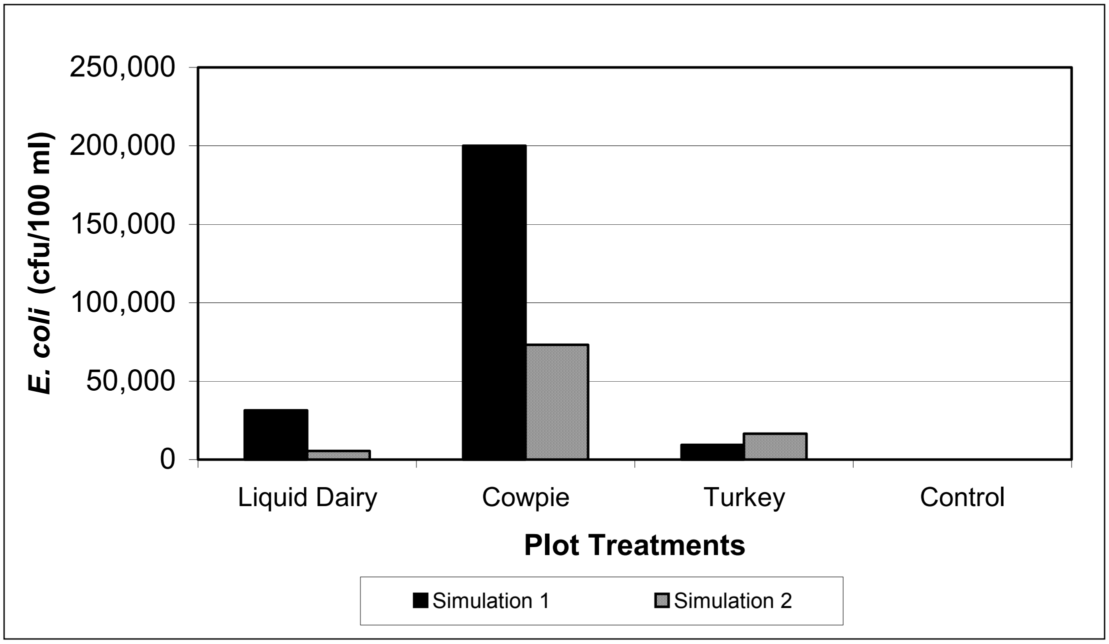

Figure 15. Flow-weighted concentrations of *E. coli* in runoff samples from transport plots

FC Concentrations from the Transport Plots

The concentrations of FC in runoff exhibited similar patterns as the E-coli among the different treatments (Figure 16). During S1, the liquid dairy and cowpie treatments had the highest average FWC of FC. During S2, the cowpie continued to produce the highest FWC of FC, but the runoff from the plots treated with turkey litter had the second highest FWC, followed by the liquid dairy treatment. The runoff FWC of FC from cowpie treatment was statistically different from all of the other treatments. There were no statistical differences in FC FWC for each treatment between the two rainfall simulations.

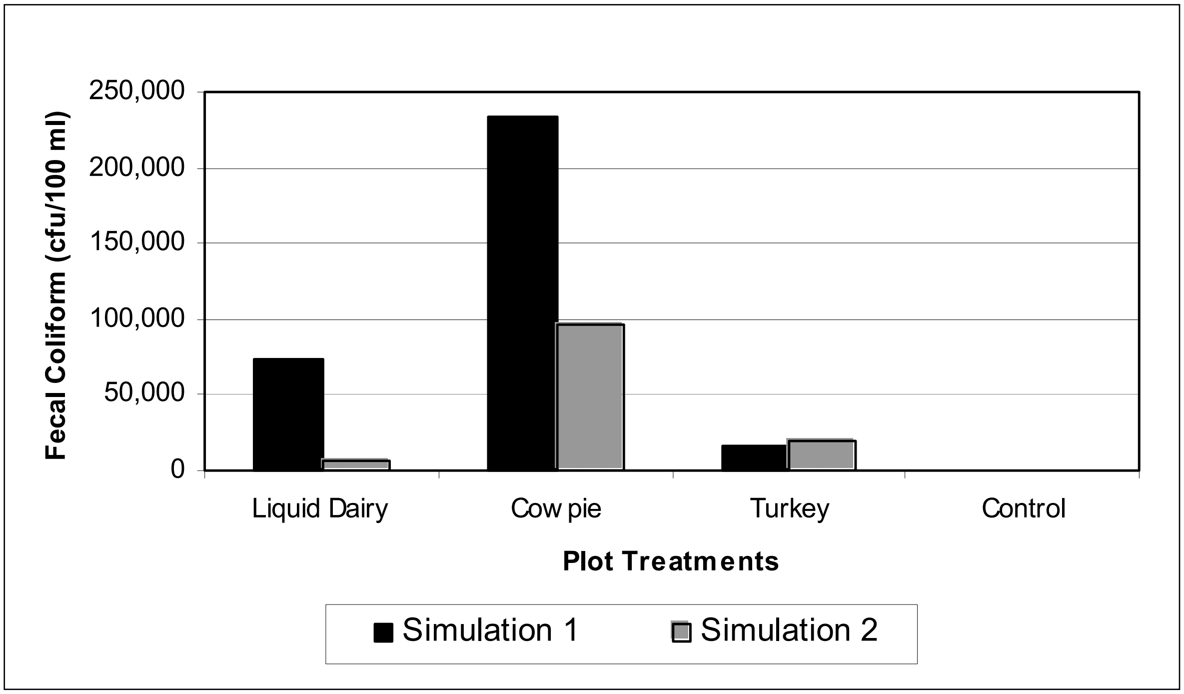

Figure 16. Flow-weighted concentrations of fecal coliform in runoff samples from transport plots

Federal standard for primary contact for FC is 200 CFU/100 mL, much less than the levels present in runoff from the manure treated plots. Baxter-Potter and Gilliland (1988) reported that the typical range of FC present in runoff from pastureland were 1,000 to 57,000 CFU/100 mL. The average value for the two simulations from the pasture treated with cowpies in this study was 1.65×10^5 CFU/100 mL. The cattle stocking density is not provided in the previous studies; therefore, it is not possible to make a meaningful comparison of the results. Furthermore, this study was designed to evaluate bacterial losses from edge of the field in small plots under intensive rainfall conditions. The bacterial concentrations reported in this study are expected to be much higher than those produced under natural rainfall from large pasture fields or watersheds. FC concentrations from grazed pasture in south central Nebraska contained concentrations of 1.21×10^5 CFU/100 mL (Schepers and Doran, 1980), which is similar to the results obtained from this study. Fecal bacteria concentration in runoff from grazed pasture is dependent on both the stocking density and the proximity of the cattle to streams. Cattle loafing in shaded or feeding areas produce high concentrations of cowpies in a smaller area and therefore higher bacteria concentrations in runoff. McCaskey et al. (1971) found FC concentrations to range from 1.4 to 21.7×10^6 CFU/100 mL in runoff from dairy waste applied to pasture plots by a tank wagon. They also reported that runoff from the control area had FC concentrations of 9.9×10^5 CFU/100mL. These values are much greater than the concentrations of 4.0×10^4 CFU/100 mL measured in our study.

Enterococcus Concentrations from the Transport Plots

Figure 17 presents the *Enterococcus* results from Table 22 in a graphical form. *Enterococcus* concentrations in runoff were slightly lower than the *E. coli* and FC concentrations for all treatments. *Enterococcus* is a subgroup of fecal streptococcus. *Enterococcus* is used as indicator bacteria because it is often present in recreational water bodies when human illness occurs (USDA, 2000) and is most often used as a fecal indicator in marine waters. Federal standard for primary contact *Enterococcus* is 33 CFU/100 mL, much less than the levels present in runoff from the manure treated plots.

During S1 simulation the cowpie treatments had the highest average FWC of *Enterococcus*, followed by the liquid dairy and turkey litter treatments. During S2, the cowpie treatment continued to produce the highest FWC of *Enterococcus*, but the runoff from the plots treated with turkey litter had the second highest FWC, followed by the liquid dairy. There were no statistical differences in enterococcal FWC for each treatment between the two rainfall simulations or among the different treatments.

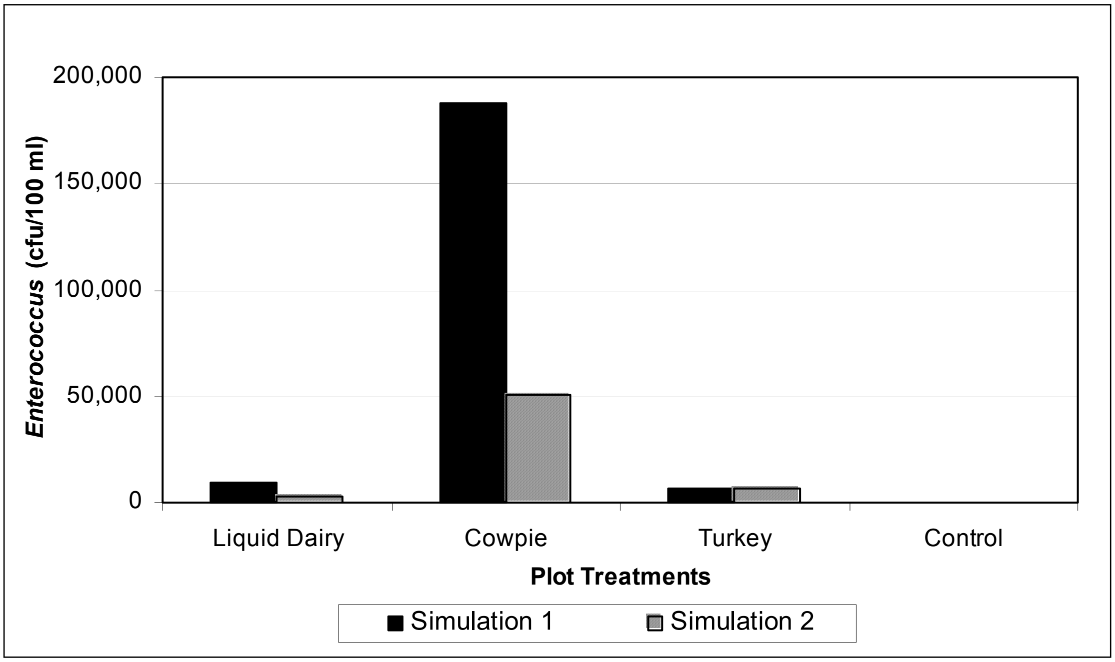

Figure 17. Flow-weighted concentrations of *Enterococcus* in runoff samples from transport plots

4.2.6 Percent of Source Bacteria Transported

By comparing the bacteria in the source manure that was applied to the transport plots (Table 21) to the total bacterial load in runoff from the transport plots, the percent of the source bacteria that was transported to the edge of the field in overland flow was calculated. The total bacteria in runoff from the transport plots were calculated by multiplying the bacteria concentration in the runoff by the total volume of runoff at each sample point. Table 23 shows the percent of source bacteria transported to the edge of field in overland flow.

Table 23. Percent of source bacteria that is present in overland flow

Treatment	% *E. coli* transported from waste	% fecal coliform transported from waste	% *Enterococcus* transported from waste
Liquid Dairy	0.06%	0.09%	0.02%
Cowpies	~1.98%[*]	~2.35%	~1.69%
Turkey	157.25%	208.72%	55.60%

[*] Actual values may be less than or greater than the presented percentages because the cowpie source manure and the runoff samples from one of the plots treated with cowpies during S1 were not diluted sufficiently.

The liquid dairy treatment had the lowest percentage of the source manure present in runoff, ranging from 0.02% for *Enterococcus* to 0.09% for fecal coliforms. This result was similar to that from the release plots and is most likely because the dairy manure was in liquid form and was able to infiltrate into the soil before the rainfall simulation began. For the cowpie treatment the percentage of the source manure present in runoff ranged from 1.69% for *Enterococcus* to 2.53% for fecal coliforms. As previously mentioned, the cowpie source manure and the runoff samples from one of the plots treated with cowpies during S1 were not diluted sufficiently. Therefore, the percent transported plots may be more or less than the presented values. The turkey treatment had more bacteria transported to the edge of the field in runoff than the amount applied to the plots in the source manure. The poultry litter that was applied to the plots may have been from a section of the pile that had more bacteria in it than the litter collected for the bacterial analysis. Another possibility is background concentrations, although unlikely since the higher concentrations in runoff were not observed on the plots treated with cowpies and liquid dairy manure.

4.2.7 Percent of Released Bacteria Present at the Edge of the Field

To determine a relationship between the bacteria release (amount available for transport) and transport, the average concentrations of all three farms from the release plots were compared to the average FWC of S1 and S2 from the transport plots. The average FWC of S1 and S2 from the transport plots was used because there were no significant differences in FWC between the two rainfall events. By comparing the concentrations from the release plots to the concentrations from the transport plots, the percent of the bacteria initially released by rainfall that is transported to the edge of the field in overland flow was determined. Table 24 shows the percent of the released bacteria that is transported in overland flow.

Table 24. Percent of released bacteria that were present in overland flow

Manure Treatment	% Released *E. coli* present in overland flow	% Released fecal coliform present in overland flow	% Released *Enterococcus* present in overland flow
Liquid Dairy	50.8%	31.7%	85.6%
Cowpie	83.7%	94.5%	121.3%
Turkey	75.2%	41.1%	137.7%
Control	0.2%	0.4%	0.1%

The cowpie treatment had the highest percentage of released bacteria transported to the edge of the field with percentages ranging from 95 % for FC to 121% for *Enterococcus*. The turkey treatment follows with percentages ranging from 41% to 138%. The differences among the three species are related to the survival characteristics of the bacteria. *Enterococcus* is able to survive longer in the environment than FC and *E. coli* because it is able to survive at higher temperatures, in high salt concentrations, and at high pH levels (Hagedorn, 2003b). The transport concentrations may be higher than the release concentrations because of background bacteria present in the soil.

4.2.8 Summary of Bacteria Transport Study

In summary, the results from the transport plots indicate that during a runoff event that occurred soon after land application of waste, the cowpie treatment had the highest bacterial transport rate, followed by the liquid dairy manure and turkey litter treatments. The average bacteria flow-weighted concentrations in runoff samples from the plots treated with cowpies were 137,000 CFU/100 mL for *E. coli*, 165,000 CFU/100 mL for FC, and 119,000 CFU/100 mL for *Enterococcus*. Runoff from plots treated with liquid dairy treatment had greater fecal bacteria concentrations in runoff during the first rainfall event (S1), which was applied within 24 hours after manure application. These concentrations however were reduced during the second rainfall event (S2), which occurred one day after the initial rainfall. The turkey treatment resulted in the opposite effect. During S1, the bacteria concentrations remained low, but increased during S2. This result was most likely explained by the composition and transport characteristics of the waste. The cowpies were easily broken apart by the impact of the raindrops and bacteria were transported off of the plots. The bacteria in liquid dairy manure infiltrated into the soil before the rainfall event, reducing the available bacteria in the waste while the bacteria in the turkey litter were more easily transported off the plots with the higher runoff volumes during S2.

The percent of source bacteria transported to the edge of the field was highest from the turkey litter treatment, but this was due to variability in the bacteria concentrations in the turkey litter. The cowpie treatment had the highest percentage of released bacteria transported to the edge of the field (ranging from 95 % for FC to 121% for *Enterococcus*), followed by the turkey litter treatment (41% to 138%).

4.2.9 Temporal distribution of Bacterial concentrations

The time distribution of bacteria concentrations with respect to the runoff hydrograph was investigated to determine the relationship between the flow and the sample concentrations. Individual samples were taken from the transport plots every 3 to 9 minutes during the rainfall simulations. The individual samples were collected and analyzed for bacteria concentrations to evaluate the temporal distribution of bacteria concentrations. Two patterns were most commonly observed in the flow/concentration relationship. The most common pattern indicated that the concentrations increased with flow rates, as shown in Figure 18.

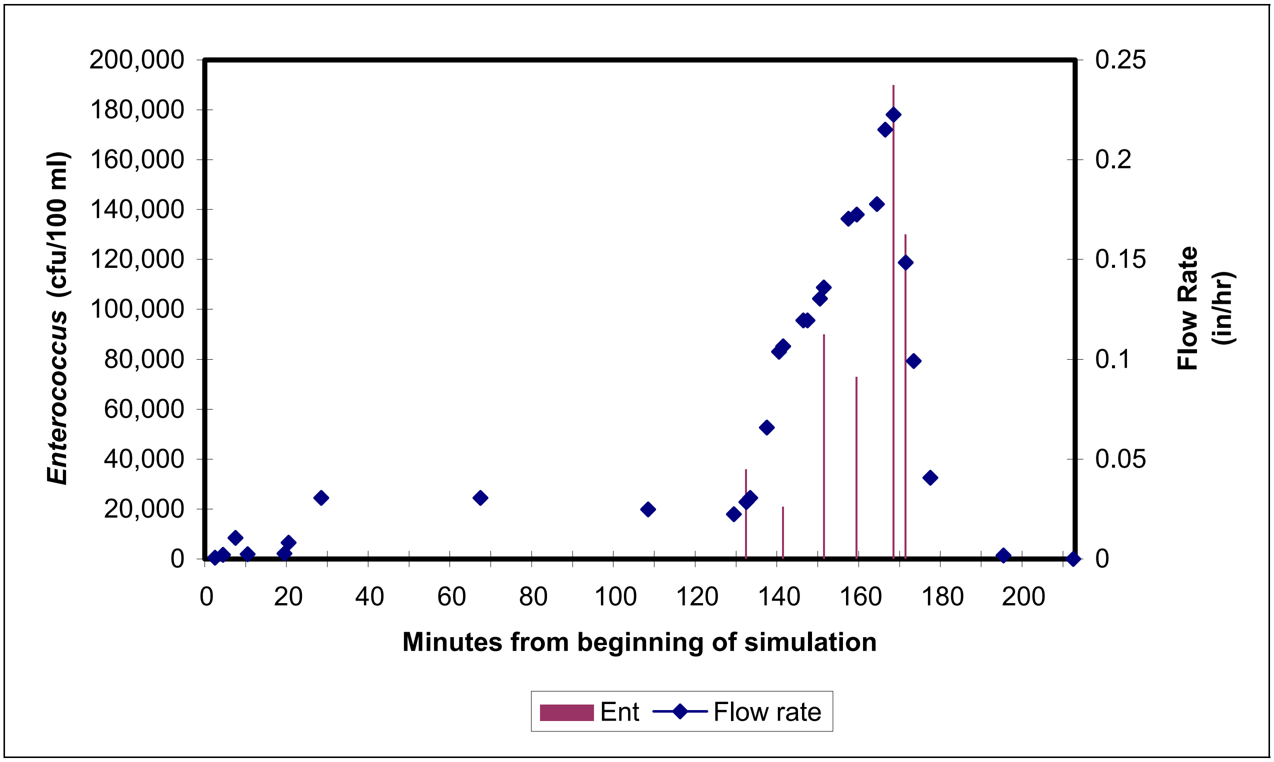

Figure 18. A comparison of the flow rate and *Enterococcus* concentrations in runoff from the transport plots (T2R1 cowpie treatment)

Another common pattern observed was that the first sample, taken immediately after runoff began, had the highest concentration of bacteria but it decreased in the subsequent samples (Figure 19). At times, the bacteria concentration in the last sample, which was taken about three minutes after the rain stopped, increased again. These results indicate that substantial dilution was taking place and therefore, higher concentrations were observed during low runoff rate periods (beginning and end of hydrograph).

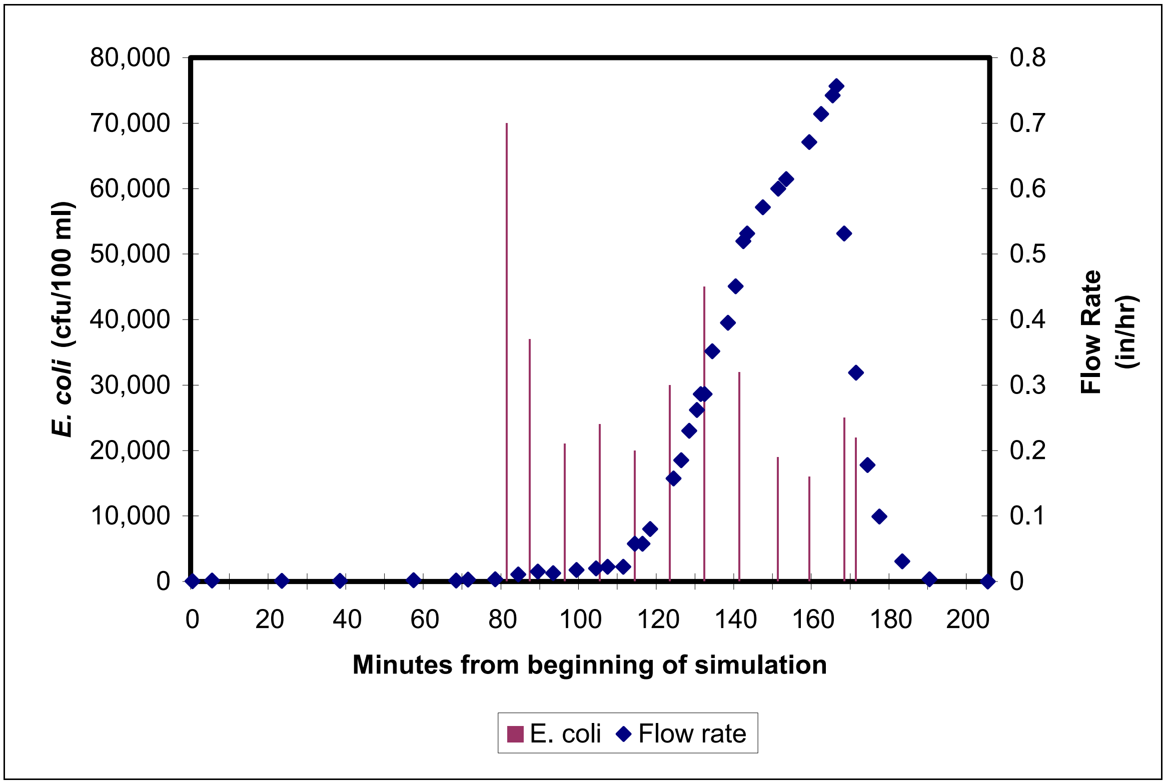

Figure 19. A comparison of the flow rate and *E. coli* concentrations in runoff from the transport plots (T5R1 liquid dairy treatment)

The relationship between flow and bacteria concentration appears to be dependent upon the indicator species and the type of manure the plots receive. The plots treated with dairy manure followed both of the patterns presented in Figures 19 and 20. During S1, the FC concentrations were highest during the peak flow, while the *E. coli* concentrations peaked immediately after the start of runoff and gradually decrease as the runoff event continued. During S2, the plots treated with liquid dairy manure followed the pattern presented in Figure 19 for all indicator species. The concentrations in runoff from the plots treated with cowpies followed the pattern presented in Figure 18 for all indicator species during S1. However, during the S2 simulations, they followed the pattern presented in Figure 19 with a peak occurring at the last sampling time for all indicator species. During the second simulation (S2), the cowpies may have broken apart from the impact of the raindrops, accounting for the higher runoff concentrations during the high flow rates. For the plots treated with turkey litter, the bacteria concentrations were highest during peak flow (Figure 18) for all three indicator species, for both rainfall simulations. These patterns are summarized in Table 25.

Table 25. Temporal distribution of indicator species and manure treatments

Indicator Species	**Liquid Dairy**		**Cowpie**		**Turkey**	
	S1	**S2**	**S1**	**S2**	**S1**	**S2**
E. coli	Figure 19*	Figure 19	Figure 18†	Figure 19	Figure 18	Figure 18
FC	Figure 18	Figure 19	Figure 18	Figure 19	Figure 18	Figure 18
Enterococcus	Figure 19	Figure 19	Figure 18	Figure 19	Figure 18	Figure 18

*bacteria concentrations decreased with time
†concentrations corresponded to flow rate

From Figures 19 and 20, it appears that the occurrence of the peak bacteria concentration is related to the runoff rate. In some cases the peak bacteria concentration may occur at the time of peak flow (Figure 18). In these situations, best management practices that reduce peak runoff rates, such as no-till farming, may be most suitable for reducing bacterial loading into waterways. The peak bacteria concentration could also occur immediately after runoff begins (Figure 19), which would indicate that best management practices that reduce the first flush effects, such as detention basins, would reduce bacterial transport.

4.2.10 Bacterial Source Tracking

Because of the limited resources available to the project and the large number of pastured cattle located throughout Virginia only the cowpie treatment was selected for a limited Bacterial Source Tracking (BST) study. Even though the same species of *Enterococcus* can be found in several different host species, major variation in the distribution of the different enterococcal species among hosts have been found (Aarestrup et al., 2002). The goal of this study was to identify the enterococcal species present in dairy manure and determine which species have the highest potential to be transported by runoff.

The commercial Biolog System was the BST method selected to evaluate the species of enterococcal isolates collected from the source manure and in runoff. The Biolog System is often used in clinical laboratories, where the genus of an unknown colony must be correctly identified more than 90% of the time (Biolog Technical Manuel, 1999). The Biolog library consists of 6 to 8 isolates that correspond with each species in the software library. Biolog compares the unknown isolates to those in the library to determine the similarity to each. Once the similarity is determined, the system provides a percent probability that the correct species has been identified. Some isolates were identified, but because of the large degree of variability in isolates existing in the environment a percent probability of correct identification could not be provided. Table 26 shows the enterococcal species identified in this study and the average percent probability of correct identification.

Table 26. The average percent probability of correct identification of enterococcal species in source manure and runoff identified by the Biolog System

Species	Total number Isolates Identified	Average Percent Probability of Correct Identification	Number identified with no Percent Probability of Correct Identification
Enterococcus faecalis	46	100%	2
Enterococcus mundtii	36	93%	8
Enterococcus gallinarum	17	91%	4
Enterococcus faecium	16	71%	11
Enterococcus casseliflavus	8	94%	2
Enterococcus hirae	5	98%	1
Enterococcus flavescens	4	89%	2
Enterococcus sulfureus	2	0%	2
Enterococcus solitarius	2	88%	1
Enterococcus avium	1	98%	0
Enterococcus columbae	1	0%	1
Enterococcus raffinosus	1	0%	1
Enterococcus saccharolyticus	1	100%	0

Twenty isolates were taken from plates that represent the runoff conditions at the beginning of runoff, at the peak runoff point, and at the end of the runoff event. Isolates were taken from each of these points for both rainfall simulations so that a comparison could be made between dry and wet soil conditions. In addition, twenty isolates were taken from the source plate to identify the enterococcal species present in the source manure (Table 27). Twenty isolates is a very small percentage of the total number of colonies present in the runoff and source manure samples. Ideally, 5% of the total count from the samples should be evaluated for source tracking. Because of the expense associated with the Biolog System and the high bacteria concentrations in the runoff and source manure samples, this was not possible.

Table 27. Enterococcal species identified in dairy cowpies (source manure) using the Biolog system

Enterococcal Species present in Source Manure	Number Identified	Percent Identified
Enterococcus mundtii	11	55%
Enterococcus gallinarum	4	20%
Enterococcus faecium	2	10%
Enterococcus faecalis	2	10%
Enterococcus solitarius	1	5%

The source manure is dominated by the *Enterococcus mundtii*, a species that is commonly related to cattle manure and also tends to die off quickly. The presence of *Enterococcus faecalis* in cattle source manure is somewhat controversial. The results from this study agree with findings from Rutkowski and Sjogren (1987) where *Enterococcus faecalis* was found to be present in cattle manure, but disagree with studies conducted by Pourcher et al. (1991) and Wheeler et al. (2002). A study by Bernstein et al. (2002) also used the Biolog method to test 50 isolates from cow manure in California. Their study found *Enterococcus faecalis* to be 2% of the total isolates identified. Thirty-eight percent of the isolates were identified as *Enterococcus casseliflavus,*31% were identified as *Enterococcus flavescens*, and 12% were identified as *Ent gallinarum*. An unpublished study by Hagedorn (C. Hagedorn, unpublished data, 2003. Blacksburg, Va.:

Virginia Tech) used the Biolog method to test 53 isolates from cow manure in Delaware and found only 2% of the isolates tested were identified as *Enterococcus faecalis*. Fifty-three percent of the isolates were identified as *Enterococcus mundtii*, 21% were identified as *Enterococcus gallinarum*, 9% were identified as *Enterococcus* c*asseliflavus* and *Enterococcus faecium*, 4% were identified as *Enterococcus flavescens*, and 2% were identified as *Enterococcus durans*. Although a small percentage of the available enterococcal isolates were identified, the results appear to be consistent with previous results obtained using the Biolog System.

In addition to evaluating the source manure to determine the existing species, it is also important to consider the transport capacities of the different species. Even if an enterococcal species is present in the source manure, it may not be transportable in surface runoff. The Biolog System was used to identify enterococcal species present in runoff at different stages of the runoff events. Enterococcal isolates were picked from the microfilters which represented samples taken from the beginning of the runoff event, the peak runoff point, and the end of the runoff event. The first rainfall simulation (S1) occurred within 24 hours of the initial manure application and represents a worst case scenario. Table 28 presents the number of colonies identified as each species in runoff samples collected at various stages of the runoff hydrograph.

Table 28. Enterococcal species present during the first rainfall simulation (S1) which occurred less than 24 hours after land application of waste

Enterococcal Species present in runoff at the beginning of S1	**Number Identified**	**Percent Identified**
Enterococcus faecalis	14	70%
Enterococcus gallinarum	1	5%
Enterococcus casseliflavus	1	5%
Enterococcus sulfureus	1	5%
Enterococcus avium	1	5%
Enterococcus saccharolyticus	1	5%
Enterococcus columbae	1	5%
Enterococcal Species present in runoff at the S1 Peak runoff	**Number Identified**	**Percent Identified**
Enterococcus faecalis	9	45%
Enterococcus mundtii	5	25%
Enterococcus gallinarum	4	20%
Enterococcus sulfureus	1	5%
Enterococcus cricetus	1	5%
Enterococcus Species present in runoff at the end of S1	**Number Identified**	**Percent Identified**
Enterococcus mundtii	8	40%
Enterococcus gallinarum	6	30%
Enterococcus faecalis	3	15%
Enterococcus solitarius	1	5%
Enterococcus flavenscens	1	5%
Enterococcus casseliflavus	1	5%

The trends present during the runoff event indicated immediate high numbers of *Enterococcus faecalis* in the sample taken immediately after the start of the runoff event which gradually declined with time as runoff continued (Table 28). On the other hand, *Enterococcus mundtii* , which was not identified in the initial sample, was present in higher numbers in the two subsequent runoff samples. *Enterococcus gallinarum* detection also increased from one in the initial sample to six in the third sample. These results indicate that the survival and transport of various species are significantly impacted by runoff characteristics. *Enterococcus faecalis* may be more transportable during initial phase of the runoff; however, *Enterococcus mundtii* and *Enterococcus gallinarum* are apparently transported more readily during higher runoff rates.

The trends during the second runoff event (S2 simulation) (Table 29) were somewhat opposite of those observed for the first runoff event. At the beginning of the simulation, *Enterococcus faecium* was the predominant species present in the runoff, but decreased in samples taken during the peak runoff and at the end of the runoff event. *Enterococcus mundtii* was highest during the second runoff sample taken at the peak flow rate, but was not present in the sample collected at the end of runoff event. *Enterococcus faecalis* was present in lower number during the beginning of the runoff event, but increases during the peak flow and is highest during the end of the simulation.

Table 29. Enterococcal species present during the second rainfall simulation (S2) which occurred about 24 hours after the first rainfall event

Enterococcal Species present in runoff at the beginning of S2	Number Identified	Percent Identified
Enterococcus faecium	10	50%
Enterococcus mundtii	4	20%
Enterococcus faecalis	2	10%
Enterococcus gallinarum	1	5%
Enterococcus raffinosus	1	5%
Enterococcus casseliflavus	1	5%
Enterococcus hirae	1	5%
Enterococcal Species present in runoff at the S2 peak runoff	**Number Identified**	**Percent Identified**
Enterococcus mundtii	8	40%
Enterococcus faecalis	6	30%
Enterococcus hirae	3	15%
Enterococcus faecium	2	10%
Enterococcus casseliflavus	1	5%
Enterococcal Species present in runoff at the end of S2	**Number Identified**	**Percent Identified**
Enterococcus faecalis	10	50%
Enterococcus casseliflavus	4	20%
Enterococcus flavenscens	3	15%
Enterococcus faecium	1	5%
Enterococcus gallinarum	1	5%
Enterococcus hirae	1	5%

The analysis of combined runoff events for S1 and S2 indicated that 37% of the isolates present in runoff were identified as *Enterococcus faecalis*, 21% of the isolates present in runoff were identified as *Enterococcus mundtii*, 11% of the isolates present in runoff were identified as *Enterococcus gallinarum* and *Enterococcus faecium*. These results indicate that *Enterococcus faecalis* may be the dominant species present in runoff from grazed pasturelands. *Enterococcus faecalis* was not present in high numbers in the source sample, but was consistently found in the runoff, indicating that it may be a more transportable species of *Enterococcus*. *Enterococcus mundtii* is also a possible form of *Enterococcus* frequently found in runoff, but may die-off more quickly than other species of *Enterococcus*, which could indicate that the *Enterococcus mundtii* may not be present if additional rainfall events were preformed at a later time after the land application of the waste.

The identification of predominant species of *Enterococcus* that are associated with specific sources of fecal pollution could greatly simplify the identification of non-point source pollution. The presence of a certain species of *Enterococcus* would indicate the source of the fecal pollution and proper measures could be taken to reduce loading without the use of computer simulation models or expensive BST analysis. In order to accomplish this task, the most transportable species related to fecal pollution from different sources should be identified. This research provides a base line for dairy cattle, but much more research is needed in this area.

4.2.11 Relationship between E. coli and FC

A relationship between FC and *E. coli* was developed by the Commonwealth of Virginia, Department of Environmental Quality, and is presented in Equation 3. This translator function was developed to translate FC data into *E. coli* data and was developed based on in-stream concentrations of fecal bacteria. This equation allows for calibration of the computer simulation models using existing FC data. When the model calibration and validation are completed, the output is converted to *E. coli* so that an *E.*

coli TMDL can be developed. This translator is necessary since the bacterial standards in Virginia have recently changed from FC to *E. coli*.

$$\log_2 EC\,(cfu/100\,mL) = -0.0172 + 0.91905 * \log_2 FC\,(cfu/100\,mL) \qquad (3)$$

Because of the large number of samples taken during this study, it provides an excellent opportunity to test the accuracy of this equation. Values were tested when the correct dilution level was obtained for both FC and *E. coli*. The equation was tested by inserting the FC values obtained from this study and calculating the *E. coli* concentrations using Equation 3. A total of 104 comparisons were made using results from both the transport (S1 and S2) and release plots. The predicted *E. coli* concentrations were compared to the actual *E. coli* concentrations. The differences between the actual and predicted *E. coli* values ranged from a -61.3% underestimation to 216.8% overestimation. When the percent difference among all values was averaged, it was determined that the equation underestimated the *E. coli* concentrations by an average of 22%. The equation overestimated 12 and underestimated 88 of the 100 values for the *E. coli* concentration. The translator may not accurately predict the relationship between fecal coliform and *E. coli* from this study because the results represent concentrations present in runoff at the edge of the field while the equation was developed based on in-stream concentrations. The complete dataset and percent differences for each data point are presented in Appendix D. Figure 20 presents the results in graphical format.

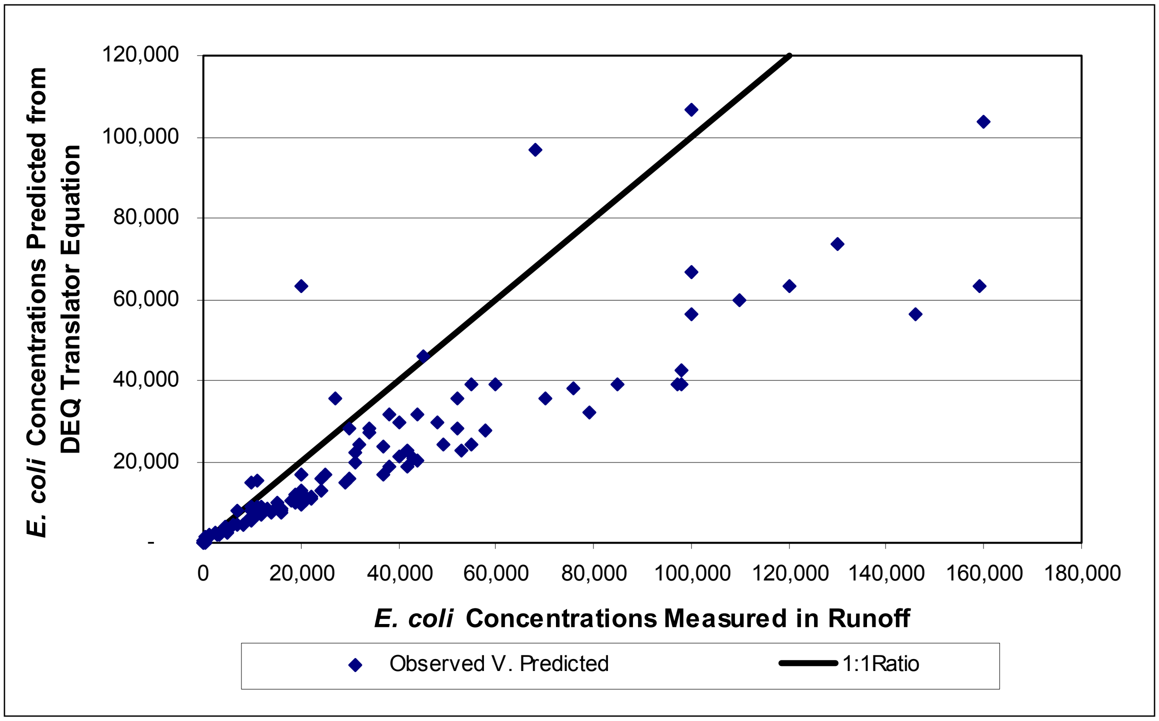

Figure 20. Comparison of *E. coli* concentrations present in runoff samples and *E. coli* concentrations predicted using the DEQ translator equation

4.3 Nutrients

This section discusses the results of the nutrient analysis on the release and transport plots. Comparisons were made between the different manure treatments to determine which one had the greatest potential to release nutrients, making them available for transport in overland flow. The results were also analyzed to determine which source manure resulted in the greatest concentrations of nutrients in overland flow. The percent of nutrients present in source manure that was released and was transported in runoff was also evaluated.

The study design represents a worst case scenario since the rainfall event occurred within 12 to 24 hours after land application of the waste. Nutrient concentrations were higher than they would be if the rainfall event had been delayed for days or weeks after the land application due to nutrient attachment to soil and due to plant uptake.

4.3.1 Source Manure

The results of the manure analysis are presented in Table 30. The values reported in the literature agree with the turkey litter concentration used in this project, but the phosphorus levels in dairy manure are quite different. The dairy manure used for this research project had lower concentrations of phosphorus than average dairy farms. The cows' diets were properly managed at the Virginia Tech Dairy to reduce phosphorus concentrations in the manure (Zhengxia et al., 2002).

Table 30. Concentrations of Phosphate, Ammonia Nitrogen, and Nitrate Nitrogen present in the source manure used in the study

Manure Source	Phosphate	Ammonia Nitrogen	Nitrate Nitrogen	Moisture Content (%)
Liquid Dairy	0.68 kg/1000L	0.64 kg/1000L	0.05 kg/1000L	96%
Cowpie	1.80 kg/t	0.90 kg/t	0.05 kg/t	83%
Turkey	19.9 kg/t	7.95 kg/t	0.19 kg/t	49%

4.3.2 Release Plots

The release plots were constructed to measure nutrient concentrations available for transport to the edge of the field in runoff, and to determine if nutrient release rates differ among pasturelands that previously received manure applications and those that had not. The release plots compared the effects of additional manure applications on soils already heavily loaded with phosphorus.

Source Manure Applied to Release Plots

The mass of each nutrient applied to the release plots is presented in Table 31. The nutrient mass applied to each plot was determined by multiplying the concentration of the nutrients in the source manure (Table 30) by the manure application rate. Since the manure was applied to the plots based on the phosphate (P_2O_5) application rate, the nitrate and ammonia application rates varied greatly, based on their concentrations in the manure. The total nitrogen was calculated by adding the nitrate, ammonia, and organic nitrogen concentrations in the source manure. The total nitrogen concentration in the source manure was then multiplied by the manure application rate.

Table 31. The total mass of the nutrients applied to the release plots

Manure Source	Phosphate	Nitrate Nitrogen	Ammonia Nitrogen	Total Nitrogen
Liquid Dairy	5.6 g (56 kg/ha)	0.41 g (4.1 kg/ha)	5.25 g (53 kg/ha)	14.6 g (146 kg/ha)
Cowpie	5.0 g (50 kg/ha)	0.16 g (1.57 kg/ha)	2.70 g (27 kg/ha)	15.2 g (152 kg/ha)
Turkey	5.5 g (55 kg/ha)	0.05 g (0.52 kg/ha)	2.23 g (22 kg/ha)	6.45 g (64 kg/ha)

The rainwater applied to the release plots was also analyzed for nutrient concentrations. The nutrient concentrations are presented in Table 32.

Table 32. Nutrient concentrations in rainwater

Rainwater	DP (mg/L)	SBP (mg/L)	Total P (mg/L)	Nitrate (mg/L)	Ammonia (mg/L)	TKN (mg/L)	TN (mg/L)
Release Plots	0.01	0.00	0.01	2.95	0.00	0.31	3.27

Phosphorus Concentrations

The released phosphorus concentrations are presented in Table 33. The phosphorus concentrations are presented for all the three farms, and the values from all three farms are averaged to compare the effects of the different treatments. The average values presented for each of the individual farms are utilized for comparison of the landuse history on the released nutrient concentrations.

Table 33. Concentrations of Dissolved Phosphorus, Sediment Bound Phosphorus, and Total Phosphorus present in runoff collected from the release plots

	Tech Research Farm					Dairy Farm				
Treatment	**Dissolved Phosphorus**		**Sediment Bound Phosphorus**		**TP***	**Dissolved Phosphorus**		**Sediment Bound Phosphorus**		**TP**
	(mg/L)	**% of TP**	**(mg/L)**	**% of TP**	**(mg/L)**	**(mg/L)**	**% of TP**	**(mg/L)**	**% of TP**	**(mg/L)**
Liquid Dairy	1.96	76%	0.63	24%	2.59	1.89	62%	1.13	38%	3.02
Cowpie	1.46	53%	1.30	47%	2.76	1.51	35%	2.76	65%	4.27
Turkey	1.08	70%	0.45	30%	1.53	1.96	62%	1.22	38%	3.19
Control	0.66	84%	0.12	16%	0.78	0.08	25%	0.24	75%	0.32
Average	**1.29**	**71%**	**0.63**	**29%**	**1.91**	**1.36**	**46%**	**1.34**	**54%**	**2.70**
	Turkey Farm					**Average of all Farms**				
Liquid Dairy	2.02	60%	1.36	40%	3.38	1.96	65%	1.04	35%	3.00
Cowpie	0.79	34%	1.54	66%	2.33	1.25	40%	1.87	60%	3.12
Turkey	0.26	45%	0.31	55%	0.56	1.10	62%	0.66	38%	1.76
Control	0.23	37%	0.38	63%	0.61	0.32	56%	0.25	44%	0.57
Average	**0.82**	**44%**	**0.90**	**56%**	**1.72**	**1.16**	**56%**	**0.95**	**44%**	**2.11**

* Total Phosphorous

Dissolved Phosphorus Treatment Effects

The average dissolved phosphorus (P_2O_4) concentration released at all three sites was highest for plots treated with the liquid dairy manure (1.96 mg/L), followed by the cowpie (1.25 mg/L) and turkey litter treatments (1.10 mg/L). Figure 21 presents the dissolved phosphorus (DP) concentrations in graphical form. Statistical analyses were performed on the release plots using the Generalized Randomized Block Design (GRBD) and the Tukey pairwise comparison (Ott and Longnecker, 2001) to determine statistical differences in DP concentrations released from the plots among different manure types. Statistical analysis indicated that the DP concentrations in liquid dairy treatment were significantly higher than the control treatment at the 0.05 significance level. The DP concentration released from the liquid dairy treatment may be higher because the waste is applied to the plots in a liquid form. The phosphate applied in the liquid form may be more easily released in the form of DP. The liquid dairy manure also would have less bioavailable phosphorus attached to organic matter particles than the cowpie and turkey litter treatments.

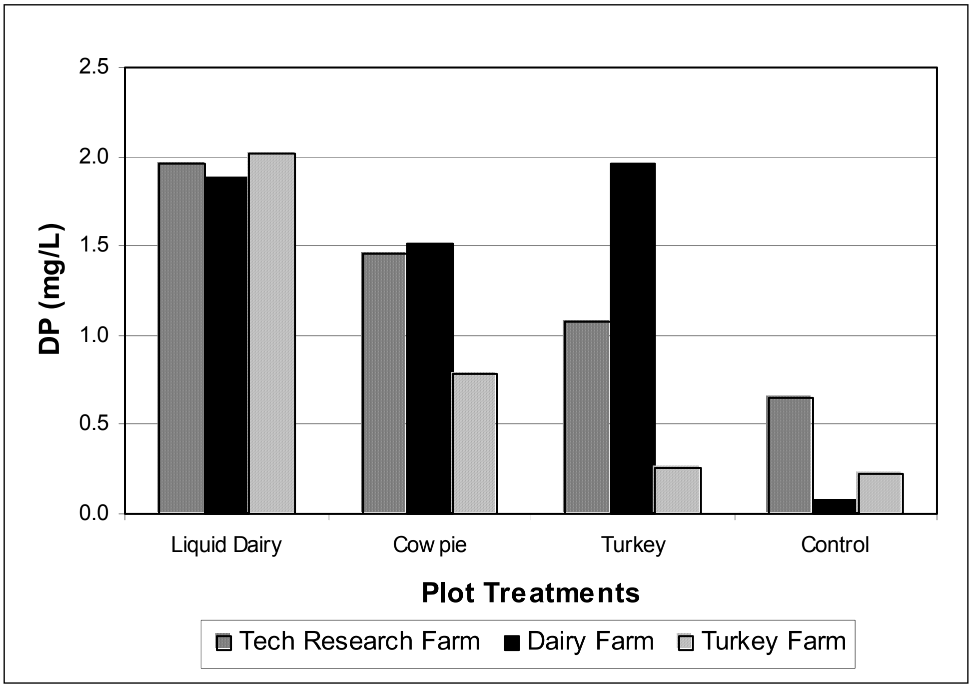

Figure 21. Dissolved Phosphorus concentrations released from the plots

Effects of Landuse History on DP Concentrations

Because the rainfall event occurred within 24 hours of the manure application, the DP released from the plots is most likely due to the animal waste applications since enough time did not pass between the manure application and the rainfall event for the phosphorus to become sediment bound. The average DP concentrations account for 71% of the total phosphorus (TP) at the Tech Research Farm where the soils had medium to low soil phosphorus levels. At the Dairy Farm and the Turkey Farms, the average DP concentrations account for 46% and 44% of the TP, respectively, which clearly indicates that a larger portion of the TP is in the dissolved form and directly related to manure applications when background soil phosphorus concentrations are low.

Source Phosphate Released from Plots

The percent of phosphate (P_2O_5) released from the source manure in the form of DP (P_2O_4) is presented in Table 34. The manure was applied to the plots based on the phosphate concentration, or the plant available phosphorus. Both dissolved phosphorus and a portion of the particulate phosphorus are available for biological uptake (Hansen et al., 2002). The total yield of DP removed from the release plots was calculated by multiplying the sample concentration by the total volume of runoff generated from each plot. The DP yield from each of the plots was averaged among the different treatments. The DP yield was then divided by the total amount of phosphate applied to the plots (Table 31) to determine the percent released from the plots.

Table 34. Percent of nutrients in source manure released from the plots

	% of Phosphate(P_2O_5) released in the form of Dissolved Phosphorus (P_2O_4)			
Manure Source	**Tech Research Farm**	**Dairy Farm**	**Turkey Farm**	**Average of all Farms**
Liquid Dairy	0.64%	0.81%	0.95%	0.80%
Cowpie	0.62%	0.58%	0.33%	0.54%
Turkey	0.33%	1.06%	0.02%	0.47%

Since the manure was applied to the plots based on the phosphate concentration in the source manure, the phosphate amount applied to all of the plots was the same. Among the three farms, the liquid dairy released the highest percentage of the P_2O_4, nearly twice that of the turkey litter. Overall, less than 1% of the source phosphate was present in runoff in the form of P_2O_4 from the release plots. Because the treatments were applied to pasturelands, the grass may have assisted in retaining the nutrients and preventing their release. That is why a grass vegetative filter strip is often recommended to reduce nutrient loading from agricultural lands.

Sediment Bound Phosphorus Treatment Effects

After land application of the waste, the release plots were rained on within 12 to 24 hours. This time period would not be sufficient for the phosphate applied to the plots to become stabilized and fixed by soil particles. Sediment bound phosphorus (SBP) released from the plots is attached to either the organic matter applied to the plots, organic matter present on the plots from previous manure applications, or eroding soil particles with high background phosphorus concentrations. Because there were no statistical differences among the TSS concentrations released from the different treatments (Table 15), the differences among the treatments were due to the attachment of SBP to organic matter particles rather than to the soils.

The average SBP concentration released at all three sites was highest from the plots treated with cowpies (1.87 mg/L), followed by the liquid dairy (1.04 mg/L) and the turkey litter (0.66 mg/L) treatments. Statistical analyses of the SBP revealed the concentrations released from the plots treated with cowpies were significantly higher than those from both the turkey litter and control plots. The cowpie treatment released higher concentrations of SBP due to the nature of the manure. The raindrops from the rainfall simulator broke apart the cowpies, increasing concentrations of organic matter particles. The SBP can be attached to either soil or organic matter particles. Figure 22 presents the SBP concentrations released from the plots.

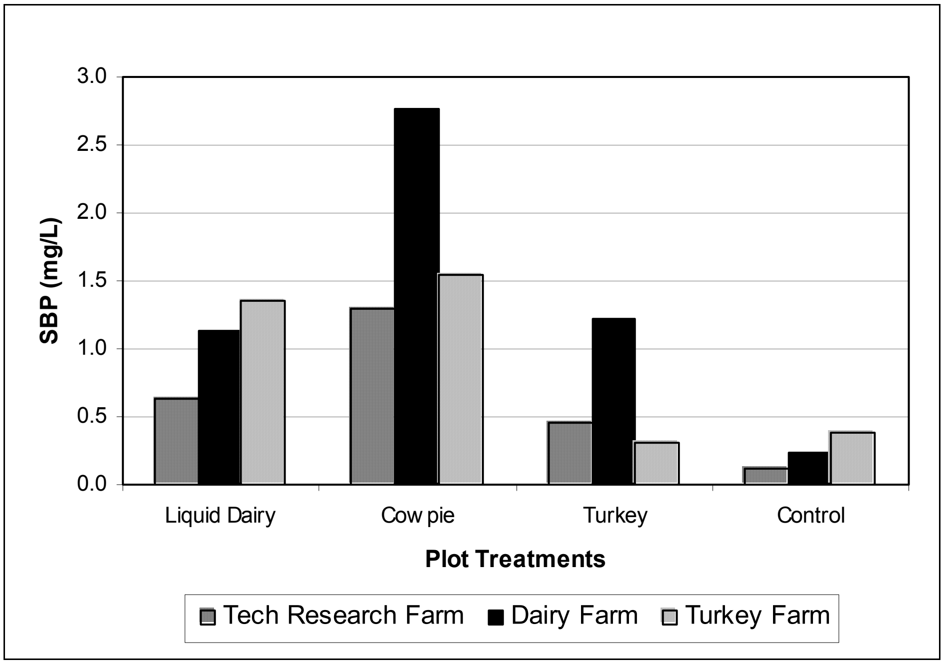

Figure 22. Sediment Bound Phosphorus concentrations released from the plots

Effects of Landuse History on SBP Concentrations

Because the rainfall event occurred within 24 hours of the manure application, the phosphate in the source manure did not have time to adhere to sediment particles or organic matter already on the plots from previous waste applications. The average released SBP concentrations account for 29% of the total phosphorus (TP) at the Tech Research Farm where the soils had medium to low residual soil phosphorus levels. At the Dairy Farm and the Turkey Farms, the average SBP concentrations for all sources account for 54% and 56% of the TP, respectively. The average SBP concentrations released from the plots at the Dairy Farm and Turkey Farm were 78% higher than the concentrations from the Tech Research Farm. This result indicates that organic matter remaining on the soil surface from previous manure applications or high background soil phosphorus concentrations increased the SBP concentration available to be transported in overland flow.

Total Phosphorus Treatment Effects

Figure 23 presents the TP concentrations released from the plots. The average total phosphorus (TP) concentration released at all three farms was highest from the plots treated with cowpies, followed by the liquid dairy and the turkey litter treatments. Statistical analysis of the TP data determined that there were no significant differences among the different plot treatments. The TP is a summation of both the DP and SBP. The TP concentrations from the liquid dairy and turkey litter treatments were 65% and 62%, respectively, DP. The TP concentrations released from the cowpie treatment was 60% SBP. When comparing the liquid dairy, cowpie, and turkey litter treatment effects, the liquid dairy and turkey litter treatments have the potential to contribute 25% and 20% higher concentrations of DP into runoff than cowpies. The liquid dairy treatment contributes a higher percentage of DP because it was applied to the pastureland in a liquid form. The phosphate applied in the liquid form may be more easily released in the form of DP, and the liquid dairy manure also would have less bioavailable phosphorus attached to organic matter particles. The turkey litter treatment had released a higher level of DP because the turkey litter source manure had a much higher DP concentration. Even though the turkey litter was applied at a lower application rate, the higher concentration in the source manure contributes to a higher percentage of DP released from the plots. The raindrops from the rainfall simulator broke apart the cowpies, increasing detachment of organic matter particles and its associated phosphate, thereby increasing SBP concentrations.

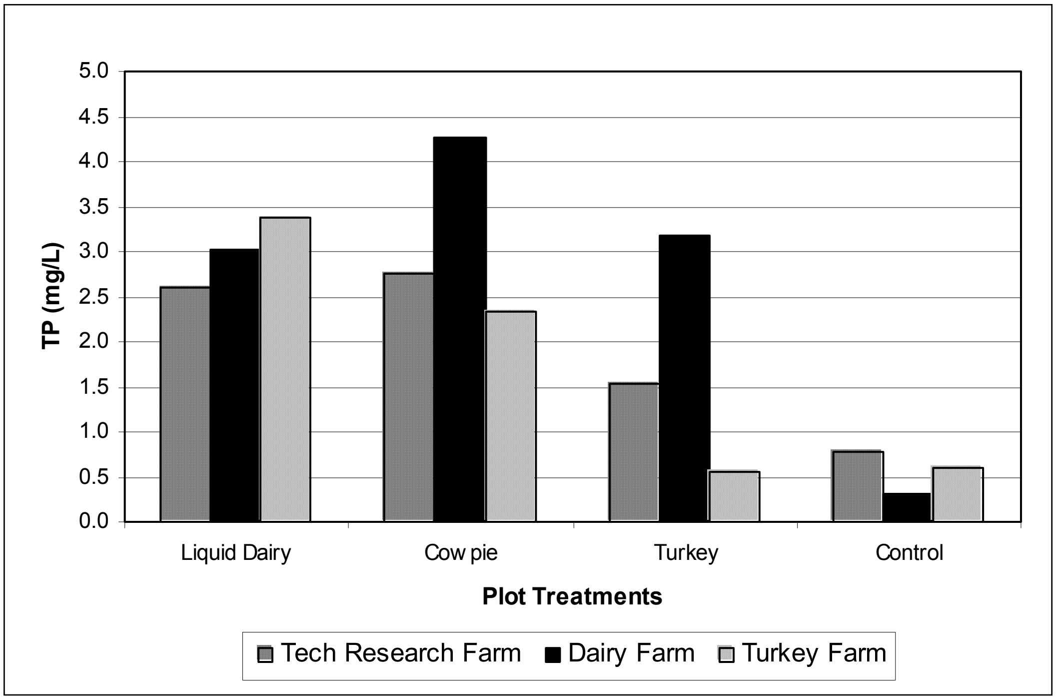

Figure 23. Total Phosphorus concentrations released from the plots

The concentrations of TP released from the plots represent the TP available to be transported by surface waters. The actual concentration of TP transported will be much lower than the concentrations released. All three treatments exhibit the potential to contribute phosphorus loading to waterbodies. The TP concentrations released from plots treated with liquid dairy manure had concentrations of 3.0 mg/L, plots treated with cowpies had concentrations of 3.12 mg/L, and plots treated with turkey litter had concentrations of 1.76 mg/L. Total phosphorus concentrations in lakes exhibiting eutrophic properties may be as low as 20 μg/L (Novotny and Olem, 1994). TP concentrations released from the release plots are from 88 to 156 times higher than TP concentrations in lakes exhibiting eutrophic properties. In a watershed with high agricultural activity, water quality can be quickly degraded through the land application of animal manure.

Nitrogen Concentrations

The released nitrogen concentrations are presented in Table 35. The nitrogen concentrations are presented for all three farms, and the values from all three farms are averaged to compare the effects of the different treatments. The average values for each of the individual farms were used to compare the landuse history to the released nutrient concentrations. The total nitrogen concentrations are the sum of TKN and nitrate concentrations.

Table 35. Concentrations of Ammonia, Nitrate, TKN, and Total Nitrogen released from the release plots

Treatment	Ammonia	Nitrate		TKN		Total N
	(mg/L)	(mg/L)	% TN	(mg/L)	% TN	(mg/L)
Tech Research Farm						
Liquid Dairy	2.76	1.75	15%	9.54	85%	11.28
Cowpie	1.23	0.09	1%	13.41	99%	13.50
Turkey	1.10	1.89	25%	5.74	75%	7.63
Control	0.65	2.75	44%	3.47	56%	6.22
Average	**1.44**	**1.62**	**21%**	**8.04**	**79%**	**9.66**
Dairy Farm						
Liquid Dairy	2.83	2.88	19%	12.62	81%	15.50
Cowpie	0.14	0.11	1%	14.31	99%	14.43
Turkey	1.86	2.00	19%	8.65	81%	10.65
Control	0.03	2.15	56%	1.71	44%	3.86
Average	**1.22**	**1.79**	**23%**	**9.32**	**77%**	**11.11**
Turkey Farm						
Liquid Dairy	3.93	3.05	22%	10.55	78%	13.60
Cowpie	0.61	0.86	8%	9.73	92%	10.59
Turkey	0.15	3.00	53%	2.71	47%	5.71
Control	0.11	2.98	67%	1.47	33%	4.45
Average	**1.20**	**2.47**	**38%**	**6.11**	**62%**	**8.59**
Average of all Farms						
Liquid Dairy	3.18	2.56	19%	10.90	81%	13.46
Cowpie	0.66	0.35	3%	12.49	97%	12.84
Turkey	1.04	2.30	29%	5.70	71%	7.99
Control	0.26	2.63	54%	2.22	46%	4.84
Average	**1.28**	**1.96**	**26%**	**7.83**	**74%**	**9.78**

The Virginia Department of Conservation and Recreation (VDCR, 1995) recommends that 45 to 67 kg/ha (40 to 60 lb/ac) of nitrogen be applied to pastures with orchardgrass, fescue, and clover. If organic (manure) nutrient sources are used, up to 135 kg/ha (120 lb/ac) nitrogen may be applied. The turkey litter treatment did not meet the recommended nitrogen application rates, but the liquid dairy manure and cowpie treatments both met or exceeded the recommended application rates. The total nitrogen applied to the turkey litter treated plots was 64 kg/ha, 146 kg/ha was applied to the liquid dairy treated plots, and 152 kg/ha was applied to the cowpie treated plots.

Nitrate Treatment Effects

Nitrate was applied to the plots treated with liquid dairy at an application rate 8.2 times higher than the plots treated with turkey litter and 2.7 times higher than the plots treated with cowpies (Table 31). The differences in nitrate application rates occurred because the manure was applied to the plots based on the phosphate concentration in the source manure.

The average nitrate released from all of the farms (Figure 24) was highest from the control plots (2.63 mg/L). The next highest concentrations were released by the liquid dairy (2.56 mg/L), turkey litter (2.30 mg/L), and then cowpie (0.35 mg/L) treatments. Statistical analyses of the nitrate concentrations in runoff from the release plots revealed that nitrate released by the cowpie treatment was significantly lower than all other treatments at the 0.05 level. The liquid dairy and turkey treatments have similar concentration in runoff indicating that the turkey litter may more easily release nitrate, or that background concentrations are contributing to the release of nitrate from the plots treated with turkey litter. Because the control plots have released nitrate concentrations similar to the concentrations released from the turkey litter and liquid dairy plots, background concentrations appear to be contributing, but these levels are low. Another contributing factor may be the nitrate present in the rainwater applied to the plots. The cowpie treatment covered much of the surface area, so that background concentrations in the soil may not have been released from the plots treated with cowpies.

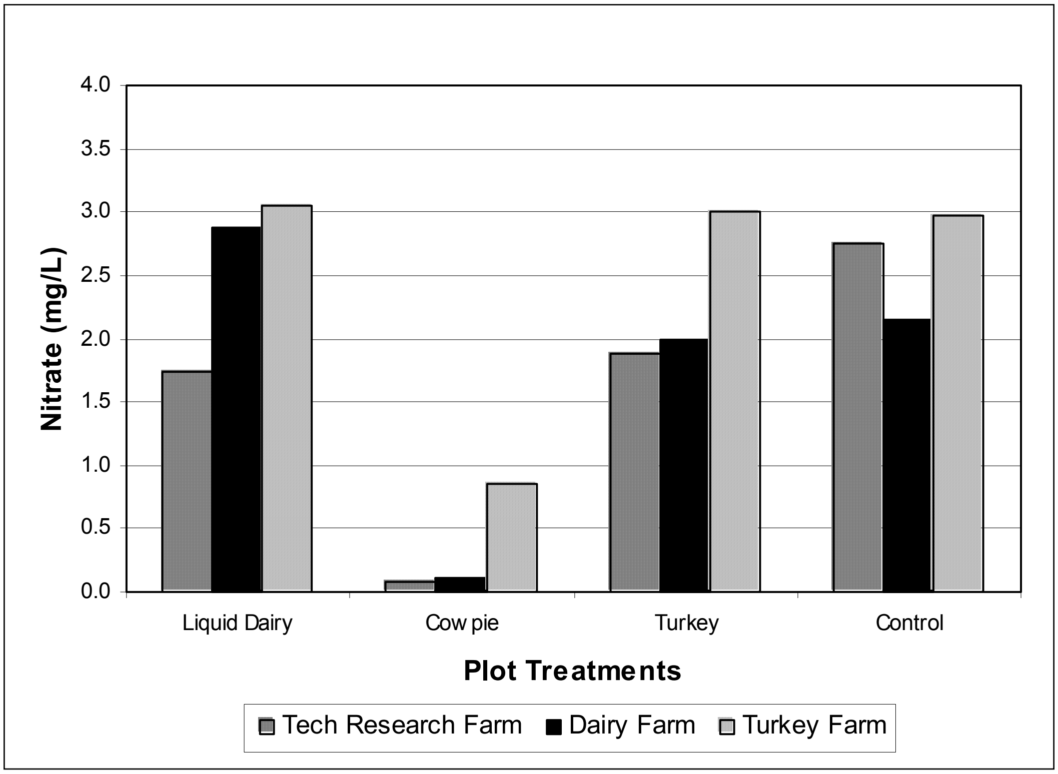

Figure 24. Nitrate concentrations released from the plots

Overall, the average nitrate concentrations accounted for 26% of the total nitrogen released from the plots. Among the three farms, a slightly higher percent of the total nitrogen was from nitrate at the Turkey Farm. There appear to be higher background nitrate concentrations at the Turkey Farm; possibly resulting from previous manure applications or the presence of cattle in the pastureland.

Because the control plots had higher nitrate concentrations in runoff than the treated plots, it appears that background concentrations in soils may affect release concentrations even more than the different manure treatments. The release of nitrate from pastureland applied with manure does not appear to be related to the different manure treatments. Instead, background soil conditions and the presence of cattle at the Dairy Farm and Turkey Farm contribute to higher concentrations than land application of animal manure.

The percent of nitrate released from the source manure is presented in Table 36. The manure was applied to the plots based on the phosphate concentration, so the nitrate application rates were variable. The nitrate loading from the release plots was calculated by multiplying the sample concentration by the total volume of runoff generated from each plot. The nitrate loading was then divided by the total amount of nitrate applied to the plots (Table 31) to determine the percent released from the plots.

Table 36. Percent of nitrate in source manure released from the plots

	Nitrate			
Manure Source	**Tech Research Farm**	**Dairy Farm**	**Turkey Farm**	**Average of all Farms**
Liquid Dairy	10.16%	17.94%	18.44%	15.51%
Cowpie	1.12%	0.60%	2.65%	1.82%
Turkey	54.78%	14.79%	23.43%	65.2%

Among the three farms, the nitrate in the turkey manure was most readily released from the plots with 64% of the nitrate in source manure present in the water collected from the release plots. This value may not be a true reflection of the nitrate release from the plots because the control plots had an overall average release concentration that was higher than the concentration of nitrate released from the plots treated with turkey litter. It is likely that background concentrations are contributing to the release of nitrate from the plots treated with turkey litter.

Ammonia Treatment Effects

The ammonia application rate to the plots treated with liquid dairy was two times higher than the plots treated with cowpies and turkey litter (Table 31). The ammonia concentrations released from the plots (Figure 25) were higher for the liquid dairy treatment (3.18 mg/L) than any of the other treatments. Statistical analyses performed on the ammonia concentrations determined that the liquid dairy treatment had significantly higher ammonia than any other treatments at the 0.05 level.

In freshwater, the chronic ammonia criteria require that the thirty day average not be exceeded more than once every three years. The criteria range from 2.5 mg/L to 3.2 mg/L for waters with a pH ranging from 6.5 to 7.5 (VDEQ, 2003). The range is lower for waters with a higher pH. The ammonia concentrations released from the liquid dairy plots exceeded this range, but since only a small percentage of the released ammonia could be transported to surface waters, ammonia from these manure treatments probably will not pose an environmental threat.

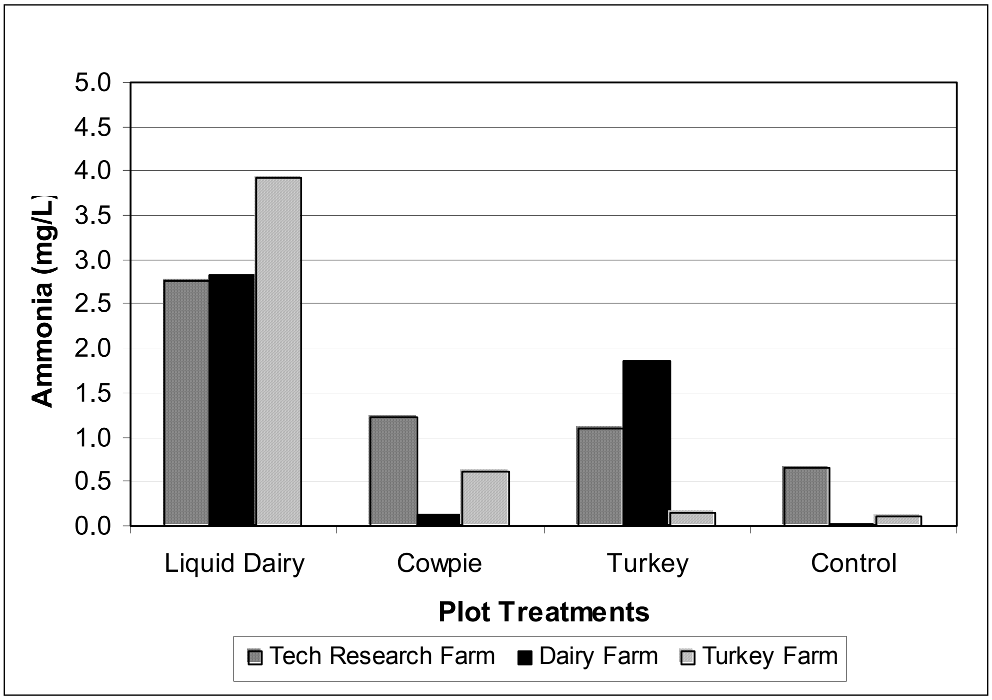

Figure 25. Ammonia concentrations released from the plots

The percent of ammonia released from the source manure is presented in Table 37. The manure was applied to the plots based on the phosphate concentration, so the ammonia application rates varied among the different treatments. The ammonia loading from the release plots was calculated by multiplying the sample concentration by the total volume of runoff generated from each plot. The ammonia loading was then divided by the total amount of ammonia applied to the plots (Table 31) to determine the percent released from the plots.

Table 37. Percent of ammonia in source manure released from the plots

	Ammonia			
Manure Source	Tech Research Farm	Dairy Farm	Turkey Farm	Average of all Farms
Liquid Dairy	0.64%	0.81%	0.95%	1.42%
Cowpie	0.62%	0.64%	0.37%	0.52%
Turkey	0.33%	1.08%	0.02%	1.15%

Among the three farms, the percent of the ammonia released from the source manure was highest for the liquid dairy manure, followed by the turkey litter and cowpie treatments. The liquid dairy was expected to have a slightly higher release rate because more ammonia was applied to the plots treated with liquid dairy manure than the plots treated with cowpies and turkey litter. Because the treatments were applied to pasturelands, the grass may have acted as buffer and retained the ammonia and prevented its release.

Total Kjeldahl Nitrogen Treatment Effects

The concentration of TKN released from the plots is presented in Figure 26. The TKN concentrations are higher than the ammonia concentrations because TKN consists of both the ammonia and organic nitrogen derived from animal or plant material. The cowpie treatment had the highest concentration in runoff (12.49 mg/L), followed by the liquid dairy (10.90 mg/L) and turkey litter treatments (5.70 mg/L). The TKN concentrations released from the plots treated with cowpies and liquid dairy manure were significantly higher than those from the control. The cowpie treatment TKN concentrations were also significantly higher than the turkey litter treatment at the 0.05 significance level.

There do not appear to be differences in the percent of TN contributed by TKN among the farms with different landuse histories. An average of 74% of the total nitrogen from the plots that received a manure application came from TKN (liquid dairy – 81%, cowpies – 97%, turkey litter – 71%), while a high percentage of the nitrogen from the control plots were due to nitrate (54%). This result is because the manure applied to the plots is composed of organic matter and therefore, much of the nitrogen applied to the plots is in the organic form.

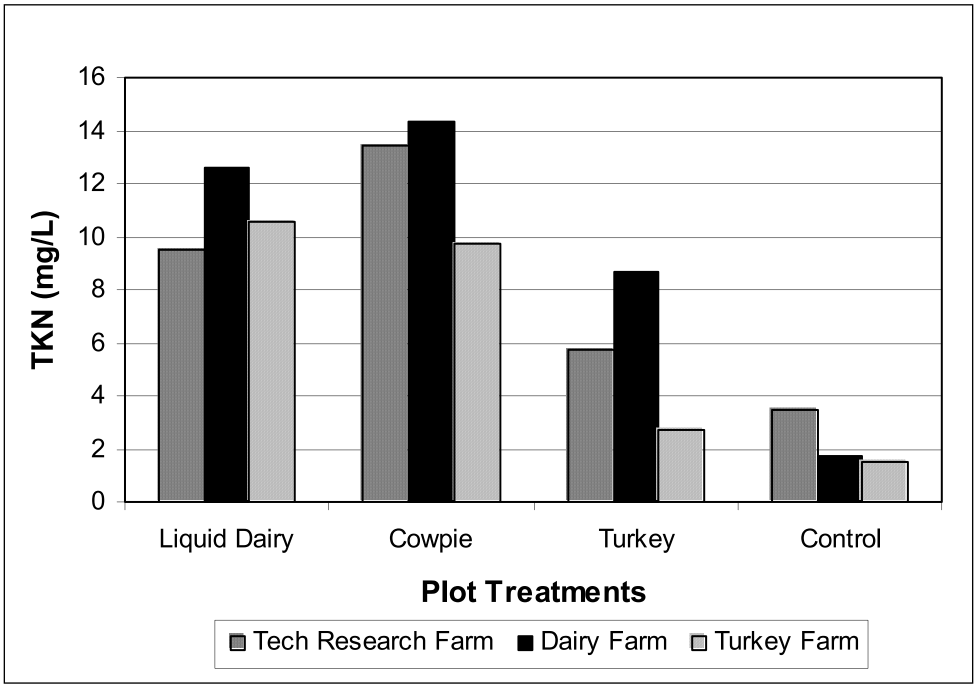

Figure 26. TKN concentrations released from the plots

Total Nitrogen Treatment Effects

Total nitrogen is the sum of TKN and nitrate. Total nitrogen concentrations were highest from plots treated with liquid dairy manure (13.46 mg/L), followed by cowpies (12.84 mg/L), turkey litter (7.99 mg/L), and the control (4.84 mg/L). Some of the TN released from the plots is due to the TN concentration present in the rainwater (Table 32).

The percent of TN released from the source manure is presented in Table 38. Among the three farms, the turkey litter released the highest percent of the source nitrogen with 1.91% released from the plots. Although the turkey litter was applied at a lower application rate, the concentration of nitrate and ammonia in the litter was higher, resulting in a higher percentage released from the plots as compared with the other treatments.

Table 38. Percent of total nitrogen in source manure released from the plots

	Total Nitrogen			
Manure Source	Tech Research Farm	Dairy Farm	Turkey Farm	Average of all Farms
Liquid Dairy	1.17%	2.12%	1.97%	1.75%
Cowpie	1.92%	2.01%	1.29%	1.74%
Turkey	1.46%	4.11%	0.16%	1.91%

Effects of Landuse History on Nitrogen Concentrations

Landuse history appears to have very little impact on the concentrations of nitrogen released from the plots. Nitrogen in the bio-available form from previous waste applications has most likely either been utilized by the plants, or leached through the soil profile.

4.3.3 Summary of Nutrient Release Study

The concentrations of nutrients released from the plots represent the nutrients available to be transported by surface waters. The actual concentrations of nutrients transported will be much lower than the concentrations released. All three manure treatments exhibited the potential to contribute phosphorus loading to waterbodies. The TP concentrations released from the cowpie and liquid dairy treatments were highest among the different treatments with 3.12 mg/L and 3.00 mg/L, respectively. Organic matter remaining on the soil surface from previous manure applications increased the SBP concentrations available to be transported to the edge of the field by 78%.

The TN concentrations in runoff from the transport plots were highest from the plots treated with cowpies. The impact of the rainfall drops on the cowpies broke them apart and caused high concentrations of organic nitrogen to be transported from the plots. The concentration of TN in runoff from the plots treated with cowpies was 9.8 mg/L, three times higher than the liquid dairy and turkey litter treatments.

4.3.4 Transport Plots

The transport plot field experiments were designed to measure nutrient concentrations transported to the edge of the field in runoff. The results were evaluated to determine if nutrient transport rates differ among different manure types applied to pastureland.

Source Manure Applied to Transport Plots

The mass of each nutrient applied to the transport plots is presented in Table 39. The nutrient mass applied to each plot was determined by multiplying the concentration of the nutrients in the source manure (Table 30) by the manure application rate. Since the manure was applied to the plots based on the phosphate application rate, the nitrate and ammonia application rates varied based on their concentrations in the manure. The nutrient concentrations in the rainwater applied to the plots is presented in Table 40.

Table 39. The total mass of the nutrients applied to the transport plots

Manure Source	Phosphate	Nitrate Nitrogen	Ammonia Nitrogen	Total Nitrogen
Liquid Dairy	306 g (56 kg/ha)	22.5 g (4.1 kg/ha)	288 g (52.5 kg/ha)	804 g (146 kg/ha)
Cowpie	275 g (50 kg/ha)	8.1 g (1.57 kg/ha)	145 g (26.5 kg/ha)	836 g (152 kg/ha)
Turkey	301 g (54.7 kg/ha)	2.9 g (0.52 kg/ha)	120 g (21.9 kg/ha)	354 g (64 kg/ha)

Table 40. Nutrient concentrations in rainwater

Rainwater	DP (mg/L)	SBP (mg/L)	Total P (mg/L)	Nitrate (mg/L)	Ammonia (mg/L)	TKN (mg/L)	TN (mg/L)
Simulation 1	0.00	0.07	0.07	0.479	0.194	3.00	3.19
Simulation 2	0.01	0.00	0.01	0.799	0.000	0.83	0.83

Phosphorus Concentrations

Runoff samples were collected every three to six minutes during the rainfall simulation. The Flow-weighted Concentrations (FWC) were calculated to determine the phosphorus concentrations in runoff from each of the transport plots. The dissolved phosphorus (DP), sediment bound phosphorus (SBP), and total phosphorus (TP) FWC are presented in Table 41. The FWC was calculated by multiplying the sample concentration by the volume of runoff that occurred during that time period. These values were then summed up and divided by the total volume of runoff from the plot to obtain the FWC.

Table 41. Flow-weighted concentrations of Dissolved Phosphorus, Sediment Bound Phosphorus, and Total Phosphorus in runoff from the transport plots

	DP[*]				SBP[†]				TP[‡]		
	S1[§] mg/L	S2[‖] mg/L	Avg[#] mg/L	% TP	S1 mg/L	S2 mg/L	Avg mg/L	% TP	S1 mg/L	S2 mg/L	Avg mg/L
Liquid Dairy	0.95	0.55	0.75ab *	85%	0.14	0.12	0.13a	15%	1.09	0.66	0.88a
Cowpie	0.90	0.78	0.84ab	53%	0.82	0.64	0.73a	47%	1.72	1.42	1.57a
Turkey	1.55	0.90	1.22a	87%	0.19	0.18	0.18a	13%	1.74	1.08	1.41a
Control	0.05	0.05	0.05b	36%	0.14	0.05	0.09a	64%	0.19	0.10	0.14a

Dissolved Phosphorus; [†] Sediment Bound Phosphorus; [‡] Total Phosphorus; [§] Simulation 1; [‖] Simulation 2; [#]Average of S1 and S2
*Values followed by the same letter do not differ at the 5% level of significance according to Tukey's pairwise comparison.

Phosphorus Yields

The phosphorus yield from the two replications was averaged to determine treatment effects on DP, SBP, and TP during the runoff events. The results are presented in Table 42. The load was calculated by multiplying the sample concentration by the volume of runoff that occurred during that time period. These values were then summed. The average load from the transport plots was calculated to determine which waste type has the greatest potential to contribute nutrient loading to waterways. Statistical differences were not found between the DP, SBP, or TP yields in runoff between the two rainfall events or among any of the different treatments.

Table 42. Average yield of Dissolved Phosphorus, Sediment Bound Phosphorus, and Total Phosphorus in runoff from transport plots

	DP*				SBP†				TP‡		
	S1§ (g/ha)	S2‖ (g/ha)	Total (g/ha)	% TP	S1 (g/ha)	S2 (g/ha)	Total (g/ha)	% TP	S1 (g/ha)	S2 (g/ha)	Total (g/ha)
Liquid Dairy	73	57	130	91%	6	8	14	9%	79	64	143
Cowpie	38	100	138	54%	35	82	117	46%	73	182	255
Turkey	58	77	135	87%	6	14	20	13%	64	91	155
Control	2	3	5	39%	3	5	8	61%	5	8	13

* Dissolved Phosphorus; † Sediment Bound Phosphorus; ‡ Total Phosphorus; § Simulation 1; ‖ Simulation 2

Dissolved Phosphorus Treatment Effects

The concentration of DP present in runoff from the different plots and simulations is presented in Figure 27. During the first simulation (S1), the concentrations of DP were highest in runoff collected from the plots treated with turkey litter (1.55 mg/L). The DP concentrations in runoff from the turkey litter treatment were 63% higher than the concentrations from the liquid dairy treatment and 72% higher than the concentrations from the cowpie treatment. During the second simulation (S2), the plots treated with turkey litter continued to have the highest DP concentrations in runoff (15% higher than the concentrations from the plots treated with cowpies and 64% higher than the concentrations from the plots treated with liquid dairy manure). Statistical analyses were performed on the FWC of DP in runoff using the repeated measure method and Tukey's pairwise comparison (Ott and Longnecker, 2001). There were significant differences between the FWC concentrations of DP during simulations S1 and S2 at the 0.05 error level. The DP concentrations in turkey litter treatment were found to be significantly higher than those from the control treatment.

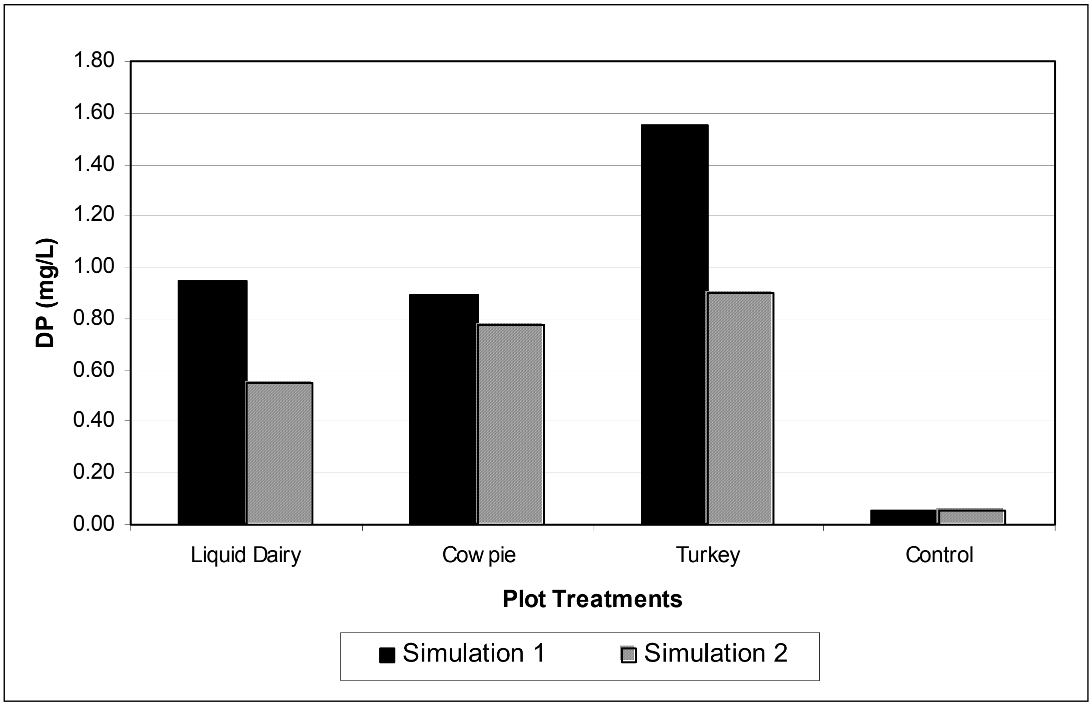

Figure 27. Flow-weighted Dissolved Phosphorus concentrations in runoff from transport plots

Among the different treatments investigated, the turkey litter had the highest concentration of DP in runoff from pasturelands treated with land applied waste during both S1 and S2. This result may have occurred because the turkey litter is light and more easily transported off the plots with runoff. Another factor contributing to the higher DP concentrations in runoff from the turkey litter treatment could be the higher phosphate concentration in the source manure (Table 30). Even thought the turkey litter was applied at a lower application rate than the other manure treatments, the higher concentration of phosphate in the source manure could increase the concentrations of DP in runoff. During heavy rainfall events, the turkey litter treatment was most likely to contribute water soluble phosphorus to downstream water bodies.

The DP concentrations in runoff from the turkey litter and liquid dairy treatments account for 87% and 85%, respectively, of the TP in runoff from the plots. To control phosphorus from pastureland treated with liquid dairy manure or turkey litter, the selected management practice should focus on reducing the DP. The DP concentration in runoff from the liquid dairy treatment may be higher because the waste is applied to the plots in a liquid form and there is less phosphate attached to organic matter, resulting in higher release rates.

The treatment yielding the greatest DP loss was the cowpie treatment, where 138 g/ha was lost during the two rainfall simulations. This loss represents 54% of the total phosphorus lost from the plots treated with cowpies. Although there were significant differences among the concentrations, the total DP yield is very similar for all three treatments. The higher yields on the liquid dairy and cowpie treated plots were the result of higher runoff volumes (Figure 11). The liquid dairy treatment increased the soil moisture content prior to the simulation, possibly accounting for the higher runoff volume during S1.

Source Phosphate in Runoff

The manure was applied to the plots based on the phosphate (P_2O_5) concentration in the source manure. The runoff from the plots was tested for DP (P_2O_4). The percent of the phosphate in runoff was calculated by dividing the mass of DP in runoff by the mass of phosphate applied to the plots. The P_2O_5 application rate to all of the plots was the same. The percent of the source manure present in runoff from different treatments were also similar (Table 43). Even though the loads differ between the two rainfall events, when the results for the two simulations were combined, the DP present in runoff were similar between the three treatments. Overall, less than 0.3% of the source phosphate was present in the form of P_2O_4 in runoff from the transport plots.

Table 43. Percent of source manure phosphate in runoff

Treatment	% of Phosphate(P_2O_5) released in the form of Dissolved Phosphorus (P_2O_4)		
	S1[*]	S2[†]	Total
Liquid Dairy	0.14%	0.11%	0.25%
Cowpie	0.08%	0.22%	0.30%
Turkey	0.11%	0.15%	0.27%

[*] Simulation 1; [†] Simulation 2

Sediment Bound Phosphorus Treatment Effects

As stated before, after land application of the waste, the transport plots were rained on within 12 to 24 hours. This time period would not be sufficient for the phosphate applied to the plots to become stabilized and fixed by soil particles because in the short term, much of the phosphorus added to the soils remains plant available (Hansen et al., 2002). SBP present in runoff is attached to the organic matter applied to the plots, organic matter present on the plots from previous manure applications, or eroding soil particles with high background phosphorus concentrations. Because the Tech Research Farm had medium to low background soil phosphorus concentrations, and no history of previous manure applications, it is most likely that the SBP in runoff was due to phosphorus attached to organic matter in the applied waste. No relationship was found between the TSS (Table 16) and SBP concentrations in runoff.

The concentrations of SBP in runoff from the transport plots are presented in Figure 28. The cowpie treatment had the highest concentrations of SBP in runoff during both simulations (0.73 mg/L), followed by the turkey litter (0.18 mg/L) and liquid dairy treatments (0.13 mg/L). There were no statistical differences between the FWC concentrations of SBP during S1 and S2 at the 0.05 error level or among any of the different treatments. The SBP concentrations in runoff from the cowpie treatment accounted for 47% of the TP in runoff from the cowpie treated plots. Although less than half of the phosphorus concentration in runoff from the cowpie plots was due to SBP, among the three treatments, the cowpies contributed the highest concentrations of SBP to runoff. The impact of the raindrops from the rainfall simulator broke apart the cowpies, increasing concentrations of organic matter particles.

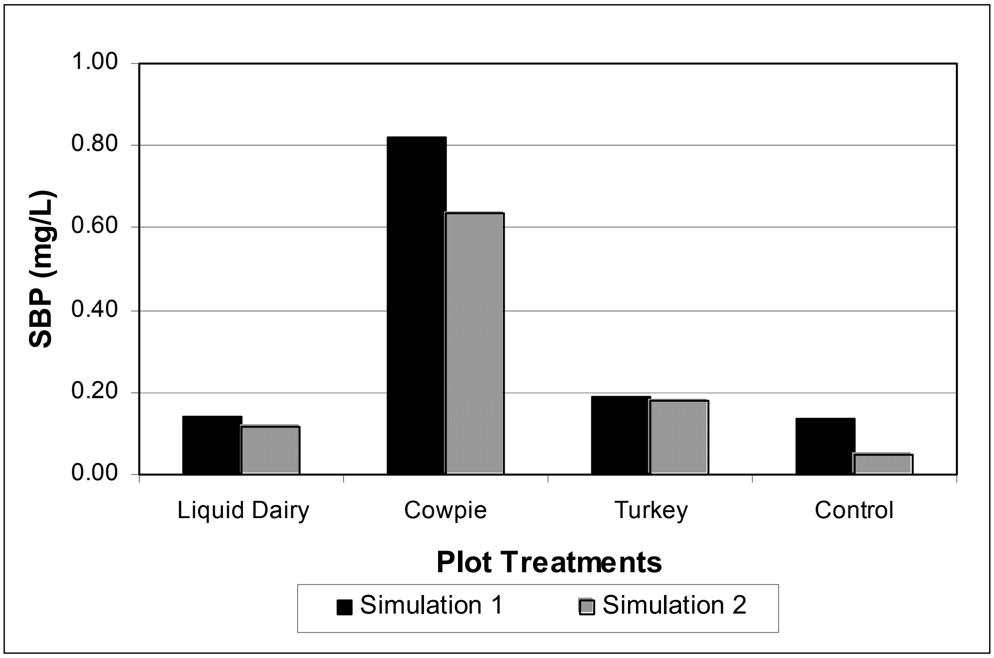

Figure 28. Flow-weighted Sediment Bound Phosphorus concentrations in runoff from the transport plots

The cowpie treatment also yielded the greatest SBP losses with a total of 117 g/ha lost during the two simulations. SBP concentrations from the different treatments decreased between the two simulations, but the yield losses increased. This result can be attributed to the higher runoff volume occurring during S2. The higher runoff volume during S2 transported more organic matter particles off the plots, resulting in the higher SBP yields.

Total Phosphorus Treatment Effects

Figure 29 presents the TP concentrations in runoff. The average concentration of TP in runoff from the two rainfall simulations was highest from plots treated with cowpies (1.57 mg/L). The concentration in runoff from the cowpie treatment was 11% higher than the concentrations in runoff from the turkey litter treatment (1.41 mg/L) and 78%

higher than the concentrations in runoff from the liquid dairy manure (0.88 mg/L). Statistical analyses revealed that the TP concentrations in runoff from S2 were significantly lower than the TP concentrations during S1. There were no statistical differences in the TP concentrations in runoff among any of the different treatments.

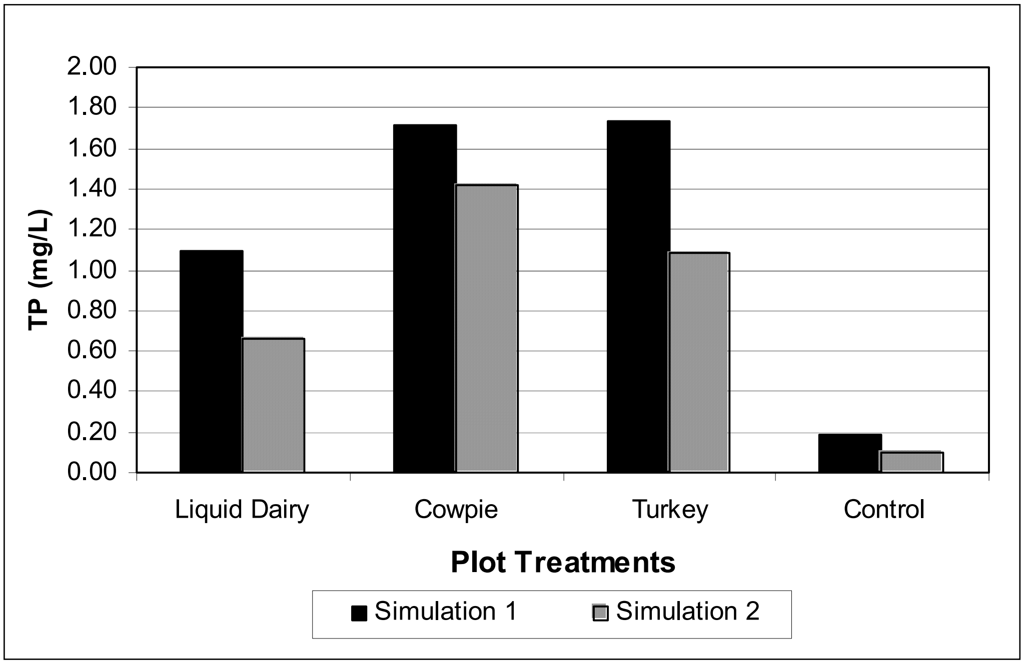

Figure 29. Flow-weighted Total Phosphorus concentrations in runoff from transport plots

The cowpie treatment contributed equally high concentrations of SBP (47% of TP) and DP (53% of TP) in runoff. The raindrops from the rainfall simulator broke apart the cowpies, increasing concentrations of organic matter particles, and therefore increasing SBP concentrations, while also releasing an equal concentration of DP into runoff. The DP concentrations in runoff from the turkey litter and liquid dairy treatments accounted for 87% and 85%, respectively, of the TP in runoff from the plots, so only a small percentage of the TP from these two treatments is in the form of SBP. As previously stated, this result is due to the characteristics of the waste applied to the plots. The liquid dairy treatment contributed a higher percentage of DP because it was applied to the pastureland in a liquid form. The phosphate applied in the liquid form may be more easily transported in the form of DP, and the liquid dairy manure also would have less bioavailable phosphorus attached to organic matter particles. The turkey litter treatment has a higher transport of DP because the phosphate is present in the turkey litter source manure at a much higher concentration and the litter is light and more easily transported with runoff. Even though the turkey litter was applied at a lower application rate, the higher concentration in the source manure contributes to a higher percentage of DP transported to the edge of the field.

The cowpies treatment also yielded the greatest TP loss with 255 g/ha lost in runoff during the two rainfall simulations. The cowpie treatment resulted in a 78% higher TP yield than the liquid dairy treatment and a 65% higher TP yield than the turkey litter treatment. The amount of phosphorus lost from a field that can contribute to increased eutrophication of downstream water bodies can be less than 2 kg $ha^{-1}yr^{-1}$ (Rehm et al., 1996). While the yield from these plots is much less than 2 kg/ha, this study only evaluated the runoff from two rainfall events and does not account for the loading over an entire year. In addition, manure is typically applied to the land twice per year.

The cowpie treatment has both the highest average TP concentration and the highest TP yield for both rainfall simulations. Total phosphorus concentrations in lakes exhibiting eutrophic properties may be as low as 20 μg/L (Novotny and Olem, 1994), which is much lower than the edge-of-field concentrations from plots treated with cowpies. In a watershed with high agricultural activity, water quality can be degraded through the land application of animal manure. In this study, all three treatments investigated contributed to phosphorus loading in surface waters and increased the risk for eutrophication.

Nitrogen Concentrations

The Flow-weighted Concentrations (FWC) of nitrogen in samples collected in runoff from the transport plots are presented in Table 44. Nitrate, ammonia, total kjeldahl nitrogen (TKN), and total nitrogen (TN) were the nitrogen components evaluated. The FWC was calculated by multiplying the sample concentration by the volume of runoff that occurred during that time period. These values were then summed up and divided by the total volume of runoff from the plot to obtain the FWC.

The manure applications were not applied based on nitrogen content, and therefore the results are affected by the waste application rate. The application rates of the nitrate, ammonia, and total nitrogen were presented in Table 39. The liquid dairy treatment had higher concentrations of both nitrate and ammonia in the source manure than the other treatments. These higher concentrations indicate that there was more potential for nitrate and ammonia to be present in higher concentrations in runoff from the plots treated with liquid dairy compared with the other manure treatments.

Table 44. Flow-weighted concentrations of Nitrate, Ammonia, and TKN in runoff from transport plots

	Ammonia			**Nitrate**				**TKN**				**TN**		
	S1[*] mg/L	**S2[†] mg/L**	**Avg[‡] mg/L**	**S1 mg/L**	**S2 mg/L**	**Avg mg/L**	**% TN**	**S1 mg/L**	**S2 mg/L**	**Avg mg/L**	**% TN**	**S1 mg/L**	**S2 mg/L**	**Avg mg/L**
LD[§]	0.97	0.74	0.9a*	0.65	1.02	0.8b	27%	2.60	1.82	2.2a	73%	3.25	2.85	3.0
CP[‖]	1.65	2.02	1.8a	0.21	0.03	0.1a	1%	10.36	9.06	9.7a	99%	10.57	9.09	9.8
TKY[#]	1.44	1.08	1.3a	0.73	1.02	0.9b	28%	2.50	1.98	2.2a	72%	3.23	3.00	3.1
CT[**]	0.18	0.18	0.2a	0.57	0.81	0.7b	30%	1.63	1.59	1.6a	70%	2.20	2.40	2.3

Simulation 1; [†] Simulation 2; [‡] Average of Simulation 1 and Simulation 2; [§] Liquid Dairy Treatment; [‖] Cowpie Treatment; [#]Turkey litter Treatment; [**]Control

*Values followed by the same letter do not differ at the 5% level of significance according to Tukey's pairwise comparison.

Nitrogen Yields

The nitrogen yield was averaged from the two replications to determine treatment effects on nitrate, ammonia, TKN, and TN during the runoff events. The results are presented in Table 45. Load was calculated by multiplying sample concentration by volume of runoff that occurred during that time period. These values were then summed. The average load from the transport plots was calculated to evaluate which waste has the greatest potential to contribute nitrogen loading to waterways.

There were significant differences between the load of nitrate during S1 and S2 simulations at the 0.05 error level, but there were no significant differences among any of the different treatments. There were no significant differences between the load of ammonia and TKN during S1 and S2 simulations or among any of the different treatments.

Table 45. Nitrate, Ammonia, TKN, and TN yield in runoff from transport plots

	Ammonia			Nitrate				TKN				TN		
	S1[*] g/ha	S2[†] g/ha	Total g/ha	S1 g/ha	S2 g/ha	Total g/ha	% TN	S1 g/ha	S2 g/ha	Total g/ha	% TN	S1 g/ha	S2 g/ha	Total g/ha
LD[‡]	73	69	142	41	93	134	29%	171	162	333	71%	212	255	467
CP[§]	71	257	327	8	2	11	1%	442	1148	1590	99%	450	1150	1601
TKY[‖]	54	91	145	24	83	107	29%	90	167	257	71%	114	249	364
CT[#]	5	19	24	15	69	83	29%	40	163	204	71%	55	232	287

Simulation 1; [†] Simulation 2; [‡] Liquid Dairy Treatment; [§] Cowpie Treatment; [‖] Turkey litter Treatment; [#]Control

Nitrate Treatment Effects

The nitrate concentrations in runoff from the transport plots are presented in Figure 30. Nitrate was applied to the plots treated with liquid dairy at an application rate 8.2 times higher than the plots treated with turkey litter and 2.7 times higher than the plots treated with cowpies (Table 39). The differences in nitrate application rates occurred because the manure was applied to the plots based on the phosphate concentration in the source manure.

The nitrate concentration was highest from plots treated with turkey litter (0.73 mg/L) during the first simulation, followed by the liquid dairy treatment (0.65 mg/L) and control treatment (0.57 mg/L). During S2, the nitrate concentration in runoff was equally high in the liquid dairy and turkey litter treatments (1.02 mg/L). Even though a much smaller amount of nitrate was applied to the turkey litter plots; the nitrate is much more concentrated in the turkey litter (Table 30), resulting in higher concentrations in runoff. Statistical analysis was performed using the repeated measure method and Tukey's pairwise comparison. There were significant differences between the FWC concentrations of nitrate during S1 and S2 simulations at the 0.05 error level. The concentration of nitrate in the runoff from the cowpie treatment was significantly lower than all other treatments.

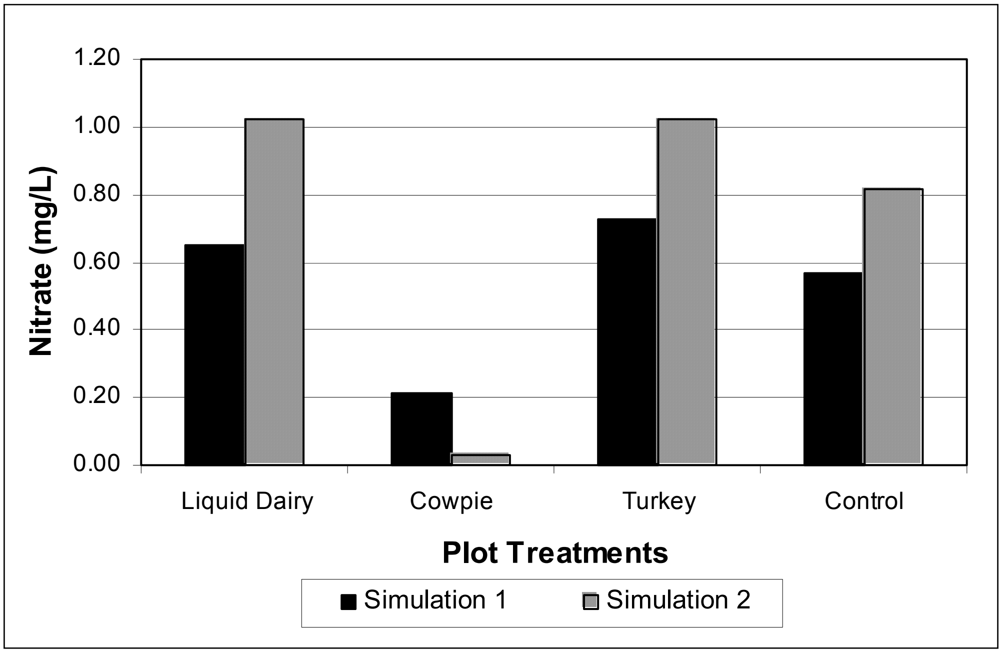

Figure 30. Flow-weighted nitrate concentrations in runoff from transport plots

The nitrate yield from the transport plots was highest from the plots treated with liquid dairy manure (134 kg/ha) because of the greater runoff volumes from those plots. Overall, the average nitrate concentrations accounted for 29% of the total nitrogen released from the turkey litter, liquid dairy manure, and control plots.

Nitrate present in the rainwater may have contributed to higher nitrate concentrations in the runoff from the plots (Table 40). However, the rainwater was applied equally to all plots, so it does not describe the variation among the different treatments. Background nitrate concentrations in soils may affect concentrations in runoff more than different manure treatments. Even though additional nitrate was applied to the cowpie plots, the control plots had seven times higher nitrate concentration in runoff than the cowpie plots. There was also very little difference between the average concentrations of nitrate in runoff from the plots treated with turkey litter and liquid dairy manure, even though the nitrate was applied to the plots treated with liquid dairy manure at a rate 8.2 times more than the plots treated with turkey litter.

The percent nitrate from the source manure present in runoff is presented in Table 46. The percent of the nitrate in runoff is calculated by dividing the mass of nitrate present in runoff by the mass of nitrate applied to the plots.

Similar to observations from the release plots, the nitrate in the turkey source manure is the most readily present in runoff with 22% of the source manure present in the runoff from the transport plots. Turkey litter more easily releases nitrate into runoff than the other treatments because nitrate is present in a higher concentration in the turkey litter. This value may not be a true reflection of the release from the plots because the control plots also had similar concentration of nitrate nitrogen in runoff.

Table 46. Percent of source manure nitrate measured in runoff

	Nitrate		
Treatment	**S1**[*]	**S2**[†]	**Total**
Liquid Dairy	1.08%	2.43%	3.52%
Cowpie	0.58%	0.17%	0.75%
Turkey	5.03%	17.02%	22.05%

[*] Simulation 1; [†] Simulation 2

Ammonia Treatment Effects

The ammonia concentrations in runoff from the transport plots are presented in graphical form in Figure 31. The average ammonia concentration in runoff was highest from the plots treated with cowpies (1.8 mg/L), followed by the turkey litter (1.3 mg/L) and the liquid dairy treatments (0.9 mg/L). The ammonia was applied to the plots treated with liquid dairy manure at twice the application rate of the other treatments (Table 7). The total ammonia yield was highest from plots treated with cowpies (with 327 g/ha), but much lower from the plots treated with turkey litter and liquid dairy manure (145 g/ha and 142 g/ha, respectively). These results indicate that ammonia is more transportable from cowpies than the other waste types, but there were no significant differences between the FWC concentrations of ammonia during S1 and S2 simulations or among any of the different treatments investigated.

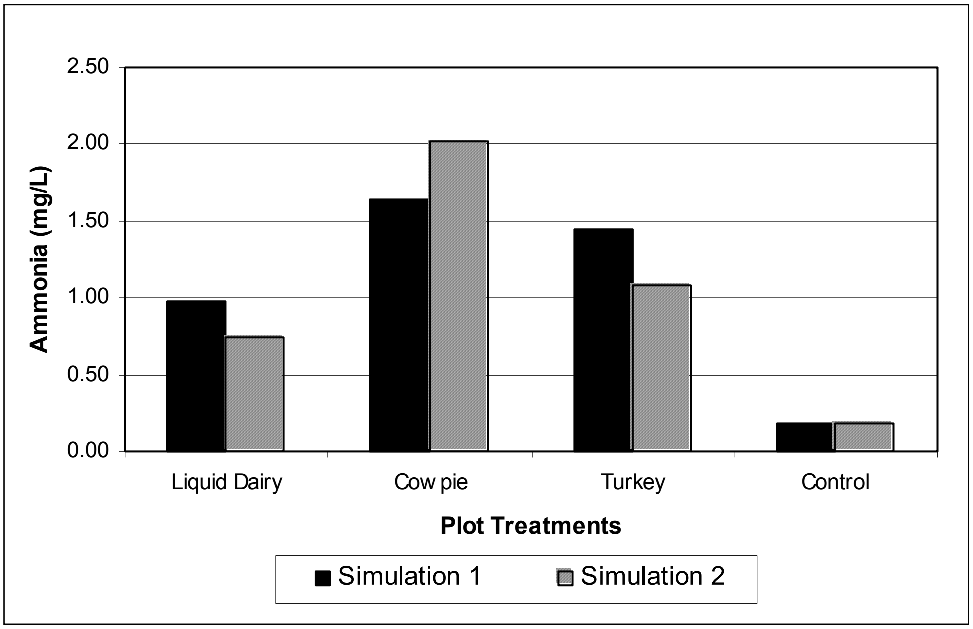

Figure 31. Flow-weighted ammonia concentrations in runoff from transport plots

In freshwater, the chronic ammonia criteria require that the thirty-day average not be exceeded more than once every three years. The standard ranges from 2.5 mg/L to 3.2 mg/L for waters with a pH ranging from 6.5 to 7.5 (VDEQ, 2003). In this study, the ammonia concentrations in runoff from the different treatments did not exceed this range. Because of the low concentrations in runoff, the ammonia from these manure treatments did not pose an environmental threat.

Source Ammonia in Runoff

The percent of ammonia from the source manure present in runoff is presented in Table 47. The percent of the ammonia in runoff was calculated by dividing the mass of nitrate present in runoff by the mass of nitrate applied to the plots.

The percent of the ammonia from the source manure present in runoff was highest from the plots treated with cowpies (0.46%), but was low for all treatments. The grass may have assisted in retaining the ammonia and preventing transport in runoff. Another factor may have been volatilization of the ammonia. Because 12 to 24 hours passed between the surface application of the waste and the rainfall simulation, sufficient time passed for some volatilization to occur.

Table 47. Percent of source manure ammonia in runoff

Treatment	Ammonia Nitrogen		
	S1[*]	S2[†]	Total
Liquid Dairy	0.15%	0.05%	0.20%
Cowpie	0.29%	0.17%	0.46%
Turkey	0.27%	0.08%	0.35%

[*] Simulation 1; [†] Simulation 2

TKN Treatment Effects

The TKN concentrations in runoff from the transport plots are presented in Figure 32. The TKN concentrations are higher since TKN included both the ammonia and organic nitrogen derived from animal or plant material. The runoff from the cowpie treatment had TKN concentrations (Figure 32) and yields (Table 45) of approximately five times higher than the liquid dairy and turkey litter treatments. The impact of the raindrops from the rainfall simulator broke apart the cowpies, increasing concentrations of organic matter particles. There were no significant differences between the FWC concentrations of TKN during S1 and S2 simulations or among any of the different treatments.

The majority of the total nitrogen from the plots were due to TKN (liquid dairy – 73%, cowpies – 99%, turkey litter – 72%, control – 70%). These percentages are high because the manure applied to the plots is composed of organic matter and, much of the nitrogen applied to the plots is in the organic form (Table 39). Some TKN present in runoff may have been from the rainwater applied to the plots.

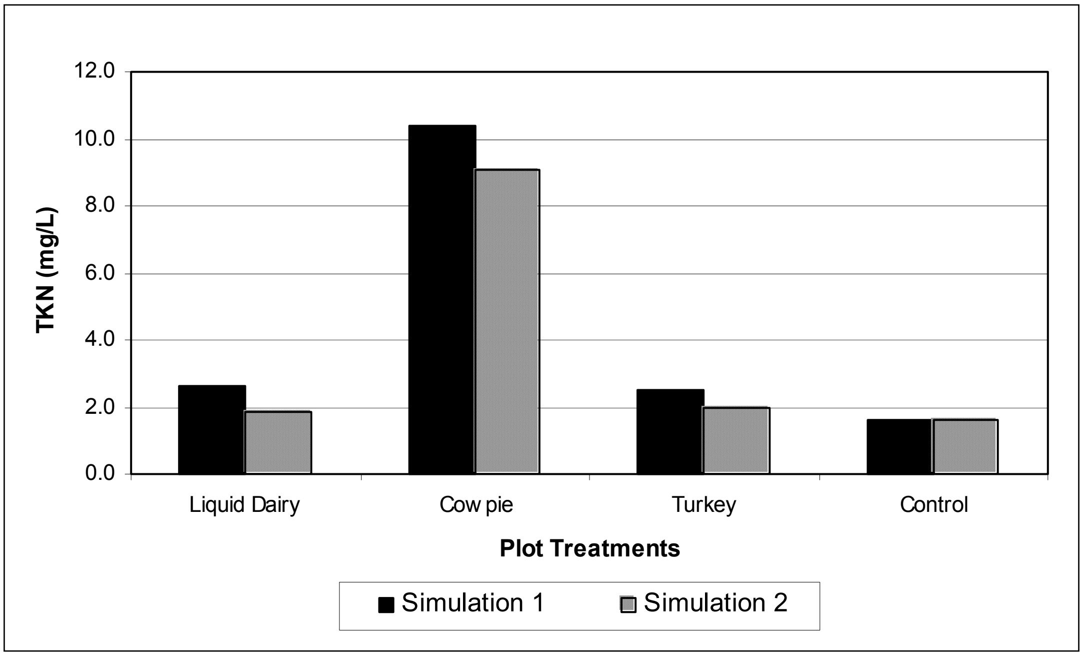

Figure 32. Flow-weighted TKN concentrations in runoff from transport plots.

Total Nitrogen Treatment Effects

The TN concentrations in runoff from the transport plots were highest from the plots treated with cowpies. The concentration of TN in runoff from the plots treated with cowpies was 9.8 mg/L; three times higher than the liquid dairy and turkey litter treatments. The majority of the TN from the cowpie treatments was due to high concentrations of organic nitrogen (TKN). The impact of the raindrops from the rainfall simulator broke apart the cowpies, which were transported by runoff and increased concentrations of organic matter particles. Of the three treatments, cowpies were most likely to contribute TN loading into surface waters.

Source Total Nitrogen in Runoff

The percent TN from the source manure present in runoff is presented in Table 48. Overall, the cowpie treatment had the highest percent of the source total nitrogen with 0.42% present in runoff from the plots.

Table 48. Percent of source manure TN in runoff

Treatment	Total Nitrogen		
	S1[*]	S2[†]	Total
Liquid Dairy	0.16%	0.10%	0.26%
Cowpie	0.32%	0.10%	0.42%
Turkey	0.19%	0.17%	0.36%

[*] Simulation 1; [†] Simulation 2

4.3.5 Summary of Nutrient Transport Study

Among the different treatments investigated, the turkey litter treatment resulted in the highest concentrations of DP in runoff during both S1 (1.55 mg/L) and S2 (0.90 mg/L). This may have occurred because the turkey litter had a higher concentration of phosphate in the source manure, and is lighter and more easily transportable by runoff. Among the three treatments, less than 0.3% of the source phosphate was present in runoff from the transport plots during both simulations combined in the form of P_2O_4.

The cowpie treatment had the highest concentrations of SBP in runoff during both rainfall events (0.73 mg/L), followed by the turkey litter (0.18 mg/L) and liquid dairy treatments (0.13 mg/L). The cowpies treatment yielded the greatest TP loss with 255 g/ha lost in runoff during the two rainfall simulations. The cowpie treatment resulted 78% higher TP yield than the liquid dairy treatment and 65% higher TP yield than the turkey litter treatment. Table 49 summarizes the nutrient yields applied, released and transported from the plots.

The phosphorus lost from a field that can contribute to increased eutrophication of downstream water bodies is reported to be less than 2 kg ha^{-1} yr^{-1} (Rehm et al., 1996). While the yield from these plots is much less than 2 kg/ha, this study only evaluates the runoff from two rainfall events and does not account for the loading over an entire year. In addition, manure is typically applied to the land twice per year. Different management practices should be implemented to reduce the phosphorus loading from pasturelands receiving different types of manure. Management practices to reduce sediment loading will most effectively reduce the SBP present in runoff from areas with high concentration of grazing cattle. When turkey litter is applied to pasturelands, the soluble form of phosphorus is more of a concern. Management practices that reduce runoff will be most effective in reducing soluble phosphorus loading.

Table 49. Summary of nutrients applied to the plots and yields released and transported off of the plots

	Phosphate (g/ha)	**Dissolved Phosphorus (g/ha)**	
Treatment	**Source Manure Applied**	**Yield[*] Released**	**Yield[†] Transported**
Liquid Dairy	55,800	447 (0.80%)	65 (0.12%)
Cowpie	50,000	272 (0.54%)	69 (0.14%)
Turkey	54,700	261 (0.47%)	67 (0.12%)
	Nitrate (g/ha)		
Treatment	**Source Manure Applied**	**Yield Released**	**Yield Transported**
Liquid Dairy	4,100	636 (15.5%)	67 (1.63%)
Cowpie	1,500	27 (1.8%)	5 (0.33%)
Turkey	520	339 (65.2%)	54 (10.4%)
	Ammonia (g/ha)		
Treatment	**Source Manure Applied**	**Yield Released**	**Yield Transported**
Liquid Dairy	52,500	747 (1.42%)	71 (0.14%)
Cowpie	26,500	137 (0.52%)	164 (0.62%)
Turkey	21,900	253 (1.15%)	72 (0.33%)
	Total Nitrogen (g/ha)		
Treatment	**Source Manure Applied**	**Yield Released**	**Yield Transported**
Liquid Dairy	146,403	2563 (1.75%)	234 (0.16%)
Cowpie	152,145	2649 (1.74%)	800 (0.53%)
Turkey	64,460	1230 (1.91%)	182 (0.28%)

[*] Yield released is an average of results from all three landuse histories. Values for each individual farm are presented in Appendix E. [†] Yield transported is an average of S1 and S2.

The eutrophication problem threshold for nitrogen concentrations in water bodies begins as low as 0.092 mg/L. Problems are likely to exist when nitrogen concentrations reach 0.92 mg/L, and severe problems are possible when the nitrogen concentrations reach 9.2 mg/L (USEPA, 1982). Total nitrogen concentrations from the transport plots exceed the threshold for likely eutrophication problems for all treatments and the total nitrogen concentrations from plots treated with cowpies exceed the threshold for severe eutrophication problems.

CHAPTER 5 SUMMARY AND CONCLUSIONS

5.1 Summary

A field study was conducted to evaluate the release and transport of bacteria and nutrients from livestock manure applied to pasturelands. Rainfall was simulated and runoff samples were collected to determine concentrations of *E. coli*, fecal coliform, *Enterococcus*, phosphorus and nitrogen present in runoff. The commercial Biolog System was used to evaluate the species of *Enterococcus* present in the source manure and in runoff from plots treated with cowpies. The goal of this study was to quantify in-field bacteria and nutrient release and transport from livestock manure during rainfall/runoff events.

5.1.1 Release Plots

The objective of the release plots (1m by 1 m) was to determine bacteria and nutrient release rates for surface applied manures, and to determine if the release rates differ between pasturelands that have and have not previously been receiving waste applications. The bacteria and nutrients released are assumed to be potentially available to be transported to the edge of the field in runoff. Four manure treatments (turkey litter, liquid dairy manure, cowpies, and none or control) and three land type treatments: pasture with a history of poultry litter application (Turkey Farm), pasture with a history of liquid dairy manure application (Dairy Farm), and pasture with no prior manure application (Tech Research Farm) were studied. Animal waste was applied to the plots based on the average phosphorus content in the waste at a rate of 56 kg/ha. A Tlaloc 3000 portable rainfall simulator, based on the design of Miller (1987), with a ½ 50WSQ Tee Jet nozzle was used to apply rain to the release plots. Rain was applied to the plots at approximately 5.08 cm/h until runoff occurred for 30 minutes. The runoff was collected down slope in a bucket and a composite sample was taken.

5.1.2 Transport Plots

The objective of the transport plots (18.3 m long by 3 m wide) was to identify differences in bacteria and nutrient transport potential of various livestock manures by comparing the concentrations of fecal bacteria and nutrients present in overland flow at the edge of the field. The transport plots were only constructed at the Virginia Tech Research Farm. The transport of bacteria from plots applied with liquid dairy, dried poultry litter, and cowpies were compared to control plots on which no animal waste was applied.

The Department of Biological Systems Engineering rainfall simulator (Dillaha et al., 1987) was used to generate storm events to produce runoff from the field plots. Rainfall was applied at a uniform rate (approximately 4.45 cm/h) to all pasture plots. A series of rainfall simulations were conducted within 24 hours after manure application. The first simulation (S1) lasted approximately 3 hours. The rainfall continued until a steady state runoff was resulted. The S1 simulation represented the bacterial transport during dry field conditions. Approximately 22 hours after the end of the first simulation, the second rainfall simulation (S2) was conducted on saturated soils.

Grab samples of runoff water were collected from the transport plots every 3 to 9 minutes during both simulated-storm events. FW-1 stage recorders were used to record the runoff hydrographs for each of the transport plots. The individual samples were collected and analyzed for bacteria and nutrient concentrations. The sample concentrations were used to calculate the bacteria and nutrient flow-weighted concentrations and the nutrient yields.

5.1.3 Bacterial Source Tracking

The objective of the Bacterial Source Tracking was to determine the enterococcal species present in dairy manure and determine which species have the highest potential to be transported with runoff. The Biolog System, a method of bacterial source tracking, was used to identify the different species of *Enterococcus* present both in the cowpie source manure and in the runoff collected from the transport plots treated with cowpies. Twenty isolates were selected for analysis from runoff samples that represented the beginning of runoff, the peak runoff rate, and the end of runoff. Isolates were taken from each of these points for both rainfall simulations so that a comparison could be made between dry and wet soil conditions. Isolates were selected from the *Enterococcus* plates used to determine the concentration of *Enterococcus* in runoff. In addition, twenty isolates were taken from the source sample to identify the enterococcal species present in the source manure.

5.2. Conclusions

5.2.1 Objective 1 Conclusions

The following conclusions could be drawn from the release plot study:

Sediment and Runoff Release

- The Dairy Farm and the Turkey Farm had 143% and 94%, respectively higher total suspended solids (TSS) concentrations released compare with the Tech Research Farm. Farms with a history of land application of waste had higher TSS concentrations available to be transported off the field during overland flow events because of the build up of residual organic material on the soil surface.

Bacterial Release

- During a short but intense rainfall event, the highest bacteria concentrations were released from the cowpie treatment (*E. coli* concentrations ranged from 37,000 to >300,000 and fecal coliform (FC) concentrations ranged from 65,000 to >300,000). Therefore, the greatest potential for bacteria to be transport with runoff into surface waters was observed for the cowpie treatments.
- The percentages of bacteria released from the cowpie treatment: 2.3% for *Enterococcus*, 3.8% for *E. coli*, and 4.1% for FC. The percentage of bacteria released from the liquid dairy treatment was less than 0.1% for all bacterial species.
- Pastureland plots with a history of previous manure applications did not have a higher percentage bacterial release compared with the pasturelands which had never received manure applications.

Nutrient Release

- Total phosphorus (TP) concentrations released from the cowpie, liquid dairy, and turkey litter treatments were 3.12 mg/L, 3.00 mg/L, and 1.76 mg/L, respectively.

The liquid dairy treatment contributed a higher percentage of dissolved phosphorus (DP), probably because it was applied to the pastureland in a liquid form and had less bio-available phosphorus attached to organic matter particles. The turkey litter treatment also had a higher release of DP because the turkey litter source manure contains a much higher DP concentration. The raindrops from the rainfall simulator broke apart the cowpies, increasing concentrations of organic matter particles, and therefore increased sediment bound phosphorus (SBP) concentrations from the cowpie treatments.

- Organic matter remaining on the soil surface from previous manure applications at the Dairy Farm and Turkey Farm increased the SBP concentrations available for transport by overland flow by 78%.
- Total nitrogen (TN) concentrations released were highest from plots treated with liquid dairy manure (13.46 mg/L), followed by cowpies (12.84 mg/L), turkey litter (7.99 mg/L), and the control (4.84 mg/L). TN in manure was applied to the liquid dairy and cowpie treatment plots at approximately the same rate (146 kg/ha and 152 kg/ha, respectively), but at more than twice the application rate of the turkey litter application rate (64 kg/ha).

5.2.2 *Objective 2 Conclusions*

The following conclusions could be drawn from the transport plot study:

Sediment and Runoff Transport

- The addition of the manure to the plots decreased TSS concentrations from the liquid dairy and turkey plots when compared to the control treatment. As bacteria and organic substances accumulate on the soil surface, they increase the filtration properties of the soil, resulting in lower TSS concentrations in runoff. The cowpies had higher moisture content than the other waste types; therefore it is possible that after the raindrop impacts disintegrated the cowpies, they were more readily carried off the plots by runoff.

Bacterial Transport

- The average bacteria flow-weighted concentrations were highest in runoff samples from the plots treated with cowpies (137,000 CFU/100 mL of *E. coli* 165,000 CFU/100 mL of FC, and 119,000 CFU/100 mL of *Enterococcus*).
- Runoff from pasture treated with liquid dairy manure had greater fecal bacteria concentrations during the initial rainfall event (S1) (31,000 CFU/100 mL for *E. coli* and 74,000 CFU/100 mL for FC), and reduced concentrations during a subsequent rainfall event (S2) (5,500 CFU/100 mL for *E. coli* and 6,800 CFU/100 mL for FC).
- The turkey treatment had lower bacteria concentrations during the initial rainfall event (S1) (9,300 CFU/100 mL for *E. coli* and 17,000 CFU/100 mL for FC), with increased concentrations during S2 (17,000 CFU/100 mL for *E. coli* and 19,000 CFU/100 mL for FC).

Nutrient Transport

- The turkey litter treatment had the highest concentration of DP in runoff (1.22 mg/L), followed by cowpies (0.84mg/L) and liquid dairy manure (0.75 mg/L). The turkey litter is light and more easily transported off the plots with runoff and had higher phosphate concentration in the source manure. During heavy rainfall events, the turkey litter treatment is most likely to contribute high concentrations of water soluble phosphorus to downstream water bodies.
- The cowpie treatment had the highest concentrations of SBP in runoff during both rainfall events (0.73 mg/L), followed by the turkey litter (0.18 mg/L) and liquid dairy treatments (0.13 mg/L).
- The TN concentrations in runoff from the transport plots were highest from the plots treated with cowpies. The concentration of TN in runoff from the plots treated with cowpies was 9.8 mg/L, three times higher than the liquid dairy and turkey litter treatments.

5.2.3 Objective 3 Conclusions

The following conclusions could be drawn from the Bacterial Source Tracking study:

Bacterial Source Tracking

- The source manure is composed of *Enterococcus mundtii* (55%), *Enterococcus gallinarum* (20%), *Enterococcus faecium* (10%), *Enterococcus faecalis* (10%), and *Enterococcus solitarius* (5%). Although only a small percentage of the available enterococcus isolates were identified, the results were similar to those from previous studies on cattle source manure using the Biolog System.
- *Enterococcus faecalis* was the dominant species present in runoff. Thirty-seven percent of the isolates present in runoff were identified as *Enterococcus faecalis*, 21% were identified as *Enterococcus mundtii*, and 11% were identified as *Enterococcus gallinarum* and *Enterococcus faecium*.

5.3 Implications and Recommendations

In recent years significant changes have occurred in the livestock industry. Animal production areas are highly concentrated, resulting in higher concentrations of waste to be applied to crop and pastureland. This comparative study determined that the cowpies have the greatest potential to contribute fecal bacteria into surface waters, followed by the land application of liquid dairy manure and then turkey litter. The composition and transport characteristics of the waste explain the differences. The cowpies were easily broken down by the impact of the raindrops and transported from the plots. The liquid dairy manure infiltrated into the soil before the rainfall event, reducing the transport capacity of the waste while the turkey litter was more easily transported off the plots with the higher runoff rates during the second rainfall event. Runoff concentrations from all treatments exceed federal fecal coliform standards of 200 CFU/100 mL for primary contact. Virginia DEQ standard for primary contact for *E. coli* is 126 CFU/100 mL. The concentrations reported in this study are much greater since they represent the edge of the field levels as opposed to in-stream concentrations. In-stream concentrations are expected to be lower due to dilution effects and die-off.

The cowpie treatment also had the greatest potential to contribute total phosphorus and total nitrogen loading into surface waters. The impact of the raindrops from the rainfall simulator broke apart the cowpies, increasing concentrations of organic matter particles. The turkey litter treatment had greater concentrations of DP in runoff because the phosphate is present in the turkey litter source manure at a much higher concentration, and the litter is light and more easily transported with runoff. Total phosphorus concentrations in lakes exhibiting eutrophic properties may be as low as 20 μg/L. In this study all three treatments could increase the risk for eutrophication due to high phosphorus concentrations in runoff. The eutrophication problem threshold for nitrogen concentrations in water bodies begins at a concentration as low as 0.092 mg/L. Problems are likely to exist when nitrogen concentrations reach 0.92 mg/L, and severe problems are possible when the nitrogen concentrations reach 9.2 mg/L (USEPA, 1982). Total nitrogen concentrations from the transport plots exceed the threshold for likely eutrophication problems for all treatments and the total nitrogen concentrations from plots treated with cowpies exceed the threshold for severe eutrophication problems.

Previous research has investigated methods of reducing bacterial and nutrients loading into surface waters. Best management practices are methods and practices for preventing or reducing nonpoint source pollution to a level compatible with water quality goals (Novotny and Olem, 1994). These management strategies may be applied to control loading of fecal bacteria and nutrients into streams. The high concentrations of bacteria and nutrients in runoff from the plots treated with cowpies imply that areas where cattle may congregate, such as shaded areas, watering or feeding areas, should be moved away from streams to increase the distance between the manure and waterways. An increased buffer zone between grazing cattle and streams will assist in reducing the loading of fecal bacteria and nutrients. In areas with high cattle density a detention basin could be installed to reduce the first flush effects and allow for organic particles and sediment to settle. When turkey litter and liquid dairy manure are applied to pasturelands, the soluble form of phosphorus is more of a concern than the sediment bound form. Once again, an increased separation distance between areas where manure is land applied and the streams will allow for bacteria and nutrients to filter out.

Nutrients present in surface waters can cause eutrophication, while bacteria may lead to deadly diseases if present in drinking water. Bacteria and nutrients in water bodies are often associated with agricultural activities. Land application of manure from confined animal production facilities and grazing animals can both be major sources of contamination in runoff. The data from this study can serve as a baseline from which the release and transport of bacteria and nutrients from agricultural watersheds to surface waters can be modeled.

5.4 Lessons Learned

The following recommendations would be made to those conducting research similar to this study:

- The turkey litter is heterogeneous in composition, with varying concentrations of bacteria in different parts of the pile. It is recommended to mix the pile thoroughly before land application, and to collect several samples of the litter from different parts of the pile for bacterial analysis.
- A larger number of plots are recommended (if possible) to increase statistical significance among the treatments.
- A pilot test prior to the rainfall simulation study would be helpful to prepare the water quality laboratory personnel on the dilution levels that may be necessary to obtain accurate concentrations of colony forming units in both the source manure and in runoff.

5.5 Recommendations for Future Study

Several recommendations can be made for the future studies on bacteria and nutrient transport from land applied livestock manure. This study focused on bacteria and nutrient transport from manure applied to pastureland; therefore, the transport properties of livestock manures applied to cropland also need to be evaluated. The impact of different methods of manure application as well as seasonal variations on bacteria and nutrient concentrations in runoff should be investigated. This information would be necessary to improve the bacteria and nutrient components of non-point source pollution models. A comparative study of the die-off rates of the bacterial indicators as related to environmental factors would also be a useful component to improve current modeling efforts.

Much additional research is also needed in the area of Bacterial Source Tracking. The evaluation of enterococcal species present in other livestock sources, wildlife, and humans would help to assist in the identification of *Enterococcus* species that are predominantly associated with specific wastes. If a strong relationship is developed to relate enterococcal species to different wastes, a sensor could be developed to identify specific species present in streams, thus, reducing the need for costly BST analysis.

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APPENDIX A   MANURE ANALYSIS

Table A.1: Animal waste analysis reports used to determine the application rates.

Sample	Liquid Dairy (lbs/1000 gal)	Liquid Dairy (lbs/1000 gal)	Turkey (lbs/ton)	Turkey (lbs/ton)	Solid Dairy (lbs/ton)
Date	10/23/2001	5/2/2002	2/2/2000	6/3/1999	9/9/2002
Ammonium Nitrogen	7.26	8.43	15.51	10.17	1.2
Total Nitrogen	15.86	18.86	46.36	16.67	9.38
Incorporated available nitrogen estimate	8.45	9.97	32.47	31.05	3.76
Surface available nitrogen estimate	4.82	5.76	26.26	26.98	3.16
Phosphorus as P_2O_5	4.57	6.84	48.44	33.14	3.81
Potassium as K_2O	20.49	22.02	24.86	23.18	2.39
Calcium	5.59	8.07	31.25	22.03	4.68
Magnesium	2.13	2.82	9.05	8.12	1.55
Sulfur	1.49	1.74	6.07	3.57	0.86
Zinc	0.06	0.06	0.45	0.31	0.04
Copper	0.15	0.14	0.06	0.03	0.01
Manganese	0.07	0.08	0.35	0.3	0.05
Sodium	3.4	0.47			0.93
Aluminum	0.46	1			0.11
Moisture	97.52%	96.35%	49.90%	21.30%	85.64%

Table A.2: Animal waste analysis reports on manure samples that were applied to the plots.

Date	10/14/2002	10/30/2002	10/30/2002	10/30/2002
Sample	Turkey (lbs/ton)	Turkey (lbs/ton)	Liquid Dairy (lbs/1000 gal)	Solid Dairy (lbs/ton)
Ammonium Nitrogen	14.6	17.2	5.34	2.4
Organic Nitrogen	29.79	31.36	9.18	7.51
Nitrate Nitrogen	0.22	0.52	0.38	0.1
Incorporated available nitrogen estimate	29.9	33.41	10.16	6.59
Surface available nitrogen estimate	25.52	28.25	8.56	5.87
Phosphorus as P_2O_5	36.37	43.06	5.71	3.39
Potassium as K_2O	31.72	42.36	14.7	7.72
Calcium	28.69	33.28	6.64	5.93
Magnesium	4.65	6.66	2.6	1.88
Sulfur	5.26	6.42	1.72	0.96
Zinc	0.26	0.31	0.05	0.03
Copper	0.03	0.05	0.15	0.01
Manganese	0.2	0.27	0.07	0.05
Sodium	8.25	11.01	2.73	1.56
Moisture	56.13%	41.61%	96.24%	79.97%
pH	8.1	8.6	7.8	6.4
Calcium Carbonate Equivalency	0	30.48	40.89	0
Soluble Phosphorus	10.35	14.71	1.87	3.53

APPENDIX B RELEASE PLOT RAINFALL SIMULATOR TESTING AND PLOT CONDITIONS

Table B.1: Nozzle Testing

	Run 1	**Run 2**	**Run 3**	**Run 1**	**Run 2**	**Run 3**	**Run 1**	**Run 2**
Date	9/19/2002	9/23/2002	9/23/2002	10/9/2002	10/10/2002	10/10/2002	10/21/2002	10/21/2002
Nozzle	1/2 50WSQ	1/2 50WSQ	1/2 50WSQ	1/2 20WSQ	1/2 20WSQ	1/2 20WSQ	1/2 30WSQ	1/2 30WSQ
Pressure	6 psi	5 psi	7 psi	7.5 psi	9 psi	14 psi	9 psi	13.5 psi
Flow	1.98 gpm	1.74 gpm	2.11 gpm	1.1 gpm	1.24 gpm	1.75 gpm	1.75 gpm	2.2 gpm
Rain gauge	Rainfall (in)	Rainfall (in)	Rainfall (in)	Rainfall (in)	Rainfall (in)	Rainfall (in)	Rainfall (in)	Rainfall (in)
1	1	0.23	0.88	0.66	1.25	1.45	2.05	1.45
2	2.15	1.82	2	0.68	0.88	1.4	1.05	1.55
3	2.75	2.65	2.7	0.64	0.86	1.3	1.15	1.6
4	2.2	2.5	2.46	0.66	0.88	1.15	1.4	1.35
5	0.8	0.4	1.02	0.9	1.5	1.25	1.8	1.45
6	2.1	1.45	1.88	0.68	0.98	1.35	1.35	1.2
7	3.05	2.8	2.95	0.54	0.88	1.5	1.05	1.4
8	3.15	3.5	3.75	0.54	0.88	1.55	1.25	1.6
9	2.5	3.2	3	0.46	0.8	1.35	1.05	1.3
10	1.55	1.85	1.83	0.64	0.98	1.2	1.4	1.2
11	2.65	2.23	2.3	0.64	0.84	1.05	1.45	1.35
12	2.6	2.62	2.37	0.46	0.78	1.2	1.2	1.45
13	2.8	3.05	3.43	0.6	0.94	1.6	1.4	1.65
14	1.8	2.3	2.18	0.5	0.78	1.5	1.15	1.4
15	1.45	1.57	1.65	0.64	0.94	1.23	1.35	1.2
16	1.4	0.36	1.16	0.76	1.15	1.14	2.4	1.85
17	3	2.91	3.01	0.5	0.74	1.04	1.5	1.65
18	2.55	2.74	2.65	0.52	0.9	1.21	1.45	1.8
19	1.75	2	1.88	0.6	0.84	1.32	1.4	1.45
20	0.6	0.36	1.05	0.6	1	1.16	1.05	1.3
Average (y)	2.0925	2.027	2.2075	0.611	0.94	1.2975	1.395	1.46
x	0.639	0.8203	0.6545	0.079	0.122	0.1345	0.23	0.148
UC	69.5%	59.5%	70.4%	87.1%	87.0%	89.6%	83.5%	89.9%

Table B.2: The calculated uniformity coefficients based on three rain gauges placed in the plots during the rainfall simulations.

Plot	RG – 1[*] (in)	RG – 2 (in)	RG – 3 (in)	Average (y)[†]	x[‡]	UC[§]
PF-1						
PF-2	3.35	3.82	2.48	3.22	0.49	84.7%
PF-3	2.25	3	2.55	2.60	0.27	89.7%
PF-4	2.6	3.2	2.55	2.78	0.28	90.0%
PF-5	2.35	2.75	2.25	2.45	0.20	91.8%
PF-6	2.45	2.95	2.48	2.63	0.22	91.8%
PF-7	3.3	3.6	3.25	3.38	0.14	95.7%
PF-8	1.95	2.35	1.9	2.07	0.19	90.9%
TKY-1	1.92	1.98	2.4	2.10	0.20	90.5%
TKY-2	3.45	4.3	3.75	3.83	0.31	91.9%
TKY-3	3.02	3.4	2.85	3.09	0.21	93.3%
TKY-4	4.6	5.35	4.25	4.73	0.41	91.3%
TKY-5	5.25	6	6	5.75	0.33	94.2%
TKY-6	2.9	3.5	3	3.13	0.24	92.2%
TKY-7	2.7	3	2.35	2.68	0.22	91.7%
TKY-8	2.5	2.9	2.4	2.60	0.20	92.3%
TKY-9	2.4	2.95	2.65	2.67	0.19	92.9%
TKY-10	3.15	3.65	3	3.27	0.26	92.2%
TKY-11	2.25	2.7	2.35	2.43	0.18	92.7%
TKY-12	2.75	2.95	2.75	2.82	0.09	96.8%
HS-1	1.7	2.25	2.05	2.00	0.20	90.0%
HS-2	1.9	2.59	2.29	2.26	0.24	89.4%
HS-3	1.68	2.03	1.85	1.85	0.12	93.6%
HS-4	1.75	2.12	2	1.96	0.14	93.0%
HS-5	2	2.7	2.5	2.40	0.27	88.9%
HS-6	1.85	2.25	2.05	2.05	0.13	93.5%
HS-7	1.95	2.35	2.03	2.11	0.16	92.4%
HS-8	1.75	2.1	1.9	1.92	0.12	93.6%
HS-9	2.9	3.2	2.7	2.93	0.18	93.9%
HS-10	2.2	2.57	2.2	2.32	0.16	92.9%
HS-11	1.85	2.45	1.95	2.08	0.24	88.3%
HS-12	2.3	2.67	2.3	2.42	0.16	93.2%

[*] Rain gauge-1 placed in the back of the plots, Rain gauge-2 placed in the middle of the plots, Rain gauge-3 placed in front of the plots; [†] Average Rainfall; [‡] Average absolute deviation from the mean rainfall depth [§] Uniformity coefficient

Table B.3: Pre-treatment of water, natural rainfall, and manure applied to the release plots.

Plot	Plot treatment	Water applied previous to simulation (gal)	Natural rainfall occurring previous to simulation	Water application date	Manure application date	Simulation date	Simulation Start time	Runoff time	Comments
TRF1[†]	LD	13.4 gal[*]	10/21 – 0.3 in	10/22/2002	10/22/2002	10/23/2002	8:06 AM	2h 45min	Simulation was halted after 2 hours, 4 min (ran out of water). Nozzle was replaced with 50S and the simulation was restarted after 2 hours, 21 min.
TRF2	TKY	13.4 gal	10/21 – 0.3 in	10/22/2002	10/22/2002	10/23/2002	12:12 PM	42 min	
TRF3	CP	13.4 gal	10/21 – 0.3 in	10/22/2002	10/22/2002	10/23/2002	2:08 PM	26 min	
TRF4	CONT	13.4 gal	10/21 – 0.3 in	10/22/2002		10/23/2002	3:42 PM	30 min	
TRF5	CP	13.4 gal		10/23/2002	10/23/2002	10/24/2002	8:57 AM	19 min	
TRF6	CONT	13.4 gal		10/23/2002		10/24/2002	11:08 AM	20 min	
TRF7	TKY	13.4 gal		10/23/2002	10/23/2002	10/24/2002	12:34 PM	35 min	
TRF8	LD	13.4 gal		10/23/2002	10/23/2002	10/24/2002	2:16 PM	21 min	Forgot to put rain gauges in until 5 - 10 min after rainfall started
TKY1[‡]	LD		10/25 – 0.63 in 10/28 – 0.79 in 10/29 – 0.61 in		10/29/2002	10/30/2002	3:41 PM	11 min	
TKY2	CONT		10/25 – 0.63 in 10/28 – 0.79 in 10/29 – 0.61 in			10/30/2002	5:07 PM	43 min	
TKY3	LD		10/29 – 0.61 in 10/30 – 0.32 in		10/30/2002	10/31/2002	8:12 AM	25 min	
TKY4	TKY		10/29 – 0.61 in 10/30 – 0.32 in		10/30/2002	10/31/2002	10:12 AM	54 min	
TKY5	CP		10/29 – 0.61 in 10/30 – 0.32 in		10/30/2002	10/31/2002	12:56 PM	1 h 19 min	Did not get much runoff due to a low spot in the plot. Jan tried to fill it in with sod.
TKY6	TKY		10/29 – 0.61 in 10/30 – 0.32 in		10/30/2002	10/31/2002	3:01 PM	30 min	
TKY7	TKY	13.4 gal		10/31/2002	10/31/2002	11/1/2002	12:39 PM	22 min	
TKY8	CP	13.4 gal		10/31/2002	10/31/2002	11/1/2002	10:11 AM	18 min	
TKY9	CP	13.4 gal		10/31/2002	10/31/2002	11/1/2002	8:42 AM	20 min	
TKY10	CONT	13.4 gal		10/31/2002		11/1/2002	7:15 AM	30 min	
TKY11	LD	13.4 gal		11/1/2002	11/1/2002	11/2/2002	8:16 AM	15 min	
TKY12	CONT	13.4 gal		11/1/2002		11/2/2002	9:34 AM	21 min	

[*] Equivalent to 2-inches of rainfall over the plots

[†] Tech Research Farm; [‡] Turkey Farm; [§] Dairy Farm

Table B.3 (continued)

Plot	Plot treatment	Water applied previous to simulation (gal)	Natural rainfall occurring previous to simulation	Water application date	Manure application date	Simulation date	Simulation Start time	Runoff time	Comments
DF1[§]	LD		11/11 – 0.94 in 11/12 – 0.85 in		11/11/2002	11/13/2002	8:17 AM	5 min	11/12/02 - rained all day. Plots HS-1, HS-2, HS-3 were covered at 6:20 am, but plots did receive some rain, approx 3/10 inch. Runoff may have occurred from all plots. Treatment had already been applied to HS-1, HS-2, HS-3.
DF2	TKY		11/11 – 0.94 in 11/12 – 0.85 in		11/11/2002	11/13/2002	9:14 AM	13 min	
DF3	TKY		11/11 – 0.94 in 11/12 – 0.85 in		11/11/2002	11/13/2002	10:20 AM	5 min	
DF4	CP		11/11 – 0.94 in 11/12 – 0.85 in		11/13/2002 AM	11/13/2002	11:34 AM	7 min	After this run, the wind knocked the simulator over. The simulator was utilized for the rest of the runs without wheels - removed ?? Inches from the total height
DF5	CONT		11/11 – 0.94 in 11/12 – 0.85 in			11/13/2002	3:27 PM	13 min	After 4 minutes of simulation, the generator ran out of gas. Rainfall was stopped for 3 minutes and then continued.
DF6	CONT		11/11 – 0.94 in 11/12 – 0.85 in			11/13/2002	4:26 PM	6 min	
DF7	TKY		11/11 – 0.94 in 11/12 – 0.85 in		11/13/2002	11/14/2002	9:13 AM	5 min	9:00 plots seemed to be thawed
DF8	CP		11/11 – 0.94 in 11/12 – 0.85 in		11/13/2002	11/14/2002	10:14 AM	4.5 min	
DF9	CP		11/11 – 0.94 in 11/12 – 0.85 in		11/13/2002	11/14/2002	11:40 AM	20 min	
DF10	LD		11/11 – 0.94 in 11/12 – 0.85 in		11/13/2002	11/14/2002	12:50 PM	10 min	
DF11	LD		11/11 – 0.94 in 11/12 – 0.85 in		11/13/2002	11/14/2002	1:52 PM	6 min	
DF12	CONT		11/11 – 0.94 in 11/12 – 0.85 in			11/14/2002	8:14 AM	10 min	Hard frost the previous night. Started simulations with a control plot

[*] Equivalent to 2-inches of rainfall over the plots

[†] Tech Research Farm; [‡] Turkey Farm; [§] Dairy Farm

APPENDIX C RUNOFF RESULTS

Table C.1: Runoff from Release Plots

Site	Treatment	Runoff Weight (lb)	Runoff (mm)
TRF1	LD	17.7	8.0
TRF2	TKY	36.9	16.7
TRF3	CP	54.4	24.7
TRF4	CONT	26.6	12.1
TRF5	CP	39.3	17.8
TRF6	CONT	27.8	12.6
TRF7	TKY	32.8	14.9
TRF8	LD	54.2	24.6
TKY1	LD	60.1	27.3
TKY2	CONT	49.2	22.3
TKY3	LD	31.5	14.3
TKY4	TKY	12.0	5.4
TKY5	CP	4.1	1.9
TKY6	TKY	6.7	3.0
TKY7	TKY	8.3	3.7
TKY8	CP	76.3	34.6
TKY9	CP	34.4	15.6
TKY10	CONT	29.6	13.4
TKY11	LD	72.7	33.0
TKY12	CONT	42.3	19.2
DF1	LD	60.0	27.2
DF2	TKY	64.1	29.1
DF3	TKY	63.7	28.9
DF4	CP	49.7	22.6
DF5	CONT	27.0	12.3
DF6	CONT	42.4	19.2
DF7	TKY	70.5	32.0
DF8	CP	49.3	22.4
DF9	CP	40.5	18.4
DF10	LD	68.8	31.2
DF11	LD	42.2	19.2
DF12	CONT	7.9	3.6

Table C.2: Transport Plot Flow Data, Simulation 1

Transport Plot Runoff Data - Simulation 1								
Plot	**T-1***	**T-2**	**T-3**	**T-4**	**T-5**	**T-6**	**T-7**	**T-8**
Treatment	**Liquid Dairy**	**Cowpie**	**Turkey Litter**	**Control**	**Liquid Dairy**	**Turkey Litter**	**Cowpie**	**Control**
Sample No.	**Flow in watershed inches**							
1	0.004	0.057	0.003	0.008	0.003	0.002	0.002	0.006
2	0.000	0.015	0.001	0.017	0.002	0.003	0.001	0.004
3	0.000	0.020	0.002	0.027	0.002	0.009	0.002	0.008
4	0.001	0.025	0.004	0.033	0.003	0.023	0.004	0.012
5	0.004	0.018	0.009	0.023	0.009	0.035	0.007	0.020
6	0.007	0.021	0.014	0.010	0.023	0.050	0.010	0.018
7	0.010		0.020		0.045	0.038	0.015	0.009
8	0.007		0.015		0.075	0.025	0.025	
9	0.002		0.017		0.089		0.047	
10					0.095		0.041	
11					0.063		0.020	
12					0.039			
Total	**0.034**	**0.156**	**0.084**	**0.119**	**0.448**	**0.185**	**0.173**	**0.078**

*Transport Plot 1

Table C.3: Transport Plot Flow Data, Simulation 2

Transport Plot Runoff Data - Simulation 2								
Plot	**T-1***	**T-2**	**T-3**	**T-4**	**T-5**	**T-6**	**T-7**	**T-8**
Treatment	**Liquid Dairy**	**Cowpie**	**Turkey Litter**	**Control**	**Liquid Dairy**	**Turkey Litter**	**Cowpie**	**Control**
Sample No.	**Flow in watershed inches**							
1	0.019	0.009	0.001	0.001	0.003	0.009	0.008	0.016
2	0.008	0.019	0.002	0.001	0.014	0.027	0.026	0.039
3	0.020	0.028	0.006	0.006	0.042	0.051	0.051	0.058
4	0.030	0.035	0.017	0.015	0.070	0.068	0.073	0.072
5	0.028	0.039	0.026	0.023	0.083	0.077	0.094	0.084
6	0.017	0.041	0.031	0.028	0.089	0.082	0.105	0.093
7	0.018	0.031	0.037	0.030	0.093	0.060	0.109	0.096
8		0.047	0.030	0.022	0.069	0.074	0.081	0.067
9			0.045	0.017	0.081		0.105	0.058
Total	**0.139**	**0.248**	**0.194**	**0.141**	**0.542**	**0.448**	**0.652**	**0.583**

*Transport Plot 1

APPENDIX D BACTERIA RESULTS

Table D.1: Bacteria concentrations and loading in runoff from each of the release plots

Site	Treatment	FC (CFU/100 mL)	*E. coli* (CFU/100 mL)	ENT (CFU/100 mL)	Runoff Weight (lbs)	Volume (mL)	FC Load (CFU)	*E. coli* Load (CFU)	ENT Load (CFU)
TRF-1	LD	22,000	15,000	14,000	17.7	8,033	1.77E+10	1.21E+10	1.12E+10
TRF-2	TKY	58,000	37,000	24,000	36.9	16,748	9.71E+10	6.20E+10	4.02E+10
TRF-3	CP	169,000	159,000	270,000	54.4	24,690	4.17E+11	3.93E+11	6.67E+11
TRF-4	CONT	2,700	600	8,000	26.6	12,073	3.26E+09	7.24E+08	9.66E+09
TRF-5	CP	150,000	146,000	>300000	39.3	17,819	2.67E+11	2.60E+11	5.35E+11
TRF-7	CONT	56,000	42,000	38,000	27.8	12,618	7.07E+10	5.30E+10	4.79E+10
TRF-7	TKY	100	100	150	32.8	14,887	1.49E+08	1.49E+08	2.23E+08
TRF-8	LD	48,000	31,000	20,000	54.2	24,600	1.18E+11	7.63E+10	4.92E+10

Table D.1 (continued)

Site	Treatment	FC (CFU/100 mL)	*E. coli* (CFU/100 mL)	ENT (CFU/100 mL)	Runoff Weight (lbs)	Volume (mL)	FC Load (CFU)	*E. coli* Load (CFU)	ENT Load (CFU)
TKY-1	LD	80,000	44,000	1,100	60.1	27,277	2.18E+11	1.20E+11	3.00E+09
TKY-2	CONT	500	<1	<1	49.2	22,330	1.12E+09	0.00E+00	0.00E+00
TKY-3	LD	45,000	38,000	1,200	31.5	14,297	6.43E+10	5.43E+10	1.72E+09
TKY-4	TKY	20,000	12,000	660	12.0	5,442	1.09E+10	6.53E+09	3.59E+08
TKY-5	CP	75,000	40,000	1,200	4.1	1,861	1.40E+10	7.44E+09	2.23E+08
TKY-6	TKY	4,000	1,200	560	6.7	3,041	1.22E+09	3.65E+08	1.70E+08
TKY-7	TKY	3,000	1,000	300	8.3	3,749	1.12E+09	3.75E+08	1.12E+08
TKY-8	CP	100,000	60,000	820	76.3	34,630	3.46E+11	2.08E+11	2.84E+09
TKY-9	CP	20,000	11,000	1,000	34.4	15,613	3.12E+10	1.72E+10	1.56E+09
TKY-10	CONT	<1	<1	<1	29.6	13,434	0.00E+00	0.00E+00	0.00E+00
TKY-11	LD	18,000	10,000	3,300	72.7	32,996	5.94E+10	3.30E+10	1.09E+10
TKY-12	CONT	<1	<1	70	42.3	19,199	0.00E+00	0.00E+00	1.34E+08

Table D.1 (continued)

Site	Treatment	FC (CFU/100 mL)	*E. coli* (CFU/100 mL)	ENT (CFU/100 mL)	Runoff Weight (lbs)	Volume (mL)	FC Load (CFU)	*E. coli* Load (CFU)	ENT Load (CFU)
DF-1	LD	>300000	100,000	200	60.0	27,232	8.17E+11	2.72E+11	5.45E+08
DF-2	TKY	38,000	30,000	120	64.1	29,093	1.11E+11	8.73E+10	3.49E+08
DF-3	TKY	68,000	34,000	120	63.7	28,911	1.97E+11	9.83E+10	3.47E+08
DF-4	CP	>300000	>300000	13,000	49.7	22,557	6.77E+11	6.77E+11	2.93E+10
DF-5	CONT	<1	<1	<1	27.0	12,254	0.00E+00	0.00E+00	0.00E+00
DF-6	CONT	<1	<1	<1	42.4	19,244	0.00E+00	0.00E+00	0.00E+00
DF-7	TKY	170,000	20,000	5,400	70.5	31,989	5.44E+11	6.40E+10	1.73E+10
DF-8	CP	>300000	>300000	6,100	49.3	22,380	6.71E+11	6.71E+11	1.37E+10
DF-9	CP	>300000	>300000	5,300	40.5	18,382	5.51E+11	5.51E+11	9.74E+09
DF-10	LD	>300000	40,000	3,000	68.8	31,226	9.37E+11	1.25E+11	9.37E+09
DF-11	LD	>300000	25,000	6,000	42.2	19,153	5.75E+11	4.79E+10	1.15E+10
DF-12	CONT	400	<1	<1	7.9	3,586	1.44E+08	0.00E+00	0.00E+00

Table D.2: Average concentration of bacteria released from all plots at all three farms for each manure treatment

Treatment	Fecal Coliform (CFU/100 mL)	*E. coli* (CFU/100 mL)	*Enterococcus* (CFU/100 mL)
Liquid Dairy	127,556	36,222	7,311
Cowpie	174,833	163,167	98,047
Turkey	43,350	17,095	4,821
Control	9,883	7,101	7,675

Table D.3: Average load of bacteria released from all plots at all three farms for each manure treatment.

Treatment	Fecal Coliform Load (CFU)	*E. coli* Load (CFU)	*Enterococcus* Load (CFU)
Liquid Dairy	3.19E+07	8.72E+06	1.42E+06
Cowpie	3.69E+07	3.46E+07	2.07E+07
Turkey	1.12E+07	3.89E+06	8.80E+05
Control	1.23E+06	8.95E+05	9.60E+05

Table D.4: Bacterial concentrations from Transport Plots, Simulation 1

Bacteria Concentrations Present in Runoff from the Transport Plots					
Transport Plots - Run 1[*]					
Plot	**Sample No.**	**Date**	**Fecal Coliform (CFU/100 mL)**	***E. coli*** **(CFU/100 mL)**	***Enterococcus*** **(CFU/100 mL)**
T-1	1	10/15/2002	100,000	55,000	2,000
T-1	2	10/15/2002	50,000	43,000	6,000
T-1	3	10/15/2002	40,000	20,000	5,000
T-1	4	10/15/2002	60,000	49,000	9,000
T-1	5	10/15/2002	70,000	52,000	4,000
T-1	6	10/15/2002	270,000	68,000	7,000
T-1	7	10/15/2002	90,000	27,000	5,000
T-1	8	10/15/2002	20,000	10,000	8,000
T-1	9	10/15/2002	12,000	9,000	7,000
T-2	2	10/15/2002	80,000	38,000	36,000
T-2	3	10/15/2002	70,000	34,000	21,000
T-2	4	10/15/2002	100,000	97,000	90,000
T-2	5	10/15/2002	300,000	100,000	73,000
T-2	6	10/15/2002	>300000	300,000	190,000
T-2	7	10/15/2002	290,000	160,000	130,000
T-3	1	10/15/2002	5,000	4,900	3,000
T-3	2	10/15/2002	18,000	7,000	5,000
T-3	3	10/15/2002	35,000	10,000	7,000
T-3	4	10/15/2002	36,000	11,000	9,000
T-3	5	10/15/2002	30,000	20,000	20,000
T-3	6	10/15/2002	55,000	31,000	25,000
T-3	7	10/15/2002	20,000	15,000	12,000
T-3	8	10/15/2002	19,000	16,000	11,000
T-3	9	10/15/2002	10,000	6,000	6,000
T-4	1	10/15/2002	<1	<1	<1
T-4	2	10/15/2002	<1	<1	<1
T-4	3	10/15/2002	<1	<1	<1
T-4	4	10/15/2002	<1	<1	<1
T-4	5	10/15/2002	<1	<1	<1
T-4	6	10/15/2002	400	100	<1
T-5	1	10/15/2002	90,000	70,000	20,000
T-5	2	10/15/2002	40,000	37,000	10,000
T-5	3	10/15/2002	26,000	21,000	11,000

[*] Analysis occurred on 10/16/2002 through 10/18/2002. Reruns were preformed 10/19/2002 through 10/23/2002. No reruns were performed on samples with CFU<1.

Table D.4 (continued)

Bacteria Concentrations Present in Runoff from the Transport Plots					
Transport Plots - Run 1[*]					
Plot	**Sample No.**	**Date**	**Fecal Coliform (CFU/100 mL)**	***E. coli*** **(CFU/100 mL)**	***Enterococcus*** **(CFU/100 mL)**
T-5	4	10/15/2002	30,000	24,000	12,000
T-5	5	10/15/2002	21,000	20,000	17,000
T-5	6	10/15/2002	70,000	30,000	16,000
T-5	7	10/15/2002	120,000	45,000	23,000
T-5	8	10/15/2002	59,000	32,000	7,000
T-5	9	10/15/2002	27,000	19,000	8,000
T-5	10	10/15/2002	18,000	16,000	7,000
T-5	11	10/15/2002	40,000	25,000	21,000
T-5	12	10/15/2002	25,000	22,000	22,000
T-6	1	10/15/2002	<1	<1	<1
T-6	2	10/15/2002	<1	<1	<1
T-6	3	10/15/2002	<1	<1	<1
T-6	4	10/15/2002	<1	<1	<1
T-6	5	10/15/2002	<1	<1	<1
T-6	6	10/15/2002	8,000	<1	<1
T-6	7	10/15/2002	2,000	<1	<1
T-6	8	10/15/2002	40,000	20,000	4,000
T-7	1	10/15/2002	150,000	100,000	46,000
T-7	2	10/15/2002	>300,000	>300,000	38,000
T-7	3	10/15/2002	>300,000	>300,000	>300,000
T-7	4	10/15/2002	>300,000	>300,000	>300,000
T-7	5	10/15/2002	>300,000	>300,000	>300,000
T-7	6	10/15/2002	>300,000	>300,000	>300,000
T-7	7	10/15/2002	>300,000	>300,000	>300,000
T-7	8	10/15/2002	>300,000	>300,000	>300,000
T-7	9	10/15/2002	>300,000	>300,000	>300,000
T-7	10	10/15/2002	>300,000	>300,000	>300,000
T-7	11	10/15/2002	>300,000	>300,000	>300,000
T-8	1	10/15/2002	<1	<1	<1
T-8	2	10/15/2002	<1	<1	<1
T-8	3	10/15/2002	<1	<1	<1
T-8	4	10/15/2002	<1	<1	<1
T-8	5	10/15/2002	<1	<1	<1
T-8	6	10/15/2002	<1	<1	<1
T-8	7	10/15/2002	600	200	100
Rainwater	1	10/15/2002	<1	<1	<1

[*] Analysis occurred on 10/16/2002 through 10/18/2002. Reruns were preformed 10/19/2002 through 10/23/2002. No reruns were performed on samples with CFU<1.

Table D.5: Bacterial concentrations from Transport Plots, Simulation 2

Bacteria Concentrations Present in Runoff from the Transport Plots

Transport Plots - Run 2*

Site	Sample #	Date	FC (CFU/100 mL)	*E. coli* (CFU/100 mL)	Ent (CFU/100 mL)
T-1	1	10/16/2002	24,000	18,000	10,000
T-1	2	10/16/2002	19,000	16,000	7,000
T-1	3	10/16/2002	15,000	12,000	8,000
T-1	4	10/16/2002	17,000	16,000	9,000
T-1	5	10/16/2002	9,200	8,000	5,000
T-1	6	10/16/2002	8,000	4,600	3,000
T-1	7	10/16/2002	4,500	2,400	1,000
T-2	1	10/16/2002	180,000	100,000	20,000
T-2	2	10/16/2002	100,000	85,000	60,000
T-2	3	10/16/2002	200,000	130,000	65,000
T-2	4	10/16/2002	170,000	120,000	42,000
T-2	5	10/16/2002	160,000	110,000	40,000
T-2	6	10/16/2002	75,000	48,000	22,000
T-2	7	10/16/2002	90,000	52,000	37,000
T-2	8	10/16/2002	98,000	76,000	90,000
T-3	1	10/16/2002	35,000	29,000	5,000
T-3	2	10/16/2002	19,000	13,000	2,000
T-3	3	10/16/2002	24,000	21,000	6,000
T-3	4	10/16/2002	17,000	14,000	4,000
T-3	5	10/16/2002	15,000	11,000	3,000
T-3	6	10/16/2002	22,000	20,000	7,000
T-3	7	10/16/2002	45,000	42,000	20,000
T-3	8	10/16/2002	23,000	19,000	7,000
T-3	9	10/16/2002	49,000	44,000	21,000
T-4	1	10/16/2002	300	100	<1
T-4	2	10/16/2002	<1	<1	<1
T-4	3	10/16/2002	<1	<1	<1
T-4	4	10/16/2002	<1	<1	<1
T-4	5	10/16/2002	<1	<1	<1
T-4	6	10/16/2002	<1	<1	<1
T-4	7	10/16/2002	<1	<1	<1
T-4	8	10/16/2002	<1	<1	<1
T-4	9	10/16/2002	500	100	<1

*Analysis occurred on 10/16/2002 through 10/19/2002. Reruns were preformed 10/19/2002 through 10/23/2002. No reruns were performed on samples with CFU<1.

Table D.5 (continued)

Bacteria Concentrations Present in Runoff from the Transport Plots

Transport Plots - Run 2*

Site	Sample #	Date	FC (CFU/100 mL)	*E. coli* (CFU/100 mL)	Ent (CFU/100 mL)
T-5	1	10/16/2002	<1	<1	<1
T-5	2	10/16/2002	<1	<1	<1
T-5	3	10/16/2002	350	270	100
T-5	4	10/16/2002	<1	<1	<1
T-5	5	10/16/2002	<1	<1	<1
T-5	6	10/16/2002	<1	<1	<1
T-5	7	10/16/2002	<1	<1	<1
T-5	8	10/16/2002	<1	<1	<1
T-5	9	10/16/2002	400	310	120
T-6	1	10/16/2002	6,000	4,400	700
T-6	2	10/16/2002	9,000	7,100	800
T-6	3	10/16/2002	4,000	3,100	500
T-6	4	10/16/2002	12,000	9,900	2,600
T-6	5	10/16/2002	10,000	8,300	2,300
T-6	6	10/16/2002	2,400	1,200	200
T-6	7	10/16/2002	3,500	2,900	700
T-6	8	10/16/2002	5,000	3,700	1,000
T-7	1	10/16/2002	52,000	40,000	2,000
T-7	2	10/16/2002	69,000	58,000	44,000
T-7	3	10/16/2002	110,000	98,000	96,000
T-7	4	10/16/2002	81,000	79,000	53,000
T-7	5	10/16/2002	37,000	24,000	16,000
T-7	6	10/16/2002	56,000	53,000	48,000
T-7	7	10/16/2002	59,000	55,000	49,000
T-7	8	10/16/2002	26,000	22,000	17,000
T-7	9	10/16/2002	100,000	98,000	95,000
T-8	1	10/16/2002	<1	<1	<1
T-8	2	10/16/2002	<1	<1	<1
T-8	3	10/16/2002	<1	<1	<1
T-8	4	10/16/2002	<1	<1	<1
T-8	5	10/16/2002	<1	<1	<1
T-8	6	10/16/2002	<1	<1	<1
T-8	7	10/16/2002	<1	<1	<1
T-8	8	10/16/2002	<1	<1	<1
T-8	9	10/16/2002	100	100	40
Rainwater	2	10/16/2002	<1	<1	<1

*Analysis occurred on 10/16/2002 through 10/19/2002. Reruns were preformed 10/19/2002 through 10/23/2002. No reruns were performed on samples with CFU<1.

Table D.6: Bacteria Flow-weighted concentrations calculated using flow data and sample concentrations

	Run 1				Run 2			
Treatment	**Plot**	**Fecal Coliforms (CFU/100 mL)**	***E. coli*** **(CFU/100 mL)**	***Enterococcus*** **(CFU/100 mL)**	**Plot**	**Fecal Coliforms (CFU/100 mL)**	***E. coli*** **(CFU/100 mL)**	***Enterococcus*** **(CFU/100 mL)**
Liquid Dairy	T1	104,132	37,303	5,782	T1	13,547	10,985	
Cow Pies	T2	169,961	101,941	77,952	T2	127,456	87,426	50,069
Turkey Litter	T3	25,425	15,831	12,970	T3	31,518	27,887	11,837
Control	T4	34	8	0	T4	61	12	0
Liquid Dairy	T5	44,013	25,285	12,899	T5	87	67	26
Turkey Litter	T6	8,014	2,720	544	T6	6,388	5,014	1,204
Cow Pies	T7	298,615	298,153	296,860	T7	64,634	59,044	50,862
Control	T8	68	23	11	T8	10	10	4

Table D.7: Bacteria Load calculated using flow data and sample concentrations

	Run 1				Run 2			
Treatment	**Plot**	**Fecal Coliforms (CFU/ha)**	***E. coli*** **(CFU/ha)**	***Enterococcus*** **(CFU/ha)**	**Plot**	**Fecal Coliforms (CFU/ha)**	***E. coli*** **(CFU/ha)**	***Enterococcus*** **(CFU/ha)**
Liquid Dairy	T1	9.11E+09	3.26E+09	5.06E+08	T1	4.78E+09	3.88E+09	2.23E+09
Cow Pies	T2	6.72E+10	4.03E+10	3.08E+10	T2	8.04E+10	5.52E+10	3.16E+10
Turkey Litter	T3	5.43E+09	3.38E+09	2.77E+09	T3	1.55E+10	1.37E+10	5.83E+09
Control	T4	1.01E+07	2.53E+06	0.00E+00	T4	2.20E+07	4.46E+06	0.00E+00
Liquid Dairy	T5	5.01E+10	2.88E+10	1.47E+10	T5	1.20E+08	9.25E+07	3.53E+07
Turkey Litter	T6	3.76E+09	1.28E+09	2.55E+08	T6	7.28E+09	5.71E+09	1.37E+09
Cow Pies	T7	1.31E+11	1.31E+11	1.30E+11	T7	1.07E+11	9.78E+10	8.42E+10
Control	T8	1.35E+07	4.48E+06	2.24E+06	T8	1.48E+07	5.90E+06	1.48E+07

Table D.8: The Virginia Department of Environmental Quality has developed a translator function to determine *E. coli* concentrations based on know FC concentrations. The results from the equation are compared to the actual *E. coli* concentrations in runoff.

Percent Difference between predicted and actual *E. coli* values							
E. coli values predicted based on VA DEQ translator function							
Plot	**Sample No.**	**Sample Collection Date**	**FC (CFU/100 mL)**	***E. coli* (CFU/100 mL)**	***E. coli* predicted from DEQ translator function**	**% difference between predicted and actual *E. coli* values**	**% difference between predicted and actual *E. coli* values (removing 4 values with >100% difference)**
Transport Plots - Run 1							
T-1[*]	1	10/15/2002	100,000	55,000	38,911	-29.3%	-29.3%
T-1	2	10/15/2002	50,000	43,000	20,578	-52.1%	-52.1%
T-1	3	10/15/2002	40,000	20,000	16,763	-16.2%	-16.2%
T-1	4	10/15/2002	60,000	49,000	24,332	-50.3%	-50.3%
T-1	5	10/15/2002	70,000	52,000	28,036	-46.1%	-46.1%
T-1	6	10/15/2002	270,000	68,000	96,943	42.6%	42.6%
T-1	7	10/15/2002	90,000	27,000	35,320	30.8%	30.8%
T-1	8	10/15/2002	20,000	10,000	8,865	-11.3%	-11.3%
T-1	9	10/15/2002	12,000	9,000	5,544	-38.4%	-38.4%
T-2	2	10/15/2002	80,000	38,000	31,696	-16.6%	-16.6%
T-2	3	10/15/2002	70,000	34,000	28,036	-17.5%	-17.5%
T-2	4	10/15/2002	100,000	97,000	38,911	-59.9%	-59.9%
T-2	5	10/15/2002	300,000	100,000	106,800	6.8%	6.8%
T-2	7	10/15/2002	290,000	160,000	103,524	-35.3%	-35.3%
T-3	1	10/15/2002	5,000	4,900	2,479	-49.4%	-49.4%
T-3	2	10/15/2002	18,000	7,000	8,047	15.0%	15.0%
T-3	3	10/15/2002	35,000	10,000	14,827	48.3%	48.3%
T-3	4	10/15/2002	36,000	11,000	15,216	38.3%	38.3%
T-3	5	10/15/2002	30,000	20,000	12,868	-35.7%	-35.7%
T-3	6	10/15/2002	55,000	31,000	22,462	-27.5%	-27.5%
T-3	7	10/15/2002	20,000	15,000	8,865	-40.9%	-40.9%
T-3	8	10/15/2002	19,000	16,000	8,457	-47.1%	-47.1%
T-3	9	10/15/2002	10,000	6,000	4,688	-21.9%	-21.9%
T-4	6	10/15/2002	400	100	243	143.4%	

[*] Transport Plot No. 1

[†] Tech Research Farm; [‡] Turkey Farm; [§] Dairy Farm

Table D.8 (continued)

Percent Difference between predicted and actual *E. coli* values							
E. coli values predicted based on VA DEQ translator function							
Plot	**Sample**	**Sample**	**FC**	***E. coli***	***E. coli***	**% difference**	**% difference**
Transport Plots - Run 1							
T-5	1	10/15/2002	90,000	70,000	35,320	-49.5%	-49.5%
T-5	2	10/15/2002	40,000	37,000	16,763	-54.7%	-54.7%
T-5	3	10/15/2002	26,000	21,000	11,282	-46.3%	-46.3%
T-5	4	10/15/2002	30,000	24,000	12,868	-46.4%	-46.4%
T-5	5	10/15/2002	21,000	20,000	9,272	-53.6%	-53.6%
T-5	6	10/15/2002	70,000	30,000	28,036	-6.5%	-6.5%
T-5	7	10/15/2002	120,000	45,000	46,009	2.2%	2.2%
T-5	8	10/15/2002	59,000	32,000	23,959	-25.1%	-25.1%
T-5	9	10/15/2002	27,000	19,000	11,681	-38.5%	-38.5%
T-5	10	10/15/2002	18,000	16,000	8,047	-49.7%	-49.7%
T-5	11	10/15/2002	40,000	25,000	16,763	-32.9%	-32.9%
T-5	12	10/15/2002	25,000	22,000	10,883	-50.5%	-50.5%
T-6	8	10/15/2002	40,000	20,000	16,763	-16.2%	-16.2%
T-7	1	10/15/2002	150,000	100,000	56,482	-43.5%	-43.5%
T-8	7	10/15/2002	600	200	353	76.6%	76.6%
Transport Plots - Run 2							
T-1	1	10/16/2002	24,000	18,000	10,482	-41.8%	-41.8%
T-1	2	10/16/2002	19,000	16,000	8,457	-47.1%	-47.1%
T-1	3	10/16/2002	15,000	12,000	6,805	-43.3%	-43.3%
T-1	4	10/16/2002	17,000	16,000	7,635	-52.3%	-52.3%
T-1	5	10/16/2002	9,200	8,000	4,343	-45.7%	-45.7%
T-1	6	10/16/2002	8,000	4,600	3,819	-17.0%	-17.0%
T-1	7	10/16/2002	4,500	2,400	2,251	-6.2%	-6.2%
T-2	1	10/16/2002	180,000	100,000	66,785	-33.2%	-33.2%
T-2	2	10/16/2002	100,000	85,000	38,911	-54.2%	-54.2%
T-2	3	10/16/2002	200,000	130,000	73,576	-43.4%	-43.4%
T-2	4	10/16/2002	170,000	120,000	63,367	-47.2%	-47.2%
T-2	5	10/16/2002	160,000	110,000	59,933	-45.5%	-45.5%
T-2	6	10/16/2002	75,000	48,000	29,871	-37.8%	-37.8%
T-2	7	10/16/2002	90,000	52,000	35,320	-32.1%	-32.1%
T-2	8	10/16/2002	98,000	76,000	38,195	-49.7%	-49.7%
T-3	1	10/16/2002	35,000	29,000	14,827	-48.9%	-48.9%
T-3	2	10/16/2002	19,000	13,000	8,457	-34.9%	-34.9%
T-3	3	10/16/2002	24,000	21,000	10,482	-50.1%	-50.1%
T-3	4	10/16/2002	17,000	14,000	7,635	-45.5%	-45.5%
T-3	5	10/16/2002	15,000	11,000	6,805	-38.1%	-38.1%

[*] Transport Plot No. 1

[†] Tech Research Farm; [‡] Turkey Farm; [§] Dairy Farm

Table D.8 (continued)

Percent Difference between predicted and actual *E. coli* values

***E. coli* values predicted based on VA DEQ translator function**

Plot	Sample No.	Sample Collection Date	FC (CFU/100 mL)	*E. coli* (CFU/100 mL)	*E. coli* predicted from DEQ translator function	% difference between predicted and actual *E. coli* values	% difference between predicted and actual *E. coli* values (removing 4 values with >100% difference)
Transport Plots - Run 2							
T-3	6	10/16/2002	22,000	20,000	9,677	-51.6%	-51.6%
T-3	7	10/16/2002	45,000	42,000	18,679	-55.5%	-55.5%
T-3	8	10/16/2002	23,000	19,000	10,080	-46.9%	-46.9%
T-3	9	10/16/2002	49,000	44,000	20,200	-54.1%	-54.1%
T-4	1	10/16/2002	300	100	187	86.8%	86.8%
T-5	3	10/16/2002	350	270	215	-20.3%	-20.3%
T-5	9	10/16/2002	400	310	243	-21.5%	-21.5%
T-6	1	10/16/2002	6,000	4,400	2,932	-33.4%	-33.4%
T-6	2	10/16/2002	9,000	7,100	4,256	-40.1%	-40.1%
T-6	3	10/16/2002	4,000	3,100	2,020	-34.8%	-34.8%
T-6	4	10/16/2002	12,000	9,900	5,544	-44.0%	-44.0%
T-6	5	10/16/2002	10,000	8,300	4,688	-43.5%	-43.5%
T-6	6	10/16/2002	2,400	1,200	1,263	5.3%	5.3%
T-6	7	10/16/2002	3,500	2,900	1,786	-38.4%	-38.4%
T-6	8	10/16/2002	5,000	3,700	2,479	-33.0%	-33.0%
T-7	1	10/16/2002	52,000	40,000	21,334	-46.7%	-46.7%
T-7	2	10/16/2002	69,000	58,000	27,667	-52.3%	-52.3%
T-7	3	10/16/2002	110,000	98,000	42,473	-56.7%	-56.7%
T-7	4	10/16/2002	81,000	79,000	32,060	-59.4%	-59.4%
T-7	5	10/16/2002	37,000	24,000	15,604	-35.0%	-35.0%
T-7	6	10/16/2002	56,000	53,000	22,837	-56.9%	-56.9%
T-7	7	10/16/2002	59,000	55,000	23,959	-56.4%	-56.4%
T-7	8	10/16/2002	26,000	22,000	11,282	-48.7%	-48.7%
T-7	9	10/16/2002	100,000	98,000	38,911	-60.3%	-60.3%
T-8	9	10/16/2002	100	100	68	-31.9%	-31.9%

[*] Transport Plot No. 1

[†] Tech Research Farm; [‡] Turkey Farm; [§] Dairy Farm

Table D.8 (continued)

Percent Difference between predicted and actual *E. coli* values

***E. coli* values predicted based on VA DEQ translator function**

Plot	Sample No.	Sample Collection Date	FC (CFU/100 mL)	*E. coli* (CFU/100 mL)	*E. coli* predicted from DEQ translator function	% difference between predicted and actual *E. coli* values	% difference between predicted and actual *E. coli* values (removing 4 values with >100% difference)
Release Plots							
TRF1[†]	1	10/23/2002	22,000	15000	9,677	-35.5%	-35.5%
TRF2	1	10/23/2002	58,000	37000	23,586	-36.3%	-36.3%
TRF3	1	10/23/2002	169,000	159000	63,025	-60.4%	-60.4%
TRF4	1	10/23/2002	2,700	600	1,407	134.6%	
TRF5	1	10/24/2002	150,000	146000	56,482	-61.3%	-61.3%
TRF6	1	10/24/2002	56,000	42000	22,837	-45.6%	-45.6%
TRF7	1	10/24/2002	100	100	68	-31.9%	-31.9%
TRF8	1	10/24/2002	48,000	31000	19,821	-36.1%	-36.1%
TKY1[‡]	1	10/30/2002	80,000	44000	31,696	-28.0%	-28.0%
TKY3	1	10/31/2002	45,000	38000	18,679	-50.8%	-50.8%
TKY4	1	10/31/2002	20,000	12000	8,865	-26.1%	-26.1%
TKY5	1	10/31/2002	75,000	40000	29,871	-25.3%	-25.3%
TKY6	1	10/31/2002	4,000	1200	2,020	68.3%	68.3%
TKY7	1	11/1/2002	3,000	1000	1,550	55.0%	55.0%
TKY8	1	11/1/2002	100,000	60000	38,911	-35.1%	-35.1%
TKY9	1	11/1/2002	20,000	11000	8,865	-19.4%	-19.4%
TKY11	1	11/2/2002	18,000	10000	8,047	-19.5%	-19.5%
DF2[§]	1	11/12/2002	38,000	30000	15,991	-46.7%	-46.7%
DF3	1	11/13/2002	68,000	34000	27,299	-19.7%	-19.7%
DF7	1	11/14/2002	170,000	20000	63,367	216.8%	
Average % Difference between predicted and actual *E. coli* values						**-22.4%**	**-30.2%**

[*] Transport Plot No. 1

[†] Tech Research Farm; [‡] Turkey Farm; [§] Dairy Farm

APPENDIX E NUTRIENT RESULTS

Table E.1: Nutrient concentrations and loading in runoff from each of the release plots

Site	Treatment	TSS (mg/L)	Ortho-P (mg/L)	Total P (mg/L)	Nitrate (mg/L)	Ammonia (mg/L)	TKN (mg/L)	Runoff Weight (lb)	Volume (mL)	Load TSS (mg)	Load Ortho-P (mg)	Load Total P (mg)	Load Nitrate (mg)	Load Ammonia (mg)	Load TKN (mg)
TRF1[*]	LD	65	1.51	2.10	0.156	2.491	7.69	17.7	8,033	522	12.1	16.9	1.3	20.0	61.8
TRF2	TKY	55	2.04	2.89	0.645	2.073	9.03	36.9	16,748	921	34.1	48.3	10.8	34.7	151.3
TRF3	CP	73	1.49	2.46	0.037	1.281	15.51	54.4	24,690	1,802	36.7	60.7	0.9	31.6	382.9
TRF4	CONT	45	0.24	0.26	2.264	0.217	2.23	26.6	12,073	543	2.9	3.2	27.3	2.6	26.9
TRF5	CP	70	1.43	3.06	0.134	1.184	11.32	39.3	17,819	1,247	25.5	54.5	2.4	21.1	201.7
TRF7	CONT	30	1.07	1.29	3.234	1.091	4.70	27.8	12,618	379	13.5	16.3	40.8	13.8	59.3
TRF7	TKY	8	0.12	0.18	3.129	0.132	2.44	32.8	14,887	119	1.8	2.7	46.6	2.0	36.4
TRF8	LD	107	2.41	3.07	3.334	3.031	11.39	54.2	24,600	2,632	59.4	75.6	82.0	74.6	280.1

[*] Tech Research Farm; [†] Turkey Farm; [‡] Dairy Farm

Table E.1 (continued)

Site	Treatment	TSS (mg/L)	Ortho-P (mg/L)	Total P (mg/L)	Nitrate (mg/L)	Ammonia (mg/L)	TKN (mg/L)	Runoff Weight (lb)	Volume (mL)	Load TSS (mg)	Load Ortho-P (mg)	Load Total P (mg)	Load Nitrate (mg)	Load Ammonia (mg)	Load TKN (mg)
TKY1[†]	LD	147	2.76	4.44	2.973	4.344	16.16	60.1	27,277	4,010	75.3	121.2	81.1	118.5	440.8
TKY2	CONT	18	0.28	0.86	2.978	0.163	1.38	49.2	22,330	402	6.2	19.1	66.5	3.6	30.9
TKY3	LD	61	1.38	2.07	3.122	2.535	4.69	31.5	14,297	872	19.7	29.6	44.6	36.2	67.0
TKY4	TKY	63	0.16	0.19	3.073	0.084	1.70	12.0	5,442	343	0.9	1.0	16.7	0.5	9.2
TKY5	CP	131	0.12	0.70	2.339	0.424	4.42	4.1	1,861	244	0.2	1.3	4.4	0.8	8.2
TKY6	TKY	29	0.56	1.13	3.057	0.357	2.58	6.7	3,041	88	1.7	3.4	9.3	1.1	7.8
TKY7	TKY	348	0.04	0.37	2.879	0.000	3.86	8.3	3,749	1,305	0.2	1.4	10.8	0.0	14.5
TKY8	CP	129	1.06	2.50	0.196	0.646	10.13	76.3	34,630	4,467	36.6	86.5	6.8	22.4	350.8
TKY9	CP	133	1.19	3.80	0.035	0.755	14.64	34.4	15,613	2,077	18.6	59.3	0.5	11.8	228.6
TKY10	CONT	58	0.08	0.31	3.018	0.016	1.31	29.6	13,434	779	1.1	4.1	40.5	0.2	17.7
TKY11	LD	124	1.93	3.62	3.062	4.923	10.79	72.7	32,996	4,092	63.6	119.5	101.0	162.4	356.0
TKY12	CONT	79	0.32	0.66	2.939	0.141	1.72	42.3	19,199	1,517	6.1	12.7	56.4	2.7	33.0

[*] Tech Research Farm; [†] Turkey Farm; [‡] Dairy Farm

Table E.1 (continued)

Site	Treatment	TSS (mg/L)	Ortho-P (mg/L)	Total P (mg/L)	Nitrate (mg/L)	Ammonia (mg/L)	TKN (mg/L)	Runoff Weight (lb)	Volume (mL)	Load TSS (mg)	Load Ortho-P (mg)	Load Total P (mg)	Load Nitrate (mg)	Load Ammonia (mg)	Load TKN (mg)
DF1[‡]	LD	183	0.29	0.78	3.055	1.142	4.35	60.0	27,232	4,983	7.8	21.2	83.2	31.1	118.4
DF2	TKY	120	1.72	2.06	1.930	1.383	5.23	64.1	29,093	3,491	50.0	60.0	56.1	40.2	152.1
DF3	TKY	153	1.83	3.80	1.339	1.267	6.77	63.7	28,911	4,423	53.0	109.9	38.7	36.6	195.7
DF4	CP	262	1.55	3.99	0.096	0.301	14.19	49.7	22,557	5,910	35.0	90.0	2.2	6.8	320.1
DF5	CONT	64	0.08	0.60	1.894	0.000	1.32	27.0	12,254	784	1.0	7.3	23.2	0.0	16.2
DF6	CONT	51	0.11	0.28	2.152	0.086	1.27	42.4	19,244	981	2.0	5.4	41.4	1.6	24.3
DF7	TKY	131	2.34	3.69	2.721	2.938	13.95	70.5	31,989	4,191	74.9	118.1	87.0	94.0	446.2
DF8	CP	189	1.71	5.59	0.190	0.054	17.75	49.3	22,380	4,230	38.3	125.2	4.3	1.2	397.2
DF9	CP	117	1.26	3.22	0.054	0.055	11.00	40.5	18,382	2,151	23.2	59.1	1.0	1.0	202.2
DF10	LD	146	2.12	3.27	2.511	3.451	14.07	68.8	31,226	4,559	66.1	102.0	78.4	107.8	439.2
DF11	LD	169	3.26	5.02	3.086	3.906	19.44	42.2	19,153	3,237	62.3	96.1	59.1	74.8	372.3
DF12	CONT	71	0.05	0.07	2.399	0.000	2.56	7.9	3,586	255	0.2	0.2	8.6	0.0	9.2

[*] Tech Research Farm; [†] Turkey Farm; [‡] Dairy Farm

Table E.2: Average Nutrient Loading from Release Plots

Tech Research Farm	Dissolved Phosphorous (g/ha)	Nitrate (g/ha)	Ammonia (g/ha)	Total Nitrogn (g/ha)
Liquid Dairy	357	416	473	1,709
Cowpie	311	17	264	2,923
Turkey	180	287	183	938
Control	82	341	82	431
Dairy Farm	**Dissolved Phosphorous (g/ha)**	**Nitrate (g/ha)**	**Ammonia (g/ha)**	**Total Nitrogn (g/ha)**
Liquid Dairy	454	736	712	3,100
Cowpie	322	25	30	3,065
Turkey	593	606	570	2,647
Control	11	244	5	166
Turkey Farm	**Dissolved Phosphorous (g/ha)**	**Nitrate (g/ha)**	**Ammonia (g/ha)**	**Total Nitrogn (g/ha)**
Liquid Dairy	529	756	1,057	2,879
Cowpie	185	39	116	1,959
Turkey	9	123	5	105
Control	45	545	22	272

Table E.3: Nutrient concentrations from Transport Plots, Simulation 1

Nutrient Concentration Present in Runoff from the Transport Plots								
Transport Plots - Simulation 1								
Site	Sample No.	Date	TSS	Ortho-P	Total P	Nitrate	Ammonia	TKN
T-1	1	10/15/2002	343	0.41	0.48	0.924	2.037	4.06
T-1	2	10/15/2002	37	0.04	0.52	0.549	0.098	1.68
T-1	3	10/15/2002	222	0.38	0.76	0.566	0.325	2.14
T-1	4	10/15/2002	112	0.56	0.69	0.570	0.461	2.47
T-1	5	10/15/2002	84	0.69	1.00	0.584	0.567	2.30
T-1	6	10/15/2002	1	0.72	1.00	0.574	0.650	2.39
T-1	7	10/15/2002	34	0.74	0.91	0.589	0.547	2.06
T-1	8	10/15/2002	30	0.71	0.87	0.589	0.508	2.10
T-1	9	10/15/2002	9	0.75	0.75	0.554	0.694	1.67
T-2	2	10/15/2002	588	0.05	0.37	0.596	0.198	2.64
T-2	3	10/15/2002	200	0.36	0.62	0.618	0.960	4.47
T-2	4	10/15/2002	97	0.50	1.30	0.033	1.056	6.87
T-2	5	10/15/2002	107	0.62	1.23	0.351	1.128	6.94
T-2	6	10/15/2002	14	0.56	1.58	0.024	0.932	9.56
T-2	7	10/15/2002	4	0.76	1.22	0.449	1.099	9.31
T-3	1	10/15/2002	90	0.78	1.08	0.543	0.522	2.16
T-3	2	10/15/2002	79	0.87	1.40	0.559	0.499	2.79
T-3	3	10/15/2002	67	1.07	1.33	0.614	0.283	2.43
T-3	4	10/15/2002	75	0.94	1.33	0.826	0.580	2.44
T-3	5	10/15/2002	47	0.97	1.35	0.919	0.752	2.36
T-3	6	10/15/2002	44	1.06	1.10	0.821	1.115	2.20
T-3	7	10/15/2002	40	1.21	1.47	0.710	1.209	2.07
T-3	8	10/15/2002	38	1.21	1.31	0.726	1.182	2.07
T-3	9	10/15/2002	12	1.36	1.44	0.728	1.243	2.00
T-4	1	10/15/2002	373	0.08	0.32	0.634	0.195	2.24
T-4	2	10/15/2002	158	0.08	0.27	0.659	0.196	1.74
T-4	3	10/15/2002	91	0.09	0.24	0.661	0.196	1.65
T-4	4	10/15/2002	74	0.09	0.18	0.661	0.203	1.44
T-4	5	10/15/2002	49	0.10	0.25	0.673	0.222	1.56
T-4	6	10/15/2002	17	0.13	0.20	0.672	0.250	1.32
T-5	1	10/15/2002	81	0.93	1.03	0.886	1.108	3.39
T-5	2	10/15/2002	85	1.00	1.30	0.781	1.065	3.08
T-5	3	10/15/2002	85	0.90	1.06	1.028	1.054	3.47
T-5	4	10/15/2002	81	0.93	1.15	0.810	1.054	3.18
T-5	5	10/15/2002	78	0.90	1.09	0.867	1.041	3.60
T-5	6	10/15/2002	73	1.04	1.07	0.710	0.968	3.09
T-5	7	10/15/2002	71	1.13	1.42	0.678	1.035	3.38
T-5	8	10/15/2002	55	1.25	1.29	0.691	1.130	3.43

Table E.3 (continued)

Nutrient Concentration Present in Runoff from the Transport Plots								
Transport Plots - Simulation 1								
Site	**Sample No.**	**Date**	**TSS**	**Ortho-P**	**Total P**	**Nitrate**	**Ammonia**	**TKN**
T-5	9	10/15/2002	46	1.31	1.42	0.662	1.241	2.76
T-5	10	10/15/2002	43	1.33	1.39	0.687	1.363	2.57
T-5	11	10/15/2002	38	1.33	1.36	0.652	1.419	2.58
T-5	12	10/15/2002	19	1.02	1.08	0.589	1.134	1.70
T-6	1	10/15/2002	80	1.30	1.36	0.646	1.342	2.60
T-6	2	10/15/2002	44	1.75	2.05	0.653	1.598	2.90
T-6	3	10/15/2002	42	1.68	1.95	0.699	1.678	2.92
T-6	4	10/15/2002	44	1.81	1.99	0.724	1.894	3.05
T-6	5	10/15/2002	35	1.95	2.11	0.671	1.855	2.97
T-6	6	10/15/2002	36	1.93	2.18	0.701	1.787	2.75
T-6	7	10/15/2002	31	2.00	2.08	0.698	1.817	2.85
T-6	8	10/15/2002	21	2.20	2.46	0.720	1.901	2.75
T-7	1	10/15/2002	103	0.68	0.91	0.442	1.748	6.24
T-7	2	10/15/2002	108	0.81	1.02	0.156	1.761	7.26
T-7	3	10/15/2002	93	0.97	2.03	0.292	1.904	12.52
T-7	4	10/15/2002	97	1.03	1.95	0.016	2.076	13.79
T-7	5	10/15/2002	82	1.17	2.03	0.045	2.260	12.83
T-7	6	10/15/2002	93	1.15	2.39	0.009	2.147	13.03
T-7	7	10/15/2002	90	1.24	2.14	0.006	2.277	13.64
T-7	8	10/15/2002	88	1.34	2.66	0.006	2.512	15.72
T-7	9	10/15/2002	95	1.46	2.58	0.014	2.393	16.16
T-7	10	10/15/2002	92	1.56	2.66	0.024	2.862	14.71
T-7	11	10/15/2002	51	1.56	2.85	0.011	3.113	15.85
T-8	1	10/15/2002	82	0.03	0.03	0.460	0.294	2.33
T-8	2	10/15/2002	105	0.01	0.21	0.514	0.271	1.84
T-8	3	10/15/2002	97	0.00	0.18	0.466	0.205	1.54
T-8	4	10/15/2002	69	0.00	0.19	0.459	0.174	2.05
T-8	5	10/15/2002	71	0.01	0.17	0.462	0.137	1.56
T-8	6	10/15/2002	65	0.00	0.12	0.482	0.078	1.48
T-8	7	10/15/2002	22	0.04	0.10	0.475	0.157	1.25
Rainwater	1	10/15/2002	39	0.00	0.07	0.479	0.194	3.00

Table E.4: Nutrient concentrations from Transport Plots, Simulation 2

Nutrient Concentration Present in Runoff from the Transport Plots								
Transport Plots – Simulation 2								
Site	**Sample No.**	**Date**	**TSS**	**Ortho-P**	**Total P**	**Nitrate**	**Ammonia**	**TKN**
T-1	1	10/16/2002	84	0.40	0.62	0.940	0.782	1.99
T-1	2	10/16/2002	50	0.50	0.72	0.966	0.930	1.74
T-1	3	10/16/2002	59	0.42	0.51	0.987	0.930	1.77
T-1	4	10/16/2002	35	0.41	0.48	0.959	0.691	1.55
T-1	5	10/16/2002	448	0.31	0.65	0.967	0.480	2.19
T-1	6	10/16/2002	101	0.31	0.48	0.900	0.430	1.57
T-1	7	10/16/2002	30	0.29	0.34	0.847	0.388	1.21
T-2	1	10/16/2002	398	0.00	0.34	0.876	0.112	1.96
T-2	2	10/16/2002	69	0.45	0.94	0.042	1.203	6.17
T-2	3	10/16/2002	66	0.52	0.89	0.157	1.400	6.36
T-2	4	10/16/2002	57	0.56	0.89	0.020	1.412	7.10
T-2	5	10/16/2002	37	0.61	1.22	0.002	1.824	7.57
T-2	6	10/16/2002	31	0.62	1.16	0.001	1.813	7.39
T-2	7	10/16/2002	31	0.62	1.15	0.001	1.742	7.40
T-2	8	10/16/2002	15	0.63	1.00	0.000	1.389	6.82
T-3	1	10/16/2002	84	0.39	0.47	0.963	0.622	1.93
T-3	2	10/16/2002	78	0.52	0.83	1.093	0.641	1.81
T-3	3	10/16/2002	80	0.40	0.66	1.347	0.572	2.06
T-3	4	10/16/2002	40	0.71	1.29	1.198	1.202	2.18
T-3	5	10/16/2002	32	0.79	0.88	1.142	1.065	1.97
T-3	6	10/16/2002	28	0.83	1.05	1.076	1.036	1.94
T-3	7	10/16/2002	26	0.84	1.05	1.036	1.027	1.85
T-3	8	10/16/2002	17	0.82	0.88	0.975	0.984	1.71
T-3	9	10/16/2002	3	0.81	1.04	0.961	0.924	1.52
T-4	1	10/16/2002	187	0.02	0.08	0.770	0.197	1.71
T-4	2	10/16/2002	199	0.00	0.26	0.788	0.080	1.61
T-4	3	10/16/2002	142	0.05	0.19	1.080	0.124	1.76
T-4	4	10/16/2002	55	0.09	0.19	1.050	0.153	1.48
T-4	5	10/16/2002	41	0.08	0.17	0.956	0.164	1.29
T-4	6	10/16/2002	27	0.09	0.12	0.911	0.221	1.28
T-4	7	10/16/2002	23	0.07	0.09	0.872	0.142	1.12
T-4	8	10/16/2002	18	0.06	0.07	0.861	0.064	1.20
T-4	9	10/16/2002	4	0.08	0.08	0.860	0.046	1.13

Table E.4 (continued)

Nutrient Concentration Present in Runoff from the Transport Plots								
Transport Plots – Simulation 2								
Site	Sample No.	Date	TSS	Ortho-P	Total P	Nitrate	Ammonia	TKN
T-5	1	10/16/2002	80	0.26	0.44	0.924	0.540	2.13
T-5	2	10/16/2002	82	0.29	0.54	1.089	0.543	3.49
T-5	3	10/16/2002	51	0.50	0.54	1.150	0.652	2.25
T-5	4	10/16/2002	28	0.73	0.90	1.150	0.848	2.11
T-5	5	10/16/2002	27	0.77	0.80	1.150	0.882	2.08
T-5	6	10/16/2002	24	0.76	0.88	1.134	0.848	2.08
T-5	7	10/16/2002	24	0.72	0.74	1.115	0.813	1.66
T-5	8	10/16/2002	17	0.67	0.74	1.083	0.717	1.63
T-5	9	10/16/2002	41	0.90	0.93	0.977	1.074	1.45
T-6	1	10/16/2002	30	1.07	1.14	1.006	1.295	2.21
T-6	2	10/16/2002	6	0.64	1.01	1.047	0.713	2.51
T-6	3	10/16/2002	49	0.86	1.21	0.897	1.264	2.13
T-6	4	10/16/2002	23	1.07	1.15	1.077	1.245	2.08
T-6	5	10/16/2002	22	1.05	1.33	0.965	1.283	3.07
T-6	6	10/16/2002	18	1.05	1.05	1.035	1.150	1.86
T-6	7	10/16/2002	17	0.99	1.00	0.990	1.095	1.68
T-6	8	10/16/2002	9	1.08	1.25	0.955	1.103	1.80
T-7	1	10/16/2002	114	0.54	0.77	0.267	1.599	5.53
T-7	2	10/16/2002	72	0.88	1.71	0.018	2.336	10.09
T-7	3	10/16/2002	54	0.85	1.60	0.009	2.136	11.00
T-7	4	10/16/2002	57	0.96	1.55	0.000	2.613	10.77
T-7	5	10/16/2002	66	1.04	1.71	0.009	2.696	10.94
T-7	6	10/16/2002	51	0.98	1.77	0.004	2.513	11.30
T-7	7	10/16/2002	63	1.03	2.11	0.000	2.561	12.95
T-7	8	10/16/2002	55	1.03	1.82	0.001	2.563	10.67
T-7	9	10/16/2002	46	1.04	1.99	0.001	2.551	11.31
T-8	1	10/16/2002	89	0.05	0.06	0.725	0.287	1.95
T-8	2	10/16/2002	37	0.06	0.67	0.096	0.702	11.32
T-8	3	10/16/2002	31	0.05	0.10	0.784	0.221	1.44
T-8	4	10/16/2002	30	0.05	0.05	0.754	0.168	1.39
T-8	5	10/16/2002	27	0.02	0.04	0.764	0.180	1.17
T-8	6	10/16/2002	19	0.02	0.04	0.767	0.244	1.22
T-8	7	10/16/2002	21	0.02	0.02	0.753	0.200	1.12
T-8	8	10/16/2002	16	0.01	0.01	0.737	0.171	1.13
T-8	9	10/16/2002	7	0.02	0.02	0.726	0.156	1.13
Rainwater	2	10/16/2002	1	0.01	0.01	0.799	0.000	0.83

Table E.5: Nutrient Flow-weighted concentrations calculated using flow data and sample concentrations

Treatment	Plot	Run 1 TSS (mg/L)	DP (mg/L)	SBP (mg/L)	TP (mg/L)	Nitrate (mg/L)	Ammonia (mg/L)	TKN (mg/L)	Run 2 TSS (mg/L)	DP (mg/L)	SBP (mg/L)	TP (mg/L)	Nitrate (mg/L)	Ammonia (mg/L)	TKN (mg/L)
Liquid Dairy	T1	71.04	0.67	0.19	0.86	0.62	0.73	2.38	136.41	0.37	0.16	0.53	0.94	0.64	1.74
Cowpies	T2	265.98	0.38	0.52	0.90	0.40	0.74	5.74	51.90	0.57	0.46	1.02	0.05	1.52	6.87
Turkey Litter	T3	40.03	1.16	0.18	1.34	0.75	1.06	2.15	24.12	0.79	0.21	1.00	1.05	1.00	1.82
Control	T4	101.46	0.09	0.14	0.23	0.66	0.21	1.60	33.54	0.07	0.05	0.12	0.92	0.14	1.26
Liquid Dairy	T5	48.80	1.23	0.09	1.32	0.68	1.22	2.82	30.46	0.73	0.07	0.80	1.10	0.84	1.91
Turkey Liltter	T6	34.60	1.95	0.19	2.14	0.70	1.82	2.86	20.89	1.00	0.15	1.16	0.99	1.16	2.14
Cowpies	T7	87.36	1.41	1.12	2.53	0.02	2.56	14.98	57.39	0.99	0.82	1.81	0.01	2.52	11.25
Control	T8	68.97	0.01	0.14	0.15	0.47	0.16	1.66	24.60	0.03	0.05	0.08	0.71	0.23	1.91

Table E.6: Nutrient Load calculated using flow data and sample concentrations

		Run 1							Run 2						
Treatment	**Plot**	**TSS (mg/L)**	**DP (mg/L)**	**SBP (mg/L)**	**TP (mg/L)**	**Nitrate (mg/L)**	**Ammonia (mg/L)**	**TKN (mg/L)**	**TSS (mg/L)**	**DP (mg/L)**	**SBP (mg/L)**	**TP (mg/L)**	**Nitrate (mg/L)**	**Ammonia (mg/L)**	**TKN (mg/L)**
Liquid Dairy	T1	621.6	5.8	1.7	7.5	5.4	6.3	20.8	4814.1	13.0	5.8	18.8	33.2	22.5	61.4
Cow Pies	T2	10522.9	15.1	20.7	35.8	15.8	29.1	227.2	3275.8	35.8	28.9	64.7	3.4	95.8	433.9
Turkey Litter	T3	854.7	24.7	3.9	28.6	16.1	22.7	45.9	1187.4	39.0	10.3	49.3	51.9	49.1	89.5
Control	T4	3057.0	2.8	4.1	6.9	19.9	6.2	48.3	1203.8	2.7	1.7	4.4	32.9	4.9	45.3
Liquid Dairy	T5	5553.4	140.4	10.1	150.5	77.1	139.0	321.2	4194.7	100.1	9.6	109.7	152.1	115.6	263.0
Turkey Liltter	T6	1623.8	91.4	9.0	100.4	32.8	85.5	134.0	2379.6	114.4	17.4	131.8	113.3	132.4	244.2
Cow Pies	T7	3828.3	61.7	49.2	110.9	1.0	112.0	656.3	9504.7	164.1	135.3	299.4	1.1	417.8	1862.3
Control	T8	1358.5	0.2	2.7	2.9	9.3	3.1	32.7	3621.7	4.2	7.3	11.5	104.6	33.9	281.1

VITA

Michelle Lynn (Peterie) Soupir was born on July 17, 1977 in Wichita, Kansas to Jerome and Nancy Peterie. She married Steven Soupir on August 10, 2002. She received a Bachelor of Science in Biological and Agricultural Engineering from Kansas State University in 1999. As an undergraduate at Kansas State, Michelle was named the National ASAE Student Engineer of the Year and was granted membership in Alpha Epsilon, Tau Beta Pi, Order of Omega, Gamma Sigma Delta and Golden Key honor societies. She placed second in the ASAE K.K. Barnes Undergraduate Paper Competition in 1998. She also participated in the National Science Foundation Research Experience for Undergraduate Program at Virginia Tech during the summer of 1999. Following graduation, she was employed as an Environmental Engineer for Camp Dresser and McKee, Inc in Kansas City, Missouri. In August 2001, she began work as a graduate research assistant in the Biosystems Engineering Department at Virginia Tech to continue her studies towards a Masters of Science degree. While working on her M.S. degree, Michelle was awarded a College of Engineering Pratt Fellowship and the William R. Walker Graduate Fellow Award from the Virginia Water Resources Research Center. Michelle has been awarded a USDA National Need Fellowship and a Cunningham Fellowship from Virginia Tech to continue her studies towards a Ph.D. degree in the fall of 2003.